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Update on the management of patients with intermediate and poor-risk testicular germ cell tumors and new biological insights

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1. Introduction

Germ cell tumors (GCT) constitute the most common solid tumor in men between the ages of 15 and 40 years. The incidence has increased over the last two decades, however, mortality rates remain low and most men are cured [1,2].

Testicular germ cell tumors (TGCT) account for approximately 1% of all newly diagnosed male cancers worldwide, and in 2012, there was estimated to be over 10,000 deaths from this disease [1]. Interestingly, the incidence of testicular cancer in low to middle income countries is about 20% of that observed in more developed countries [3].

The causes of the increasing incidence are not clear. Some authors postulate that the increase is due to environmental exposures such as dietary changes or pollution [2], while others consider that the geographical variability of incidence is due to access to the health system and failure to establish the diagnosis [4]. Some recognized predisposing factors in the development of GCT are cryptorchidism, Klinefelter's syndrome, human immunodeficiency virus infection, exposure to estrogen [5] or family history [6,7].

Approximately 90% of GCT arise in the testicles, though extragonadal primary tumors of the retroperitoneum, mediastinum, and pineal gland may occur. In this review we will focus on metastatic germ cell tumors of adolescents or young adults with primary testicular tumor.

According to the new WHO classification (2017) TGCT are classified in two groups: seminomas and non-seminomas. Within the non-seminomas, four different varieties can be distinguished: Embryonal carcinoma, yolk sac tumor, choriocarcinoma, and teratoma [8]. Around 40% of seminomas and 20% of non-seminomas are pure; the majority of the cases are diagnosed as mixed forms with a combination of the different aforementioned histologies.

While most extragonadal tumors are more challenging to treat, in general, GCT have an excellent overall prognosis with 5-year survival rates of > 95% in developed countries [9].

Adequate risk assessment is essential to determining optimal treatment. The International Germ Cell Cancer Collaborative Group (IGCCCG) established a prognosis-based classification system which has been applied to clinical practice and clinical trial design since 1997. Metastatic GCT are classified as low (60%), intermediate (25%) and high (15%) risk based on the primary site, elevation of alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG) and lactic dehydrogenase (LDH), and the presence of non-pulmonary visceral metastases such as liver, bone and brain [10] Table 1. Extrapulmonary metastases lead to a worse prognosis. Specifically, Feldman et al highlighted, in a retrospective study, brain metastasis as a factor of poor prognosis and the need for multimodal management [11].

Each risk group classification correlates with response to chemotherapy and cure [10].

Even in the metastatic setting, with suitable treatment, a 5-year survival rate is expected in 90% of patients with good risk IGCCCG classification at time of diagnosis. But the percentage drops considerably in those of intermediate risk up to 80% and up to 50–60% in poor risk [9,12].

The aim of this article is to present a thorough overview of the recent advances in management of intermediate- and poor-risk metastatic TGCT and also discuss how emerging knowledge of tumor biology may aid to improve outcomes in poor prognostic group patients.

2. First line treatment

Clinical guidelines recommend 4 cycles of BEP (bleomycin, etoposide,cisplatin) or 4 cycles of VIP (etoposide, ifosfamide, cisplatin) for patients who may not tolerate bleomycin as first-line treatment in patients of intermediate or high risk [13,14]. In phase III randomized trials (ECOG, SWOG and CALGB) BEP was compared to VIP without any differences in terms of efficacy identified, although VIP was associated with poor toxicity profile and more side effects [15].

Up to 50% of patients with poor-risk disease experience failure of first-line platinum chemotherapy [16]. To improve these results, in terms of response and survival, different approaches have been evaluated, such as the intensification of chemotherapy treatment, the addition of new drugs to the BEP combination, or testing new combinations of drugs.

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Table 1
First line of treatment.

Trial	Year	Study design	Phase	Sample size	Treatment arms	Results
Husband et al.	1992	Prospective	II	53	Single arm: 2 cycles of POMB, then alternating ACE/POMB for a minimum of 7 cycles and 2–4 cycles after the tumor markers had become normal.	The overall CR rate was 62% [95% confidence interval (CI) 49–75%), and for patients with large or very large volume disease the CR rate was 56% (95% CI 41–71%). The overall 5 year survival was 61%, Comparison with previous studies suggests that POMB/ACE chemotherapy is not superior to BEP, even in patients with adverse prognostic factors.
Kaye et al.	1998	Prospective	III	380	Two arms: -BEP/EP - BOP/VIP-B	There was no significant difference in proportion of CR (57% with BEP/EP and 54% with BOP/VIP-B; $p = 0.69$). There was no significant difference in time to first disease progression, or failure-free or overall survival ($p = 0.21$, 0.101, and 0.190, respectively).
Motzer et al.	2007	Prospective	III	219	Two arms: - 4 cycles of BEP - 2 cycles of BEP followed by 2 cycles of HDCT	CR 55% to BEP and 56% to BEP + HDCT. The 1 year durable CR was 48% after BEP alone and 52% after BEP + HDCT ($p = 0.53$) Secondary endpoint, unsatisfactory marker decline after 2 cycles of BEP ($N = 69$): 1 year durable CR was 61% for HDCT versus 34% for BEP alone ($p = 0.03$).
Mardiak et al.	2007	Prospective	II	24	Single arm: 4 cycles of T-BEP	CCR or PR with negative tumor markers was achieved in 13 patients (54.2%; CI 95%: 34.3–74.1%). Median survival was not achieved and median time-to-progression is 9.5 months. Patients treated with 1st line T-BEP didn't achieve higher response rate or time to progression. Median survival was not achieved and 75% of the patients survive more than 30.8 months
Tryakin et al.	2011	Prospective	II	51	Single arm: T-BEP: the number of cycles (4–6) was dependent on the normalization of tumor markers	The PFS rate at 1 year after chemotherapy was 58% (29 of 50 patients)
Wit et al.	2012	Prospective	III	337	Two arms: - 4 cycles of T-BEP - 4 cycles of BEP	PFS at 3 years was 79.4% in the T-BEP group vs 71.1% in the BEP group (HR 0.73; CI, 0.47 to 1.13; $p = 0.153$). The study recruited a smaller-than-planned number of patients and included 7.7% ineligible patients. When ineligible patients were excluded, the analysis of all eligible patients demonstrated a 12% superior 3-year PFS with T-BEP, which was statistically significant
Feldman et al.	2014	Prospective	II	60	Single-arm: 4 cycles of TIP once every 3 weeks.	CR 68%; FR 80%; 3-year overall survival 91%, Intermediate-risk survival 100% and poor risk survival 87%
Fizazi et al.	2014	Prospective	III	263	After patients had received 1 cycle of BEP, AFP and hCG were assessed. Three arms: - Patients with favorable decline continued BEP - Patients with an unfavorable decline were randomized to receive: -BEP -Dose-dense regimen (paclitaxel, BEP plus oxaliplatin (2 cycles), followed by cisplatin, ifosfamide and bleomycin (2 cycles)	The 3-year PFS unfavorable tumor marker decline was 59% in the dose-dense regimen vs 48% in the BEP group with a HR of 0.66.
Huddart et al.	2015	Prospective	II	89	Two arms: −4cycles of BEP −6 cycles of CBOP/BEP	The 1-year PFS rates were 43% for BEP and 65% for CBOP/BEP; HR: 0.59 (95% CI, 0.33–1.06). Two-year survival rates were 61% for BEP and 67% for CBOP/BEP (HR: 0.78 [95% CI, 0.41–1.50]).

CR: Complete response; FR: Favorable Response; BEP: bleomycin, etoposide, and cisplatin; CBOP-BEP: carboplatin, bleomycin, vincristine, cisplatin-BEP; BOP / VIP-B: bleomycin, vincristine, cisplatin / etoposide, ifosfamide, cisplatin and bleomycin; POMB / ACE: cisplatin, vincristine, methotrexatee, bleomycin, actinomycin D, cyclophosphamide, etoposide; T-BEP: paclitaxel-bleomycin, etoposide and cisplatin.

The addition of carboplatin to an induction-type regimen has been studied in a phase 2 trial compared to BEP. Here, CBOP-BEP (carboplatin, bleomycin, vincristine, cisplatin-BEP) showed no benefit in progression-free survival (PFS) or in overall survival (OS) and but was associated with greater toxicity [17].

The addition of ifosfamide to BEP compared with BEP was tested in another randomized trial. BOP / VIP-B (bleomycin, vincristine, cisplatin / etoposide, ifosfamide, cisplatin and bleomycin) was compared to BEP/EP and there was no evidence of an improvement in response rate or survival [18].

Among other drugs, actinomycin D and methotrexate were tested with BEP components in a single-arm trial. POMB / ACE (cisplatin, vincristine, methotrexatee, bleomycin, actinomycin D, cyclophosphamide, etoposide) was not superior to BEP when compared to historical results of previous studies [19]. To date, randomized clinical trials of this regimen compared to BEP have not been done.

Another combination of drugs such as T-BEP (paclitaxel-bleomycin, etoposide and cisplatin) has shown promising results, although it has not yet shown any benefit compared to BEP in patients with intermediate and poor prognosis GCT [20–22].

In the case of patients with intermediate prognosis, a phase 3 study did not demonstrate superiority of T-BEP. In this study, some patients of good and intermediate risk were included erroneously. These patients were excluded and a new analysis was performed. In this post hoc analysis, a 12% superiority was demonstrated in 3-year PFS with T-BEP, which was statistically significant [22].

With regard to patients with poor prognosis, the studies performed are phase 2. The toxicity was high with T-BEP and no higher response rates or time to progression were achieved [20,21], although in one of the studies the overall survival was surprisingly long [20].

Combination regimens such as TIP are accepted as a salvage therapy in second line treatment [13,14,23]. Recently, TIP has also been explored with promising results and an acceptable safety profile in patients with intermediate and poor prognosis in the first line setting. A prospective, multicentre, single-arm phase II trial for previously untreated and poor or intermediate risk patients evaluated the safety and efficacy of 4 cycles of TIP (paclitaxel 240 mg/m^2 over two days, ifosfamide 6 g/m^2 over 5 days with mesna support, and cisplatin 100 mg/m^2 over 5 days) once every 3 weeks with granulocyte colony-stimulating factor support (G-CSF). Patients with residual or viable tumour after surgery received two additional cycles of TIP. Sixty-eight percent achieved a complete response (CR) and 80% of favourable responses (FR) were described, including CR and partial responses (PR) and biomarkers normalization [24]. When compared to reported retrospective series [25], an improvement in response rate was observed. By comparison, the Memorial Sloan Kettering Cancer Center (MSKCC)-led Intergroup randomized trial showed a 55% CR and 60% FR rate after 4 cycles of standard BEP.

Furthermore, TIP reached higher survival rates with an OS of 91% at 3-years, including 100% 3-year OS for intermediate risk and 87% for poor risk patients. Conversely, 2-year OS was 83% for intermediate risk and 69% for high risk patients treated with BEP [24,25]. However, a well-designed randomized clinical trial comparing TIP vs BEP as a first line therapy is mandatory before establishing a new standard of care in this population. Currently, a study in patients with previously untreated intermediate- and poor-risk GCTs is ongoing (NCT01873326) [26].

The response to high dose chemotherapy (HDCT) with autologous stem cell transplantation in patients pretreated with relapsed GCT has led to the examination of this approach as a possible first-line treatment in poor prognosis patients.

Between 1990 and2000, the use of chemotherapy was investigated in several phase 2 trials with promising results [27], which have not been confirmed in phase 3 studies.

For example, a large phase 3 trial published in 2007 by Motzer et al. where patients were randomized to receive either 4 cycles of standard BEP or two cycles of BEP followed by two cycles of HDCT (high-dose carboplatin, etoposide, and cyclophosphamide). The CR rate was 55% for BEP and 56% for BEP plus HDCT. The 1-year durable CR rate was 48% after BEP alone and 52% after BEP plus HDCT ($p = 0.53$). Notably, the adverse events were higher for BEP plus HDCT group. [25].

Another important prospective randomized trial compared high dose sequential VIP to four cycles of BEP, but also failed to demonstrate a benefit for HDCT in OS, CR rates or PFS [28].

In conclusion, the routine inclusion of HDCT in the first-line treatment has not shown an improvement in outcomes and, therefore, is not used in the first-line setting.

The first-line treatment studies are summarized in Table 1.

2.1. Tumor markers kinetics for personalized treatment

As aforementioned, serum tumor biomarkers are used in assigning prognostic risk groups as well as in diagnosis, staging, and assessing response to treatment. AFP, HCG and LDH are the main biomarkers currently used in TGCT.

Ninety percent of non-seminomatotous GCT present with increased serum biomarkers and up to 30% of seminomas present with an elevated HCG [29–31].

AFP is associated to non-seminomatous GCT. Therefore, if there is an increased AFP in pure testicular seminoma, it is assumed that an undetected focus of non-seminoma is likely present [29–31]. LDH is less specific and its concentration is normally proportional to tumor volume [29–31].

In an effort to improve outcomes in the first-line setting, some studies have evaluated the prognostic value of tumor marker kinetics during first line chemotherapy treatment.

In a phase 3 trial patients were randomized to receive 4 cycles of BEP or 2 cycles of BEP followed by 2 cycles of HDCT. This trial included a secondary analysis aimed at finding out if there were differences in the 1-year durable CR regarding the treatment received among patients with an unsatisfactory decrease of the marker after 2 cycles of BEP (no normalization of serum tumor markers). The 1-year durable CR proportion was 61% in patients who received HDCT versus 34% in patients who received BEP alone ($p = 0.03$). The results of this secondary analysis demonstrated that patients with early chemotherapy resistance, demonstrated by an unsatisfactory decline of serum tumor markers, experienced a higher durable CR when treatment was switched to HDCT. The conclusions of this study were limited due to small size and non-randomization, however the authors support a personalized-treatment strategy development for GCTs based on tumor markers kinetics [25].

Following the same line of research, Fizazi et al. published the results of a randomized, phase III, multicenter, international clinical trial (GETUG 13), evaluating the role of tumor marker decline in patients with poor-risk GCT [32]. In this study, AFP and HCG were assessed between days 18 and 21 after one cycle of BEP. Patients with a favorable decline continued BEP for another 3 cycles; patients with an unfavorable decline were randomized to receive either 3 cycles of BEP or a dose-dense regimen consisting of paclitaxel, BEP, plus oxaliplatin (2 cycles), followed by cisplatin, ifosfamide and bleomycin (2 cycles) with G-CSF support. (The definition of favorable and unfavorable pattern of decrease in serum tumor markers was established from a logarithmic formula described in the supplementary material of the article and supported by an online application system in a Gustave-Roussy web) [32].

The 3-year PFS in patients with an unfavorable tumor marker decline was 59% in the dose-dense regimen versus 48% in the BEP group (HR 0.66 [0.44–1.00], 95% CI, $p = 0.05$). This represents a reduction of 34% in the risk of progression or death. In addition, in patients with an unfavorable tumor marker decline, only 6% of patients in the dose-dense arm required HDCT with an autologous stem cell transplant, compared to 16% of patients in the BEP arm ($p = 0.0015$) and this endpoint is itself meaningful.

More grade 3–4 neurotoxicity (8% versus 1%) and grade 1–3 auditory toxicity (48% versus 29%) occurred in the dose-dense arm, with no excess febrile neutropenia or toxic deaths.

The authors concluded that this study ought to change clinical practice, and that personalization of treatment according to the kinetics

of tumor marker decline in patients with poor prognosis is viable and clinically beneficial. Whether using dose-dense chemotherapy in those with suboptimal tumor marker decline is the optimal strategy remains unknown and ideally should be validated in a prospective clinical trial [32]. Today, this is the standard of treatment in France, but in other parts of the world it is not widely adopted [33].

Lastly, other cancer biomarkers based on microRNA, cell circulating mitochondrial DNA or DNA methylation are being explored but have yet to be validated and implemented in clinical practice [29].

3. Second line of treatment

Approximately 10–30% of GCT exhibit resistance to platinum-based regimens. However, roughly 50% of patients will be cured with second-line salvage therapy.

Nowadays, clinical guides recommend as second-line treatment options VeIP (vinblastine, ifosfamide, and cisplatin), TIP and/or HDCT [13,14]. However, the optimal selection of patients has yet to be fully defined [24,25].

These regimens have not been compared, so the choice of one over the others is often based on prognostic factors, the patient`s performance status at the time of disease relapse, and physician experience. In clinical practice, patients with unfavorable prognostic features, including extragonadal primary site, incomplete response to first-line therapy, high-volume disease, high levels of serum markers, IGCCCG intermediate- or poor-risk classification at diagnosis, or platinum refractory disease, should be referred to expert centers for HDCT or clinical trials [13,14]

HDCT is an aggressive regimen mostly based on carboplatin and etoposide combinations that has been especially explored in poor risk patients (Table 2). HDCT has demonstrated its curative benefit in second or successive lines from retrospective studies. A single-center study from Indiana University showed disease-free survival (DFS) rates at 48 months of 70% with second-line and 45% with third-line therapy [34]. DFS rates were 51% for poor-risk patients, and 68% and 45% for cisplatin-sensitive and -refractory subgroups, respectively [34].

Recently, Indiana University has reported results from a patient population different from the previous study published in 2007 [34], but with the same treatment regimen. To date, it is the largest retrospective analysis of cisplatin-refractory GCT treated with HDCT with a recruitment of 364 patients. This study showed comparable PFS at 2 years comparable to that published in the year 2007. Additionally, several prognostic variables were identified: previous platinum-sensitivity, primary tumor location (gonadal or extragonadal), prognostic risk classification by IGCCCG system, HCG levels and the number of line in which HDCT was used [35].

As mentioned above, it is unclear whether HDCT improves outcomes over standard dose salvage treatment as second-line therapy [13,14]. In an attempt to answer this question, a prospective phase I/II trial evaluated salvage therapy with HDCT and paclitaxel and ifosfamide as induction chemotherapy in patients with previously treated GCTs and poor prognostic features [36] (Table 2). There were 50% complete and 8% partial responses with negative markers; 5-year DFS was 47% and OS was 52% [36]. The authors concluded that this regimen may be a new standard of treatment in patients with poor prognostic features. However, from our point of view, prospective randomized phase III clinical trials with HDCT alone as control group are mandatory to confirm these results.

Sequential HDCT has also been considered although the results have not been favorable. Pico et al. published a randomized phase 3 clinical trial comparing 4 cycles of VIP or VeIP or 3 cycles of this combination and 1 cycle of HDCT (high-dose carboplatin, etoposide and cyclophosphamide). The single cycle of HDCT after standard chemotherapy had no effect on treatment outcomes but it should be noted that as a limitation there was no initial classification according to the IGCCCG risk classification. Recently, Lorch et al. explored differences between single and sequential HDCT, but the study was stopped prematurely because

Table 2
HDCT as second line of treatment.

Trial	Year	Study design	Phase	Sample size	Treatment arms	Results
Einhorn et al.	2007	Retrospective		184	Single-arm: HDCT.	A 48-month follow-up showed a benefit of 70% in DFS in the second line and 45% in the third line. In sensitive and refractory platinum of 68 and 45% respectively, and in the poor prognosis of 51%
Feldman et al.	2010	Prospective	I/II	108	Salvage therapy with HDCT and paclitaxel and ifosfamide as induction chemotherapy: 3 cycles of TI followed by 3 cycles of HDCT	There were 50% and 8% partial responses with negative markers; 5-year DFS was 47% and OS was 52%.
Lorch et al.	2012	Prospective		211	Two arms: - 1 cycle of VIP + 3 cycles of HDCT - 3 cycles of VIP + 1cycle of HDCT	Differences have not been conclusive and the study was stopped prematurely because of excess treatment-related mortality in one of the study arms
Adra et al.	2016	Retrospective		364	Single-arm: HDCT.	A 3.3 year follow-up showed 2-year PFS was 60% and 2-year-OS was 66%. As second line 2-year PFS of 63%,and as third line 49%

CR: Complete response; DFS: Disease Free Survival; PFS Progression Free Survival; HDCT: High Dose Chemotherapy; VIP: etoposide, ifosfamide, cisplatin.

of excess treatment-related mortality in one of the study arms and reached inconclusive results regarding efficacy [37] (Table 2).

There are several ongoing studies to assess the best second line regimen, including the TIGER trial, a prospective, randomized, international phase III trial comparing standard-dose chemotherapy (TIP) with high-dose chemotherapy in patients with relapsed GCT (NCT02375204) [38].

Late relapses (> 2 years after treatment) occur in 2–6% of patients [39]. In these cases the guidelines recommend the surgical approach if feasible, with chemotherapy as an alternative [13,14]. Chemotherapy seems to have a minor curative potential in these patients [40] and, for this reason, referral of late-relapsing patients to high-volume institutions ensures the best chances of cure. Suboptimal primary treatment increases the risk of late relapses [41], so a good approach is important from the beginning

4. Subsequent lines of treatment

GCT patients that have progressed after 2 or more lines of treatment should be encouraged to participate in clinical trials. If not possible, several treatment schedules have been explored in these poor prognosis patients (Table 3). Current evidence in this patient population is based on retrospective case series and phase 2 trials. Below is the evidence in support of the most commonly used drugs and combinations in routine clinical practice.

Two-drug combinations, such as gemcitabin–oxaliplatin or gemcitabine-paclitaxel, and three-drug regimens, such as gemcitabine–paclitaxel–oxaliplatin or gemcitabine-paclitaxel-cisplatin, have been explored. Gemcitabine and oxaliplatin doublet therapy was studied in a phase II trial with 35 patients previously treated with platinum-containing regimens. Eighty-nine percent of the patients had failure to HDCT. An objective response rate (ORR: CR + PR) of 46% with an acceptable toxicity profile was described [42].

In addition, the combination of gemcitabine and paclitaxel has been studied in a phase II trial with 28 patients who had previously received platinum-based regimens with progressive disease after two or more lines of treatment in 75% of cases. This trial reported an ORR of 21.4% with an acceptable toxicity profile [43].

Regarding three-drug regimens, a phase II study with GOP (gemcitabine, oxaliplatin and paclitaxel) in 41 patients with non-seminomatous GCT was conducted. Eighty-one percent of these patients were classified as intermediate or poor risk based on IGCCCG classification. All patients had cisplatin-refractory disease and had previously received a median of two lines of treatment (range 1–3). There was an ORR of 51%, and 39% of these obtained a complete or marker-negative partial response [44].

Furthermore, a retrospective review of 75 patients with good (45%), intermediate (20%) and poor (32%) risk GCT, receiving gemcitabine, paclitaxel and cisplatin as third or fourth-line therapy was published. It reported 8 CR, 29 PR and 13 stable disease (SD) with this regimen. Moreover, the ORR was 49% with a 2-year PFS of 14.8% and OS of 29.5%. Of note, the 5-year OS in disease-free patients was 60.3% [45]

The combination of irinotecan, paclitaxel and oxaliplatin (IPO) in patients with relapsed GCT was also reviewed. IPO was used in 72 patients (29 as second-line, 43 as third-line), with a large proportion of the patients receiving HDCT after IPO. [46]. In this study, the 2-year PFS was 30.2% and the 3-year OS was 33.4%. Only 3% of patients achieved a CR, while 41% and 18% of patients achieved a PR with negative and positive serum tumor markers, respectively. Progressive disease (PD) was reported in 20% of patients [46]. In the second-line setting, 2-year and 3-year PFS were 43.5% and 49.1%, while in third-line they were 21.0% and 23.9%, respectively [46].

Other drugs such as nedaplatin, a second-generation cisplatin derivative, have been tested in combination with paclitaxel and ifosfamide in Japanese patients with cisplatin-refractory or following multiple-lines in relapsed GCT. The ORR was 62.9%, with an OS at a median follow up of 34 months of 59.3% and a median time to progression of 12 months [47]. The major toxicity was hematological, with grade ≥3 hematological adverse events in 94.1% of the patients in the second line setting. It is difficult to extrapolate these results from Japanese patients to other countries and patient populations, and therefore more studies should be developed exploring the use of this drug [47].

These results in advanced/refractory disease are encouraging and should stimulate further research on new pathways and therapies in this group of patients with poor prognosis.

5. Future perspectives

In recent years, an increased understanding about the pathogenesis of TGCT has been gained regarding genetic alterations in germ cell differentiation genes, steroid signaling, and other sex-determining genes [48–51]. A number of further questions remain unanswered and the lack of novel therapies for refractory patients is a reality.

Several epigenetic alterations including DNA methylation and histone modifications in TGCT have been identified [52–56]. Some authors suggested that the degree of DNA methylation might determine tumor cells' sensitivity to chemotherapy [52,57]. Therefore, a clinical trial exploring the combination of cisplatin and SGI-110, a hypomethylating drug, is ongoing (Table 4). *Poly-ADP-ribose polymerase* (PARP) proteins are involved in base excision repair, one of the major DNA repair system in cells, and PARP is overexpressed in TGCT compared to normal testis cells [57,58]. Thus, a proof-of-concept study to define efficacy of gemcitabine, carboplatin and veliparib in patients

Table 3
Third and subsequent lines of treatment.

Agent	Author	Year	Phase	Sample size	Indication	Results
Gemcitabine + Oxaliplatin	Kollmannsberger	2004	II	35 patients	Refractory GCTs	Overall response rate 46% 3 CR
Gemcitabine + Paclitaxel	Hinton	2001	II	28 patients	Refractory GCTs	Overall response rate 21% 3 CR
Gemcitabine + Vinorelbine	Chen	2009	Case Report	2 patients	Extragonadal refractory GCTs	1PR, 1SD
Gemcitabine + Oxaliplatin + Paclitaxel	Bokemeyer	2008	II	41 patients	Refractory GCTs	Response rate 51% Complete or partial marker-negative responses 39%
Gemcitabine + Paclitaxel + Cisplatin	Necchi	2014	Retrospective	75 patients	Refractory GCTs	Response rate (CR + PR) 49% 8 CR, 29 PR,13 SD
Irinotecan + Paclitaxel + Oxaliplatin + /-HDTC	Badreldin	2016	Retrospective	72 patients	Refractory GCTs	2 years PFS 30.2% 3-year OS 33.4%
Paclitaxel + Ifosfamide + Nedaplatine	Nakamura	2015	Retrospective	65 patients	Refractory GCTs	Response rate 62,9% OS at 34months 59.3% PFS12 months

CR: complete response; PR: partial response: SD: stable disease.

Table 4
Ongoing Clinical trials in germ cell tumors treatment.

NCT	Phase	Study design	Drugs	Indication	Previous treatment	Comments
NCT02429466	1	Single arm	Cisplatin and SGI-110 (Guadecitabine)*	Refractory GCTs	Cisplatin-based CT	Currently recruiting. Estimated study completion date: May 2018.
NCT01851200	2	Single arm	BrentuximabVedotin**	CD30-positive refractory GCTs	2/3 lines platin-based CT	Ongoing, not recruiting. Estimated study completion date: September 2017.
NCT02499952	2	Single arm	Pembrolizumab	Refractory GCTs	Cisplatin-based CT	Ongoing, not recruiting. Estimated study completion date: January 2019.
NCT02860819	2	Single arm	Gemcitabine, Carboplatin and Veliparib***	Refractory GCTs	High-dose CT	Currently recruiting. Estimated study completion date: December 2018.
NCT02478502	2	Single arm	Cabazitaxel	Refractory GCTs	Cisplatin-based CT	Currently recruiting. Estimated study completion date: June 2018.
NCT02115165	2	Single arm	Cabazitaxel	Refractory GCTs	Cisplatin-based CT	Currently recruiting. Estimated study completion date: May 2020.
NCT01782339	2	Single arm	Actinomycin D, Paclitaxel, Methotrexate and Oxaliplatin	Refractory GCTs with poor prognosis	Platin-based CT	Currently recruiting. Estimated study completion date: November 2018.
NCT02375204	3	Arm A: TIP. Arm B: TI-CE and SC reinfusion.	Paclitaxel, Ifosfamide, Cisplatin, Carboplatin, Etoposide.	Refractory GCTs	1-line cisplatin-based CT	Currently recruiting. Estimated study completion date: March 2018.

* Hypomethylating drug.
** CD30-directed antibody-drug conjugate (IgG1 antibody specific for human CD30 and amicrotubule-disrupting agent, monomethyl auristatin E)
*** PARP inhibitor.
CT: chemotherapy. GCTs: Germ Cell Tumors. NCT: Number Clinical Trial. PARP:Poly (ADP-ribose) polymerase.SC: stem cell. TI-CE: Paclitaxel and ifosfamide with leukapheresis, followed by Carboplatin and Etoposide and stem cell reinfusion. TIP: Paclitaxel, Ifosfamide and Cisplatin.

with refractory GCT is currently recruiting (Table 4). Gene expression regulation by microRNA (miRNA) as a hallmark of cancer is often deregulated in GCT. It is known that the proportion of patients with seminomas and embryonal carcinomas presenting with elevated tumor markers is low. In this context, patterns of miRNA deregulation are highly characteristic and may therefore be used to provide enhanced prognostic and diagnostic accuracy in these tumor types [57,59–61]. However, the clinical significance of miRNAs in TGCT as predictive biomarkers for drug response remains to be verified by more clinical and translational studies.

Development of immunotherapeutic strategies in GCT are in their infancy. CD30, a member of the tumor necrosis factor-receptor superfamily, is usually found on the surface of activated T cells but has also been detected on a variety of cell types of hematopoietic origin, especially embryonal carcinoma. Moreover, its expression is usually retained even after multiple chemotherapy lines and CD30 IHC expression by resected residual disease after chemotherapy has been reported to have a prognostic value [62]. Brentuximabvedotin (BV) is an antibody-drug conjugate consisting of the chimeric anti-CD30 antibody chemically conjugated to a monomethylauristatin E, a synthetic analog of dolastatin (antitubulin agent). Early signals of clinical activity have been reported with the use of BV in heavily pretreated patients with GCT [63,64]. These data provided the rationale for developing a phase 2 trial with BV for the salvage therapy of advanced and CD30-positive GCT (Table 4). In addition, PD-L1 expression has been documented in the majority of cases of both seminoma (73%) and non-seminoma (64%) [65]. These results provided the rationale for the design of clinical trials with the use of the immune checkpoint inhibitors in patients with GCT; one specifically evaluating the role of the PD-L1 inhibitor, pembrolizumab, in relapsed GCT is currently underway (Table 4).

6. Molecular biology

Several hypotheses have attempted to explain the exceptional sensitivity of TGCT to chemotherapy treatment. Some authors consider that the chemosensitivity can be explained by the low mutational burden of TGCT [57]. Other authors propose that the reason is the low expression of DNA repair genes [66], while others postulate that the reason is the normal expression (or frequently high expression) of p53 [67], or epigenetic mechanisms related to the hypomethylation of these tumors [68].

In the near future, the ability to identify patients of intermediate and poor risk or who will develop platinum resistance based on molecular biology data will be realizable in clinical practice, potentially allowing a personalized treatment strategy from the time of diagnosis.

Recently, Bagrodia et al. hypothesized if patients molecularly-identified as platinum-refractory could be considered for an aggressive treatment in first line [69].

To date, this is the largest series of GCT treated with a cisplatin-containing chemotherapy regimen analyzed by next-generation sequencing (NGS) methods. The initial NGS was performed on 19 tumors. Subsequently, a validation was performed to confirm the results obtained, with 161 samples used (70% non-seminomatous and 30% seminomatous). In the first cohort, nine tumors were cisplatin sensitive and 10 were resistant. Among the 10 patients with cisplatin resistant disease, 2 had TP53 alterations and 3 had amplification of MDM2 (an E3 ubiquitin-protein ligase that negatively regulates TP53). Thus, 5 of 10 cisplatin resistant tumors harbored alterations within the TP53/MDM2 pathway compared with none of the cisplatin sensitive tumors. In the validation cohort, TP53 mutations/deletions were found in 16% of resistant versus 0% of sensitive tumors. Taken together, TP53/MDM2 alterations were found in 24% of resistant compared to 2.6% of sensitive samples. After a follow-up of nearly 40 months, it was observed that patients with TP53/MDM2 pathway alterations had a PFS significantly shorter than those without alterations. In addition, in the

multivariate analysis including the international prognostic classification, the presence of a TP53/MDM2 alteration was an independent predictor of disease progression following first line cisplatin-based chemotherapy (HR 1.83 [1.12–2.98]; 95% CI, $p = 0.016$). Potential targetable alterations were identified in 55% of platinum resistant samples. That said, the frequency of each specific alteration was very low. Inhibitors of MDM2 are currently being studied in the in vitro setting. Despite the identification and validation that the TP53 / MDM2 pathway may lead to platinum resistance, there was a large proportion of patients in which the resistance to cisplatin was not explained by deep sequencing techniques. The authors concluded that, while more studies are needed, genomic sequencing will help in the stratification of these patients in the future. Finally, the presence of actionable genomic alterations in nearly half of cisplatin–resistant GCT in this cohort suggests that novel treatment approaches may be possible for these patients [69].

Another study using whole-exome and transcriptome sequencing showed that a primary somatic feature of GCT is highly recurrent chromosome arm level amplifications and reciprocal deletions (reciprocal loss of heterozygosity) variations. It was found that these alterations are significantly enriched compared to 19 other cancer types. In addition, by functional measurement of apoptotic signaling (BH3 profiling), they found that primary testicular tumors have high mitochondrial priming that facilitates chemotherapy-induced apoptosis. Finally, by phylogenetic analysis of testicular tumors with chemotherapy resistance, they showed how testicular tumors gain additional reciprocal loss of heterozygosity that is associated with loss of pluripotency markers (NANOG and POU5F1) in chemoresistant teratomas or transformed carcinomas [70].

These recent studies have identified the convergence of cancer genomics, mitochondrial priming and GCT evolution, which may provide further insights into tumor biology and mechanisms of chemosensivity and resistance.

7. Conclusions

GCT are a heterogeneous group of neoplasms with different clinical behaviors. Despite medical advances over past decades, much remains to improve long-term results in poor risk patients. The most effective treatment for patients with intermediate and high risk metastatic GCT remains BEP in the first line setting. However, in recent years we have been able to understand that treatments such as TIP or HDCT can provide favorable benefits in this setting compared to BEP, although further studies are still needed to justify the greater toxicity profile of these regimens. Based on growing evidence, data support an adaptive treatment strategy according to serum tumor markers decline, especially in patients with unfavorable biomarker kinetics, whose relapse risk appears greater with standard treatment. Further studies should be developed to determine if treatments, such as HDCT, are an upfront option for patients with poor risk disease.

The treatment of patients with relapsed GCT remains controversial. Nowadays, the challenges are to determine which patients should receive standard salvage chemotherapy and which HDCT, and to establish the optimum sequence.

The mechanisms by which a tumor is intermediate or poor prognosis remain unclear and are likely to involve a sequence of genetic and epigenetic changes.

Thus, it is mandatory to reach a truly individualized treatment based on the genetic and phenotypic profile of each patient to achieve good oncological responses and diminish side effects and post-treatment morbidity, including reproductive health and durable quality of life, in this young patient population.

Conflict of interest

We wish to confirm that there are not conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

References

[1] GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and prevalence worldwide, Available from: http://globocan.iarc.fr/Default.aspx.

[2] T. Shanmugalingam, A. Soultati, S. Chowdhury, S. Rudman, M. Van Hemelrijck, Global incidence and outcome of testicular cancer, Clin. Epidemiol. 5 (2013) 417–427.

[3] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, CA Cancer J. Clin. 61 (April (2)) (2011) 69–90.

[4] A. Rosen, G. Jayram, M. Drazer, S.E. Eggener, Global trends in testicular cancer incidence and mortality, Eur. Urol. 60 (August (2)) (2011) 374–379.

[5] G.J. Bosl, R.J. Motzer, Testicular germ-cell cancer, N. Engl. J. Med. 337 (July (4):) (1997) 242–253.

[6] C. Turnbull, N. Rahman, Genome-wide association studies provide new insights into the genetic basis of testicular germ-cell tumour, Int. J. Androl. 34 (August (4 Pt 2)) (2011) e86–e96 discussion e96-97.

[7] M.H. Greene, C.P. Kratz, P.L. Mai, C. Mueller, J.A. Peters, G. Bratslavsky, et al., Familial testicular germ cell tumors in adults: 2010 summary of genetic risk factors and clinical phenotype, Endocr. Relat. Cancer 17 (June (2)) (2010) R109–R121.

[8] S.R. Williamson, B. Delahunt, C. Magi-Galluzzi, F. Algaba, L. Egevad, T.M. Ulbright, S.K. Tickoo, J.R. Srigley, J.I. Epstein, D.M. Berney, Members of the ISUP Testicular Tumour Panel. The World Health Organization 2016 classification of testicular germ cell tumours: a review and update from the International Society of Urological Pathology Testis Consultation Panel, Histopathology 70 (February (3)) (2017) 335–346.

[9] J. Oldenburg, S.D. Fosså, J. Nuver, A. Heidenreich, H.-J. Schmoll, C. Bokemeyer, et al., Testicular seminoma and non-seminoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 24 (October (Suppl 6)) (2013) vi125–vi132.

[10] International Germ Cell Consensus Classification, a prognostic factor-based staging system for metastatic germ cell cancers. International Germ Cell Cancer Collaborative Group, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 15 (February (2)) (1997) 594–603.

[11] D.R. Feldman, A. Lorch, A. Kramar, C. Albany, L.H. Einhorn, P. Giannatempo, et al., Brain metastases in patients with germ cell tumors: prognostic factors and treatment options – an analysis from the global germ cell cancer group, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 34 (February (4)) (2016) 345–351.

[12] F. Calabrò, P. Albers, C. Bokemeyer, C. Martin, L.H. Einhorn, A. Horwich, et al., The contemporary role of chemotherapy for advanced testis cancer: a systematic review of the literature, Eur. Urol. 61 (June (6)) (2012) 1212–1221.

[13] NCCN clinical practice guidelines in oncology [Internet]. Available from: https://www.nccn.org/professionals/physician_gls/f_guidelines.asp#testicular.

[14] Testicular Seminoma and Non-Seminoma: ESMO clinical practice guidelines | ESMO [Internet]. Available from: http://www.esmo.org/Guidelines/Genitourinary-Cancers/Testicular-Seminoma-and-Non-Seminoma.

[15] C.R. Nichols, P.J. Catalano, E.D. Crawford, N.J. Vogelzang, L.H. Einhorn, P.J. Loehrer, Randomized comparison of cisplatin and etoposide and either bleomycin or ifosfamide in treatment of advanced disseminated germ cell tumors: an Eastern Cooperative Oncology Group, Southwest Oncology Group, and Cancer and Leukemia Group B Study, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 16 (April (4)) (1998) 1287–1293.

[16] E. González-Billalabeitia, J.M. Sepúlveda, P. Maroto, J. Aparicio, J.A. Arranz, E. Esteban, et al., Consensus recommendations from the spanish germ cell cancer group on the use of high-dose chemotherapy in germ cell cancer, Eur. Urol. Focus (2016).

[17] R.A. Huddart, R. Gabe, F.H. Cafferty, P. Pollock, J.D. White, J. Shamash, et al., A randomised phase 2 trial of intensive induction chemotherapy (CBOP/BEP) and standard BEP in poor-prognosis germ cell tumours (MRC TE23, CRUK 05/014, ISRCTN 53643604), Eur. Urol. 67 (March (3)) (2015) 534–543.

[18] S.B. Kaye, G.M. Mead, S. Fossa, M. Cullen, R. deWit, I. Bodrogi, et al., Intensive induction-sequential chemotherapy with BOP/VIP-B compared with treatment with BEP/EP for poor-prognosis metastatic nonseminomatous germ cell tumor: a Randomized Medical Research Council/European Organization for Research and Treatment of Cancer study, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 16 (February (2)) (1998) 692–701.

[19] D.J. Husband, J.A. Green, POMB/ACE chemotherapy in non-seminomatous germ cell tumours: outcome and importance of dose intensity, Eur. J. Cancer Oxf. Engl. 28 (1) (1992) 86–91.

[20] J. Mardiak, T. Sálek, Z. Sycová-Milá, J. Obertová, M. Recková, M. Mego, et al., Paclitaxel, bleomycin, etoposide, and cisplatin (T-BEP) as initial treatment in patients with poor-prognosis germ cell tumors (GCT): a phase II study, Neoplasma 54 (3) (2007) 240–245.

[21] A. Tryakin, M. Fedyanin, D. Kanagavel, I. Fainstein, J. Sergeev, B. Polockij, et al., Paclitaxel + BEP (T-BEP) regimen as induction chemotherapy in poor prognosis patients with nonseminomatous germ cell tumors: a phase II study, Urology 78 (September (3)) (2011) 620–625.

[22] R. de Wit, I. Skoneczna, G. Daugaard, M. De Santis, A. Garin, N. Aass, et al., Randomized phase III study comparing paclitaxel-bleomycin, etoposide, and cisplatin (BEP) to standard BEP in intermediate-prognosis germ-cell cancer: intergroup study EORTC 30983, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 30 (March (8)) (2012) 792–799.

[23] G.V. Kondagunta, J. Bacik, A. Donadio, D. Bajorin, S. Marion, J. Sheinfeld, et al., Combination of paclitaxel, ifosfamide, and cisplatin is an effective second-line therapy for patients with relapsed testicular germ cell tumors, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 23 (September (27)) (2005) 6549–6555.
[24] D.R. Feldman, J. Hu, T.B. Dorff, K. Lim, S. Patil, K.M. Woo, et al., Paclitaxel, ifosfamide, and cisplatin efficacy for first-line treatment of patients with intermediate- or poor-risk germ cell tumors, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 34 (July (21)) (2016) 2478–2483.
[25] R.J. Motzer, C.J. Nichols, K.A. Margolin, J. Bacik, P.G. Richardson, N.J. Vogelzang, et al., Phase III randomized trial of conventional-dose chemotherapy with or without high-dose chemotherapy and autologous hematopoietic stem-cell rescue as first-line treatment for patients with poor-prognosis metastatic germ cell tumors, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 25 (January (3)) (2007) 247–256.
[26] Paclitaxel, Ifosfamide and Cisplatin (TIP) Versus Bleomycin, Etoposide and Cisplatin (BEP) for patients with previously untreated intermediate- and poor-risk germ cell tumors [Internet]. Available from: https://clinicaltrials.gov/ct2/show/NCT01873326.
[27] Salvage, dose intense and high-dose chemotherapy for the treatment of poor prognosis or recurrent germ cell tumours, Cancer Treat. Rev. 31 (May (3)) (2005) 197–209.
[28] G. Daugaard, I. Skoneczna, N. Aass, R. De Wit, M. De Santis, H. Dumez, et al., A randomized phase III study comparing standard dose BEP with sequential high-dose cisplatin, etoposide, and ifosfamide (VIP) plus stem-cell support in males with poor-prognosis germ-cell cancer. An intergroup study of EORTC, GTCSG, and Grupo Germinal (EORTC 30974), Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 22 (May (5)) (2011) 1054–1061.
[29] M.C. Mir, N. Pavan, M.L. Gonzalgo, Current clinical applications of testicular cancer biomarkers, Urol. Clin. N. Am. 43 (February (1)) (2016) 119–125.
[30] T. Nazeer, J.Y. Ro, R.J. Amato, Y.W. Park, N.G. Ordonez, A.G. Ayala, Histologically pure seminoma with elevated alpha-fetoprotein: a clinicopathologic study of ten cases, Oncol. Rep. 5 (December (6)) (1998) 1425–1429.
[31] L. Weissbach, R. Bussar-Maatz, K. Mann, The value of tumor markers in testicular seminomas. Results of a prospective multicenter study, Eur. Urol. 32 (1) (1997) 16–22.
[32] K. Fizazi, L. Pagliaro, A. Laplanche, A. Fléchon, J. Mardiak, L. Geoffrois, et al., Personalised chemotherapy based on tumour marker decline in poor-prognosis germ-cell tumours: results of the GETUG 13 phase III trial, Lancet Oncol. 15 (December (13)) (2014) 1442–1450.
[33] X. Durand, A. Fléchon, T. Murez, L. Rocher, P. Camparo, N. Morel-Journel, et al., [CCAFU french national guidelines 2016–2018 on testicular germ cell tumors], Prog. En Urol. J. Assoc. Francaise Urol. Soc. Francaise Urol. 27 (November (Suppl 1)) (2016) S147–S165.
[34] L.H. Einhorn, S.D. Williams, A. Chamness, M.J. Brames, S.M. Perkins, R. Abonour, High-dose chemotherapy and stem-cell rescue for metastatic germ-cell tumors, N. Engl. J. Med. 357 (July (4)) (2007) 340–348.
[35] N. Adra, R. Abonour, S.K. Althouse, C. Albany, N.H. Hanna, L.H. Einhorn, High-dose chemotherapy and autologous peripheral-blood stem-cell transplantation for relapsed metastatic germ cell tumors: the Indiana University Experience, J. Clin. Oncol. 35 (November (10)) (2016) 1096–1102.
[36] D.R. Feldman, J. Sheinfeld, D.F. Bajorin, P. Fischer, S. Turkula, N. Ishill, et al., TI-CE high-dose chemotherapy for patients with previously treated germ cell tumors: results and prognostic factor analysis, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 28 (April (10)) (2010) 1706–1713.
[37] A. Lorch, A. Kleinhans, A. Kramar, C.K. Kollmannsberger, J.T. Hartmann, C. Bokemeyer, et al., Sequential versus single high-dose chemotherapy in patients with relapsed or refractory germ cell tumors: long-term results of a prospective randomized trial, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 30 (March (8)) (2012) 800–805.
[38] Standard-dose combination chemotherapy or high-dose combination chemotherapy and stem cell transplant in treating patients with relapsed or refractory germ cell tumors - – full text view - – ClinicalTrials.gov [Internet]. Available from: https://clinicaltrials.gov/ct2/show/NCT02375204.
[39] M.E. Lipphardt, P. Albers, Late relapse of testicular cancer, World J. Urol. 22 (April (1)) (2004) 47–54.
[40] A. Gerl, C. Clemm, N. Schmeller, M. Hentrich, R. Lamerz, W. Wilmanns, Late relapse of germ cell tumors after cisplatin-based chemotherapy, Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 8 (January (1)) (1997) 41–47.
[41] J. Oldenburg, S.D. Fosså, Late relapse of nonseminomatous germ cell tumours, BJU Int. 104 (November (9 Pt B)) (2009) 1413–1417.
[42] C. Kollmannsberger, J. Beyer, R. Liersch, P. Schoeffski, B. Metzner, J.T. Hartmann, et al., Combination chemotherapy with gemcitabine plus oxaliplatin in patients with intensively pretreated or refractory germ cell cancer: a study of the German Testicular Cancer Study Group, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 22 (January (1)) (2004 1) 108–114.
[43] S. Hinton, P. Catalano, L.H. Einhorn, P.J. Loehrer, T. Kuzel, D. Vaughn, et al., Phase II study of paclitaxel plus gemcitabine in refractory germ cell tumors (E9897): a trial of the Eastern Cooperative Oncology Group, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 20 (April (7)) (2002) 1859–1863.
[44] J.-H. Chen, P.-Y. Chang, N.-S. Yao, Gemcitabine plus vinorelbine as an effective salvage chemotherapeutic regimen in cisplatin-refractory germ cell tumor, South Med. J. 102 (May (5)) (2009) 546–548.
[45] A. Necchi, N. Nicolai, L. Mariani, S. Lo Vullo, P. Giannatempo, D. Raggi, et al., Combination of paclitaxel, cisplatin, and gemcitabine (TPG) for multiple relapses or platinum-resistant germ cell tumors: long-term outcomes, Clin. Genitourin. Cancer 12 (February (1)) (2014) 63–69.e1.
[46] W. Badreldin, J. Krell, S. Chowdhury, S.J. Harland, D. Mazhar, V. Harding, et al., The efficacy of irinotecan, paclitaxel, and oxaliplatin (IPO) in relapsed germ cell tumours with high-dose chemotherapy as consolidation: a non-cisplatin-based induction approach, BJU Int. 117 (March (3)) (2016) 418–423.
[47] T. Nakamura, T. Ueda, M. Oishi, H. Nakanishi, A. Fujihara, Y. Naya, et al., Salvage combined chemotherapy with paclitaxel, ifosfamide and nedaplatin for patients with advanced germ cell tumors, Int. J. Urol. Off. J. Jpn. Urol. Assoc. 22 (March (3)) (2015) 288–293.
[48] G. Lottrup, A. Jørgensen, J.E. Nielsen, N. Jørgensen, M. Duno, A.M. Vinggaard, et al., Identification of a novel androgen receptor mutation in a family with multiple components compatible with the testicular dysgenesis syndrome, J. Clin. Endocrinol. Metab. 98 (June (6)) (2013) 2223–2229.
[49] A. Ferlin, F. Ganz, M. Pengo, R. Selice, A.C. Frigo, C. Foresta, Association of testicular germ cell tumor with polymorphisms in estrogen receptor and steroid metabolism genes, Endocr. Relat. Cancer 17 (March (1)) (2010) 17–25.
[50] W. Kristiansen, R. Karlsson, T.B. Rounge, T. Whitington, B.K. Andreassen, P.K. Magnusson, et al., Two new loci and gene sets related to sex determination and cancer progression are associated with susceptibility to testicular germ cell tumor, Hum. Mol. Genet. 24 (July (14)) (2015) 4138–4146.
[51] A. Jørgensen, J.E. Nielsen, K. Almstrup, B.G. Toft, B.L. Petersen, E. Rajpert-De Meyts, Dysregulation of the mitosis-meiosis switch in testicular carcinoma in situ, J. Pathol. 229 (March (4)) (2013) 588–598.
[52] H. Wermann, H. Stoop, A.J.M. Gillis, F. Honecker, R.J.H.L.M. van Gurp, O. Ammerpohl, et al., Global DNA methylation in fetal human germ cells and germ cell tumours: association with differentiation and cisplatin resistance, J. Pathol. 221 (August (4)) (2010) 433–442.
[53] T. Kawakami, C. Zhang, Y. Okada, K. Okamoto, Erasure of methylation imprint at the promoter and CTCF-binding site upstream of H19 in human testicular germ cell tumors of adolescents indicate their fetal germ cell origin, Oncogene 25 (June (23)) (2006) 3225–3236.
[54] D.J. Smiraglia, J. Szymanska, S.M. Kraggerud, R.A. Lothe, P. Peltomäki, C. Plass, Distinct epigenetic phenotypes in seminomatous and nonseminomatous testicular germ cell tumors, Oncogene 21 (May (24)) (2002) 3909–3916.
[55] G.J. Netto, Y. Nakai, M. Nakayama, S. Jadallah, A. Toubaji, N. Nonomura, et al., Global DNA hypomethylation in intratubular germ cell neoplasia and seminoma, but not in nonseminomatous male germ cell tumors, Mod. Pathol. Off. J. U S Can. Acad. Pathol. Inc. 21 (November (11)) (2008) 1337–1344.
[56] H. Ushida, T. Kawakami, K. Minami, T. Chano, H. Okabe, Y. Okada, et al., Methylation profile of DNA repetitive elements in human testicular germ cell tumor, Mol. Carcinog. 51 (September (9)) (2012) 711–722.
[57] K. Litchfield, M. Levy, R.A. Huddart, J. Shipley, C. Turnbull, The genomic landscape of testicular germ cell tumours: from susceptibility to treatment, Nat. Rev. Urol. 13 (July (7)) (2016) 409–419.
[58] F.J. Romano, S. Rossetti, V. Conteduca, G. Schepisi, C. Cavaliere, R. Di Franco, et al., Role of DNA repair machinery and p53 in the testicular germ cell cancer: a review, Oncotarget 7 (December (51)) (2016) 85641–85649.
[59] H. Ling, L. Krassnig, M.D. Bullock, M. Pichler, MicroRNAs in testicular cancer diagnosis and prognosis, Urol. Clin. N. Am. 43 (February (1)) (2016) 127–134.
[60] M.J. Murray, J.C. Nicholson, N. Coleman, Biology of childhood germ cell tumours, focussing on the significance of microRNAs, Andrology 3 (January (1)) (2015) 129–139.
[61] K.-P. Dieckmann, A. Radtke, M. Spiekermann, T. Balks, C. Matthies, P. Becker, et al., Serum levels of MicroRNA miR-371a-3p: a sensitive and specific new biomarker for germ cell tumours, Eur. Urol. 71 (February (2)) (2017) 213–220.
[62] P. Giannatempo, B. Paolini, R. Miceli, D. Raggi, N. Nicolai, E. Farè, et al., Persistent CD30 expression by embryonal carcinoma in the treatment time course: prognostic significance of a worthwhile target for personalized treatment, J. Urol. 190 (November (5)) (2013) 1919–1924.
[63] C. Albany, D.R. Feldman, L.E. Garbo, L.H. Einhorn, Antitumor activity of brentuximab vedotin in CD30 positive refractory germ cell tumors, J. Clin. Oncol. 31 (suppl 6; abstr 327) (2013) Available from: http://meetinglibrary.asco.org/content/107223-134.
[64] A. Necchi, A. Anichini, D. Raggi, P. Giannatempo, D. Magazzù, N. Nicolai, et al., Brentuximab vedotin in CD30-expressing germ cell tumors after chemotherapy failure, Clin. Genitourin. Cancer 14 (August (4)) (2016) 261–264.e4.
[65] C.D. Fankhauser, A. Curioni-Fontecedro, V. Allmann, J. Beyer, V. Tischler, T. Sulser, et al., Frequent PD-L1 expression in testicular germ cell tumors, Br. J. Cancer 113 (July (3)) (2015) 411–413.
[66] S. Usanova, A. Piée-Staffa, U. Sied, J. Thomale, A. Schneider, B. Kaina, et al., Cisplatin sensitivity of testis tumour cells is due to deficiency in interstrand-cross-link repair and low ERCC1-XPF expression, Mol. Cancer 9 (1) (2010) 248.
[67] M. Gutekunst, M. Oren, A. Weilbacher, M.A. Dengler, C. Markwardt, J. Thomale, et al., p53 hypersensitivity is the predominant mechanism of the unique responsiveness of testicular germ cell tumor (TGCT) cells to cisplatin, in: A.L. Gartel (Ed.), PLoS One, 6 2011, p. e19198.
[68] Y. Sheikine, E. Genega, J. Melamed, P. Lee, V.E. Reuter, H. Ye, Molecular genetics of testicular germ cell tumors, Am. J. Cancer Res. 2 (2) (2012) 153–167.
[69] A. Bagrodia, B.H. Lee, W. Lee, E.K. Cha, J.P. Sfakianos, G. Iyer, et al., Genetic determinants of cisplatin resistance in patients with advanced germ cell tumors, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 34 (November (33)) (2016) 4000–4007.
[70] A. Taylor-Weiner, T. Zack, E. O'Donnell, J.L. Guerriero, B. Bernard, A. Reddy, et al., Genomic evolution and chemoresistance in germ-cell tumours, Nature 540 (7631) (2016) 114–118.