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*Review*

# Therapeutic Challenges for Cisplatin-Resistant Ovarian Germ Cell Tumors

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**Abstract:** The majority of patients with advanced ovarian germ cell cancer are treated by cisplatin-based chemotherapy. Despite adequate first-line treatment, nearly one third of patients relapse and almost half develop cisplatin-resistant disease, which is often fatal. The treatment of cisplatin-resistant disease is challenging and prognosis remains poor. There are limited data on the efficacy of specific chemotherapeutic regimens, high-dose chemotherapy with autologous progenitor cell support and targeted therapies. The inclusion of patients in clinical trials is strongly recommended, especially in clinical trials on the most frequent male germ cell tumors, to offer wider therapeutic opportunities. Here, we provide an overview of current and potential new treatment options including combination chemotherapy, high-dose chemotherapy and molecular targeted therapies, for patients with cisplatin-resistant ovarian germ cell tumors.

**Keywords:** female patients; germ cell tumors; ovarian germ cell tumors; salvage therapy; refractory; high-dose chemotherapy

## 1. Introduction

The European age-standardized incidence rate for female germ cell tumors (GCTs) is 0.4 per 100,000 persons per year [1], whereas that of males is 15-fold higher [2]. GCTs derive from embryonic germ cells, which, instead of differentiating correctly, undergo malignant transformation [3]. They arise in the gonads in >90% of cases. The remaining 10% are extragonadal tumors occurring in the mediastinum, retroperitoneum and, less frequently, central nervous system sites such as neurohypophysis and the pineal gland [4]. This distribution reflects the original route followed by germ cell precursors during embryologic development.

Malignant ovarian GCTs (MOGCTs) are subdivided into dysgerminomatous tumors (the most frequent type) and non-dysgerminomatous tumors. The World Health Organization (WHO) classification of MOGCT is shown in Table 1 [5]. Dysgerminomas express features typical of primordial germ cells and may be associated with dysgenetic gonads and sexual development including the Turner syndrome, testicular feminization and triple X syndrome [6,7]. Most dysgerminomas produce lactic dehydrogenase (LDH) and β-human chorionic gonadotropin (β-hCG), as shown

in Table 2 [8]. In gonadal dysgenesis, dysgerminoma derives from a gonadoblastoma, and rarely from an intra-abdominal testis. The majority of these tumors have high mitotic activity [9].

**Table 1.** Malignant ovarian germ cell tumors classification (according to the World Health Organization (WHO) classification of tumors) [5].

| Germ Cell Tumors | Tumor Type |
|---|---|
| Primitive germ cell tumors | Dysgerminoma<br>Yolk sac tumor<br>Polyvescicular vitelline tumor<br>Glandular variant<br>Hepatoid variant<br>Embryonal carcinoma<br>Polyembryoma<br>Non-gestational choriocarcinoma<br>Mixed germ cell tumor |
| Biphasic or triphasic teratoma | Immature teratoma<br>Mature teratoma |

**Table 2.** Germ cell tumors (GCTs) and their tumor markers [8].

| GCTs | Markers | | |
|---|---|---|---|
| | **AFP** | **β-hCG** | **LDH** |
| Dysgerminoma | Normal | May be elevated | May be elevated |
| Yolk sac tumor | Elevated in all cases | Normal | May be elevated |
| Embryonal carcinoma | May be elevated | May be elevated | Elevated |
| Non-gestational choriocarcinoma | Normal | Elevated in all cases | Normal |
| Mature teratoma | Normal | Normal | Normal |
| Immature teratoma | May be elevated | Normal | Normal |
| Mixed germ cell tumor | May be elevated | May be elevated | May be elevated |

GCTs, germ cell tumors; β-hCG, β-human chorionic gonadotropin; AFP α-fetoprotein; LDH, lactic dehydrogenase.

Non-dysgerminoma tumors have multiple histological subtypes that resemble the differentiation occurring during human development. In particular, hallmarks of embryonic differentiation occur in embryonal carcinoma (EC) and teratoma (both mature and immature), while features of extraembryonic differentiations are present in yolk sac tumors and choriocarcinomas [10]. ECs, choriocarcinomas and malignant struma ovarii tumors represent the rarest forms of MOGCTs. Teratomas show various patterns of somatic differentiation and can be divided into mature subtypes, characterized by well-differentiated tissue, and immature subtypes, characterized by incomplete differentiation. They tend to have both low mitotic and apoptotic indices [11]. On occasion, mature teratomas undergo malignant transformation of differentiated tissue, requiring treatment regimens for that specific malignant tissue [12]. Yolk sac tumors and choriocarcinomas display morphologies resembling extra-embryonically differentiated tissue. The former derive from endodermal sinus and secrete α-fetoprotein (AFP) [8], while the latter are composed of cytotrophoblastic and syncytiotrophoblastic cells that typically express β-hCG, as shown in Table 2 [8,12]. ECs are uncommon among MOGCTs but quite frequent among testicular GCTs (TGCTs) [11]. They display features of primitive epithelial cells during the early stages of embryonic development. ECs have the highest mitotic and apoptotic indices of all GCT histopathologic subtypes [11,13]. Furthermore, in more than 50% of cases they express high levels of AFP, β-hCG and LDH [8].

In general, non-dysgerminomas are cisplatin (CDDP)-sensitive and are treated by a combination of surgery and chemotherapy, whereas teratomas are relatively chemo-resistant. In the majority of patients, GCTs consist of a mixture of non-dysgerminomatous histologic components, although a combination of dysgerminoma and non-dysgerminoma components is also possible. Tumor markers may thus vary between histotypes.

Dysgerminomas are most likely to be localized in the ovaries at diagnosis and are often early-stage tumors [14]. However, precise data on the incidence of these tumors by stage at diagnosis is not available due to the rarity of the disease. In women <20 years of age, GCTs account for 58% of all ovarian tumors and should always be suspected in young females with a solid ovarian mass [15,16]. The first choice of treatment is surgery, which, depending on the age and prognosis of the patient should attempt to preserve fertility. There is evidence to suggest that stage IA pure dysgerminoma should be treated with surgery alone because of the low rate of recurrence and also because patients can be successfully treated in the event of relapse [17]. Similarly, patients with stage IA grade 1 immature teratoma do not require adjuvant treatment after adequate surgery [18]. Other data propose managing all-grade immature teratoma and all-stage I dysgerminoma with close surveillance, only treating in the event of relapse [17,19]. A multimodality approach regardless of stage IA disease comprising surgery and platinum-based chemotherapy with bleomycin, etoposide and cisplatin (BEP) has obtained 5-year survival rates of up to 100% for dysgerminomas and 85% for non-dysgerminomatous MOGCTs [20–22]. Thus, given its efficacy and tolerability, BEP has become standard adjuvant therapy for women with MOGCTs [19].

Patients with de novo advanced disease require a surgical approach, and a careful balance is needed between achieving optimal cytoreduction and preventing delays in postoperative chemotherapy [19]. The majority of patients with MOGCTs relapse within the first 2 years after surgery and, given the lack of randomized controlled trials, are managed with same therapeutic strategy used for relapsed TGCTs [23]. In fact, BEP has proven effective in prolonging survival in patients with metastatic or recurrent OGCTs, obtaining remission rates of between 75% and 90% [24,25]. Despite this high response rate, there is still a proportion of patients (30–35%) who fail to respond completely or who relapse after completion of first-line chemotherapy. Among these, a fraction will have platinum-refractory disease, defined as radiologic or serologic progression within 4 weeks of prior platinum-based regimen. There are limited data on the efficacy and feasibility of specific chemotherapeutic regimens for this subgroup and prognosis remains very poor.

In the present review we provide an overview of current options and future prospects for the management of female patients with advanced GCTs, focusing on those who are not cured after first-line chemotherapy and are thus candidates for salvage treatment.

## 2. Prognostic Factors

Prognostic factors are an important element in the management of MOGCT because they can help to identify the patients who need more intensive therapeutic strategies. Despite the common origin of GCTs from primordial germ cells, prognostic factors for MOGCT differ from those of TGCTs. There is a well known prognostic classification system for male GCTs incorporating stage, histology and serum biomarkers [26], whereas there are no established prognostic parameters for MOGCTs. Bower et al. reported that OGCT patients <30 years of age treated with chemotherapy showed an improved 3-year survival [27]. However, the importance of their findings was limited by the small number of cases included in the study. A retrospective analysis by Murugaesu et al. demonstrated that initial-stage disease and elevation of both β–hCG and AFP were independent factors of poor prognosis, but that age at diagnosis was not [28]. Univariate and multivariate analyses in a retrospective study carried out by Mangili et al. showed that patient age (>45 years) and treatment in a non referral center were the most important predictors of recurrence in, whereas stage >I and yolk sac histology were independent indicators of poor prognosis [29].

A modified version of the male International Germ Cell Cancer Collaboration Group (IGCCCG) risk classification revealed that patients classified as poor risk on the basis of pre-operative and pre-chemotherapy markers had poorer progression-free (PFS) and overall survival (OS) rates [30]. However, this staging system was not able to distinguish between good and intermediate-risk patients, probably because of the small number of patients in the two groups. Another retrospective study of 42 pediatric cases of germ cell and sex cord-stromal ovarian tumors demonstrated that

tumor size and histologic type were not significantly correlated with survival [31]. Furthermore, the level of AFP at diagnosis was not significantly associated with survival or recurrence. A recent study focused on patients diagnosed with advanced stage (II–IV) MOGCT submitted to primary cytoreductive surgery [32]. The authors reported no difference in OS following stratification by histology for dysgerminoma or non-dysgerminoma. Furthermore, the presence of macroscopic residual disease following primary cancer-directed surgery did not correlate with poorer prognosis. Further studies are needed to evaluate the prognostic impact of vital residual disease, as already done for male GCTs [33].

Therefore, as shown above, currently there is not a validated prognostic classification that could help physicians in their clinical practice and further studies are requested [34]. A prognostic classification is also lacking for relapsed/refractory female GCTs. A recent large cooperative study of the International Prognostic Factors Study Group identified prognostic factors for male GCTs at first relapse capable of substantially differentiating prognosis on the basis of the prognostic score (OS rates ranged from 10–70%) [35]. A similar study among several cooperative groups is ongoing to better characterize prognostic factors and the efficacy of second-line therapies in this setting.

## 3. CDDP-Acquired Resistance

When indicated, CDDP-based chemotherapy can cure the majority of patients with GCTs. Conversely, CDDP-refractoriness is associated with aggressive disease and extremely poor prognosis. The molecular basis for this sensitivity or resistance, probably multifactorial, is still poorly understood. CDDP sensitivity appears to correlate with the frequent presence of wild-type TP53, low levels of p53 and the p53 negative feedback regulator, MDM2 (mouse double minute 2 homolog). Moreover, high levels of Oct4 and low levels of cytoplasmatic p21 contribute to the high sensitivity of GCTs to CDDP-based chemotherapy. Thus, the development of CDDP resistance is mediated through altered levels of various key factors [36]. Other mechanisms of CDDP-acquired resistance include reduced drug accumulation, increased detoxification of CDDP in cellular cytoplasm and decreased access of CDDP to DNA inside the nucleus [37]. Although numerous studies have analyzed the mechanisms involved in CDDP resistance in TGCTs, relatively little is known about such mechanisms in OGCTs. Given their common origin, they could be similar. The main mechanisms are as follows.

### *3.1. Role of p53*

CDDP sensitivity in GCTs is largely related to the role of p53 and to DNA damage response. In numerous tumor types, CDDP resistance correlates with p53 inactivation at the genetic or protein level given that tumor protein p53 (TP53) is one of the most widely mutated genes in human cancer. Surprisingly, CDDP resistance has been directly linked to TP53 mutations in only a subset of refractory TGCTs, [38]. Thus, mutations in components regulating the p53 pathway are frequent and play an important role in CDDP resistance in TGCTs. Elevated expression of the p53, MDM2 and p21, and reduced expression of octamer-binding transcription factor 4 (Oct-4) and Noxa (a target gene of p53) have been detected in intrinsic CDDP-resistant TGCTs cells prior to CDDP treatment [39–44]. p21 is activated by p53 and high expression of the former correlates with the intrinsic CDDP-resistant in TGCTs and may be involved in apoptosis inhibition [41,44,45]. MDM2 is an important antagonist of p53 and increased expression has been detected in CDDP-resistant with respect to CDDP-sensitive TGCT cells [44–46].

### *3.2. DNA Methylation*

More undifferentiated GCTs such as seminomas, which show higher sensitivity to CDDP, are hypomethylated, whereas non-seminomas have more highly methylated DNA [47]. Furthermore, different methylation profiles have been observed in sensitive and resistant nonseminomas. Koul et al. suggested that promoter hypermethylation of *RASSF1A* and *HIC1* genes are associated with CDDP resistance, while a high incidence of *MGMT* and *RARB* promoter hypermethylation may play a role

in the sensitivity of GCTs to CDDP [48]. A number of studies analyzed the effect of demethylating agents such as 5-azacytidine, 5-aza-deoxycytidine and guadecitabine in GCT cell lines, hypothesizing a correlation between global methylation status and response to chemotherapy. Further studies are needed to increase our knowledge about these promising agents [47,49–51].

### 3.3. PDGFRb/PI3K/p-AKT Pathway

Deregulation of the PDGFRb/PI3K/p-AKT pathway plays a key role in CDDP resistance [41,52,53] and is associated with the inactivation of PTEN, which also increases the mammalian target of rapamycin signaling. However, Mego et al. failed to demonstrate the efficacy of everolimus (mammalian target of rapamycin inhibitor) against heavily pretreated refractory GCTs [54]. Koster et al. found that high expression of p21 CDDP-resistant EC cells correlated with reduced levels of Oct4 protein and miR-106b seed family members [55]. Conversely, the same authors reported high levels of these miRs and Oct4 in the EC component of chemosensitive GCT patients [41]. PI3K/Akt is needed for the transfer of p21 from the nucleus to the cytoplasm to prevent CDDP-induced apoptosis. Thus, targeting cytoplasmic p21 through PI3K/Akt inhibition sensitizes cells to CDDP-induced apoptosis [55].

### 3.4. Cellular Differentiation

ECs represent an undifferentiated type of tumor that shares expression of pluripotency factors with normal embryonic stem cells. Yolk-sac tumors, choriocarcinomas and teratomas are believed to be derived from more highly differentiated cells [56]. The induction of cellular differentiation could be a way of circumventing CDDP resistance. In fact, cells have a different propensity to undergo apoptosis, probably due to a reduction in proliferation and an inhibition of cell-cycle progression, a phenomenon reported after CDDP-induced DNA damage [57,58]. Abada et al. hypothesized a link between differentiation and resistance to apoptosis in both nonmalignant primordial germ cells and neoplastic germ cells [59]. Interestingly, this has not been seen in other neoplastic diseases. The authors showed that CDDP could induce acute resistance to itself through a differentiation response in pluripotent germ cell tumor cells. Timmer-Bosscha et al. demonstrated that the use of all-trans-retinoic acid (RA) caused differentiation in CDDP-resistant GCTs but reduced apoptotic susceptibility [60]. Conversely, docosahexaenoic acid (DHA) potentiated CDDP-induced cytotoxicity and apoptosis in vitro.

## 4. Conventional Salvage Dose Regimens

Recurrent OGCT is typically detected by an increase in serum tumor markers (β-hCG, AFP and LDH) or by the appearance of new lesions at routine surveillance imaging. Patients who progress after first-line chemotherapy are commonly treated with ifosfamide and CDDP-based regimens, a strategy also used in men with relapsed GCTs [23,61]. For those with residual disease, treatment with paclitaxel, ifosfamide and CDDP (TIP) is recommended as second-line therapy [62] following the high complete response (CR) rate (70%) and low relapse rate observed in TGCTs [63]. In a phase 2 study of the Memorial Sloan Kettering Cancer Center (MSKCC), 46 patients with relapsed TGCTs were treated with TIP as second-line therapy [63]. There was a selection bias for a relatively favorable prognosis, the trial excluding patients with extragonadal GCT (generally considered to have a poor prognosis) and including only patients who obtained a CR to front-line chemotherapy and were CDDP-sensitive, thus with a better prognosis. The paclitaxel dose, given on day 1 as a 24-h infusion, was increased until the maximum tolerated dose of 250 mg/m$^2$ was reached; ifosfamide 1500 mg/m$^2$ was administered by infusion over 60 min on days 2 to 5; and CDDP 25 mg/m$^2$ was infused over 30 min on days 2 to 5. All patients received prophylactic granulocyte colony-stimulating factor (G-CSF). Thirty-two (70%) of the 46 patients obtained a CR and 29 (63%) were disease-free at a median follow-up of 69 months. The 2-year progression-free survival rate was 65%. Fourteen CR patients showed a late relapse and seven achieved a CR to chemotherapy followed by surgery. All seven were disease-free at a median follow-up of 51 months. A British Medical Research Council multicenter phase II trial evaluated the use of second-line TIP based on four courses of paclitaxel 175 mg/m$^2$ on day 1 followed by ifosfamide

1 g/m$^2$ and CDDP 20 mg/m$^2$ on days 1–5 at 3-weekly intervals [64]. Thus, lower doses of chemotherapy were administered with respect to the MSKCC TIP schedule, i.e., −30% paclitaxel, −16.7% ifosfamide and the same doses of cisplatin. In this study patients did not receive G-CSF support after initial BEP chemotherapy. Forty-three patients were enrolled, 26 (60%) of who achieved a CR with negative markers. One-year survival was 70% and failure-free survival was 36%. According to MSKCC risk group, CR was 73% in the group of 26 patients with "good-risk" disease and 41% for the 17 "poor-risk" patients. These results are inferior to those of the MSKCC study where TIP therapy was administered more intensively, at a higher dose, and with G-CSF support, supporting the hypothesis that the higher doses of chemotherapy in the TIP regimen may have had an impact on treatment efficacy. In gynecologic oncology units, the TIP regimen used for cervical cancer included three courses of paclitaxel 175 mg/m$^2$ plus ifosfamide 5 g/m$^2$ infused over 24 h and CDDP 75 mg/m$^2$ every 3 weeks [65]. This regimen is sometimes also used for female GCT. However, compared to MSKCC, the TIP regimen used much lower doses of chemotherapy than that of MSKCC, i.e., −30% paclitaxel, −16.7% ifosfamide and −25% CDDP, without G-CSF support. In light of this, the MSKCC TIP regimen should be the preferred treatment for female GCTs (as it showed the highest best efficacy in male GCT).

CDDP-resistant female GCT is so rare that no prospective studies have ever been conducted in this patient population. The only phase II study performed to date focused on patients with recurrent or advanced dysgerminoma who were CDDP-sensitive and treated with the PVB (CDDP, vinblastine, bleomycin) regimen. However, the trial was closed early because of poor accrual [66]. A retrospective review on MOGCT reported long term-survival in only 10% of patients treated with standard-dose salvage chemotherapy [28]. In male CDDP-resistant GCT, oxaliplatin, gemcitabine and paclitaxel represent the drugs of choice. In a phase 2 trial conducted on 18 male patients with CDDP-refractory non-seminomatous GCT treated with oxaliplatin and gemcitabine, 3 (17%) achieved a prolonged complete remission lasting 44, 20 and 18 months, one of whom underwent surgical resection of residual masses, obtaining a disease-free status [67]. Female patients resistant to CDDP-based treatment can also be treated with vincristine/actinomycin D/cyclophosphamide or paclitaxel/gemcitabine as salvage therapy [19], but prognosis remains poor and worse than in male GCT [34]. Within this context, obtaining a response is an important objective as it may permit surgical intervention, which would increase the chances of long-term survival [68]. No studies investigating the use of an oxaliplatin-gemcitabine regimen in female patients with GCTs have been performed to date and are warranted.

## 5. High-Dose Salvage Chemotherapy

High-dose chemotherapy (HDC) with peripheral blood progenitor cell (PBPC) support is considered an option for salvage treatment in male patients with relapsed GCT [69,70]. Multicycle HDC regimens are currently considered the best option in male GCTs and two regimens are currently used in clinical practice for female GCTs, the Indiana University regimen and the MSKCC regimen [71,72]. The former consists of a mobilizing course with G-CSFs alone or 1–2 cycles of PEI (CDDP, etoposide and ifosfamide) according to the patient's clinical conditions (chemotherapy is used for bulky and aggressive disease requiring an urgent therapeutic approach) followed by two courses of HDC (tandem regimen) comprising carboplatin 700 mg/m$^2$ and etoposide 750 mg/m$^2$ daily for 3 days, followed by PBPC infusion [71]. The MSKCC regimen consists of mobilizing chemotherapy with ifosfamide and paclitaxel followed by three HDC courses of carboplatin AUC 7–8 and etoposide 400 mg/m$^2$ daily for 3 days, followed by PBPC infusion [72]. For patients with recurrent testicular cancer, HDC can be considered superior to conventional-dose chemotherapy following the results of a multicenter retrospective analysis [69]. A few small studies have evaluated the use of salvage HDC and hematopoietic stem cell transplantation in female patients with relapsed/refractory GCTs. The European Society for Blood and Marrow Transplantation (EBMT) carried out a retrospective analysis of female patients with GCTs treated with salvage HDC [61]. Of the 51 assessable patients, 17 achieved a CR, eight a marker-negative partial remission and five stable disease (SD). There were three treatment-related deaths. 42% of patients

were progression-free following HDC at a median follow-up of 87 months [61]. Ammakkanavar et al. treated 13 patients with recurrent malignant OCGTs with the Indiana University regimen. Seven patients achieved a CR, of who four remained disease-free 12, 22, 120 and 270 months after treatment [25].

## 6. New Drugs

Over the last decade several targeted therapies for male GCTs have been evaluated but proven ineffective [73]. Such data are lacking for female patients because female GCTs were not included in these studies. An ongoing phase 2 trial (NCT02533765) is investigating the efficacy of olaparib in GCT patients (male and female) who progressed during CDDP-based regimen or progressed/relapsed after HDC or after at least two different CDDP-based chemotherapy regimens. Olaparib is an oral poly (ADP-ribose) polymerase (PARP) inhibitor. PARP inhibition is a promising therapeutic strategy for cancers characterized by specific DNA defects, in particular neoplastic cells that show a BRCA1 or BRCA2 mutation and are rendered deficient in homologous recombination repair (HR). Tumors with HR deficiency (HRD) cannot repair DNA damage, which may lead to cell death. PARP inhibition eliminates an alternative DNA repair pathway, thus promoting tumor cell death [74,75]. The biologic rationale for the study was provided by other authors who demonstrated that PARP was overexpressed in TGCTs with respect to normal testicular tissue. Mego et al. reported that PARP overexpression was an early event in male GCT development [76]. Patients with low PARP expression in primary GCTs had a better, albeit non-significant, OS than those with high PARP expression (5-year OS 89.2% vs. 78.7%; HR = 0.50, 95% CI 0.21–1.17, $p = 0.12$) [76].

In GCT tumorigenesis, aberrations of the retinoblastoma (RB) pathway are a central event [77], especially in CDDP-resistant tumors [78]. Teratomas have been shown to demonstrate high RB protein (pRB) expression [79,80]. Palbociclib is an oral inhibitor of pRB phosphorylation through the inhibition of cyclin-dependent kinases 4 and 6. Within this context, Vaughn et al. performed a single-arm phase 2 trial of palbociclib in patients with pRB-expressing refractory metastatic GCT. The 24-week PFS rate was 28% and patients with teratoma and teratoma with malignant transformation showed significantly better PFS [81].

The role of immune check-point inhibitors should also be analyzed in these rare tumors. High PD-L1 expression has been reported in TGCTs, indicating that patients could potentially benefit from immunotherapy approaches with programmed death 1 (PD-1) or PD-ligand 1 (PD-L1) inhibition [82]. Furthermore, PD-L1 expression appears to have a prognostic value in patients with GCTs, suggesting that those with high PD-L1 expression are more likely to have (more) aggressive disease and worse survival [83]. However, preliminary results from two studies of pembrolizumab and avelumab in male GCTs did not show significant results [84,85]. A single-arm phase II trial investigated pembrolizumab 200 mg i.v. q3weeks in patients with relapsed GCT who progressed after first-line CDDP-based chemotherapy and after at least one salvage regimen [84]. Twelve patients were enrolled and all had non-seminoma, one of extragonadal origin. There were no partial or complete responses. Radiographic stable disease of 19 and 28 weeks was observed in two patients, both of who had negative PD-L1 staining. In another phase II study, Mego et al. treated eight patients with multiple relapsed and/or refractory GCTs [85], with 10 mg/kg avelumab administered biweekly. Five of the eight patients had CDDP-refractory disease. All had progressed at a median follow-up of 2.6 months. Curiously, no severe immune-related adverse events were reported in either study, probably due to the short duration of exposure to the drugs.

Further studies based on patient selection and specific biomarkers are needed, including, where possible, female GCTs [86]. The main completed and ongoing studies are summarized in Table 3. The recruitment of female patients with GCTs in prospective clinical trials on salvage treatments for male patients is warranted to give female patients the opportunity of potentially benefitting intensive chemotherapeutic approaches and/or new agents.

**Table 3.** Principal studies for treatment of relapsed/refractory GCT.

| Type of Regimen | Tumor Type Assessed | Type of Study | Patients (No. of Female Patients) | Therapy (No. of Patients) | Dose Regimen | Response | Treatment-Related Death (No.) | Study | Recruitment Status |
|---|---|---|---|---|---|---|---|---|---|
| HDC | Female gonadal/extragonadal relapsed/refractory GCTs | Retrospective | 51 (51) | Carboplatin, Etoposide and cyclophosphamide (21) Carboplatin, etoposide and ifosphamide (14) Carboplatin and etoposide (13) Carboplatin, etoposide and thiotepa (3) | N.A. | 15 CR 9 PR (−) 5 PR (+) 5 SD | 3 | De Giorgi et al. [60] | Completed |
| | Female recurrent GCTs | Retrospective | 13 (13) | Carboplatin and etoposide | Carboplatin 700 mg/m2 and etoposide 750 mg/m$^2$ IV daily for 3 consecutive days | 7 CR | none | Ammakkanavar et al. [24] | Completed |
| New therapy | Male and female refractory GCTs | Phase 2 | 30 (4) | Palbociclib | 125 mg daily for 21 days Q28days | 0 CR 24-week PFS 28% | none | Vaughn et al. [80] | Completed |
| | Male relapsed GCTs | Phase 2 | 12 (0) | Pembrolizumab | 200 mg IV Q3weeks | 0 CR 0 SD | none | Adra et al. [83] | Completed |
| | Male refractory GCTs | Phase 2 | 8 (0) | Avelumab | 10 mg/kg Q14days | 12-week PFS 0%, | none | Mego et al. [84] | Completed |
| | Male and female gonadal/extragonadal relapsed/refractory GCTs | Phase 2 | 18 (0) | Olaparib | 300 mg twice daily continuously | N.A. | N.A. | NCT02533765 | Active, not recruiting |
| | Male and female refractory GCTs | 2-stage, phase 2 | N.A. | ASP1650 | Study to establish the recommended phase 2 dose | N.A. | N.A. | NCT03760081 | Recruiting |
| | Male and female gonadal/extragonadal Refractory GCTs | Randomized, 3-stage, phase 2 | N.A. | Durvalumab ± tremelimumab | Durvalumab 1500 mg IV, q4 weeks; Tremelimumab 75 mg IV, both on day 1 and q4 weeks | N.A. | N.A. | NCT03081923 | Recruiting |

GCTs, germ cell tumors; HDC, high dose chemotherapy; CR, complete response; PR (−), partial remission with negative marker; PR (+), partial remission with positive marker; SD, stable disease; PFS, progression-free survival; N.A., not available.

## 7. Conclusions

Although female GCTs represent an extremely rare disease, the majority of patients can be cured with surgery or CDDP-based treatment. For this reason, the goal of treatments must also be to preserve fertility and reduce the risk of late adverse effects to guarantee good quality of life. Salvage therapy for MOGCT remains an unmet need, with current treatments based mainly on the results of retrospective studies. Several new drugs for this setting have been investigated in recent years, but with disappointing effects.

The present review suggests that HDC is a valid therapeutic option for CDDP-resistant female GCT. However, it is clear that more effective treatments are needed in this subset of patients. In two prospective clinical trials and in major consecutive series in GCTs accepting both genders, female GCT patients represented 3–7% of the overall recruited population [72,87]. In the pediatric population where GCTs represent one of the most common solid tumors and the ovary is a frequent primary site, it is common to enroll both male and female patients in clinical trials. Two important studies in this setting reported that female patients varied from 40% to 70% of the total GCTs [88,89].

On the basis of the rarity of the disease, new clinical trials on GCTs should include both male and female patients to provide prospective data on intensified therapeutic strategies such as HDC and/or new drugs, in particular molecular targeted therapies, for female GCTs. In this respect, the role of cooperative groups at national and international level will be crucial. Moreover, collaborative studies could be performed to validate prognostic classification systems to identify patients who are more likely to benefit from salvage HDC than from other SDC regimens. For the moment, patients with CDDP-resistant MOGCTs should undergo HDC or should be included in clinical trials, when possible. Referral of patients to centers with high expertise in this field is essential.

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## References

1. Van der Hel, O.L.; Timmermans, M.; van Altena, A.M.; Kruitwagen, R.F.P.M.; Slangen, B.F.M.; Sonke, G.S.; van de Vijver, K.K.; van der Aa, M.A. Overview of non-epithelial ovarian tumours: Incidence and survival in the Netherlands, 1989–2015. *Eur. J. Cancer* **2019**, *118*, 97–104. [CrossRef] [PubMed]
2. Møller, H.; Evans, H.; Giwercman, A.; Oosterhuis, J.W. Epidemiology of gonadal germ cell cancer in males and females. *APMIS* **2003**, *111*, 43–46. [CrossRef] [PubMed]
3. Oosterhuis, J.W.; Looijenga, L.H.J. Testicular germ-cell tumours in a broader perspective. *Nat. Rev. Cancer* **2005**, *5*, 210–222. [CrossRef] [PubMed]
4. Feldman, D.R.; Chaganti, R.S.K. Epidemiology, biology, and genetics of adult male germ cell tumors. In *Urological Oncology*; Springer: London, UK, 2015; pp. 431–450.
5. Walker, R.A.; World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. *Histopathology* **2005**, *46*, 229. [CrossRef]
6. Kemp, B.; Hauptmann, S.; Schröder, W.; Amo-Takyi, B.; Leeners, B.; Rath, W. Dysgerminoma of the ovary in a patient with triple-X syndrome. *Int. J. Gynecol. Obstet.* **1995**, *50*, 51–53. [CrossRef]
7. Schwartz, I.S.; Cohen, C.J. Dysgerminoma of the ovary associated with true hermaphroditism. *Obstet. Gynecol.* **1980**, *56*, 102–106.
8. Modesitt, S.C.; Brown, J. *Germ Cell and Sex Cord-Stromal Ovarian Cancers. Gynecologic Oncology: Clinical Practice and Surgical Atlas*, 1st ed.; McGraw-Hill: New York, NY, USA, 2012; Available online: https://doctorlib.info/oncology/gynecologic-oncology-clinical-practice/14.html (accessed on 25 September 2019).
9. Euscher, E.D. Germ Cell Tumors of the Female Genital Tract. *Surg. Pathol. Clin.* **2019**, *12*, 621–649. [CrossRef]
10. Low, J.J.H.; Ilancheran, A.; Ng, J.S. Malignant ovarian germ-cell tumours. *Best Pract. Res. Clin. Obstet. Gynaecol.* **2012**, *26*, 347–355. [CrossRef]

11. Ulbright, T.M. Germ cell tumors of the gonads: A selective review emphasizing problems in differential diagnosis, newly appreciated, and controversial issues. *Mod. Pathol.* **2005**, *18*, S61–S79. [CrossRef]
12. Houldsworth, J.; Bosl, G.J.; Chaganti, R.S.K. Genetics of Adult Male Germ Cell Tumours. *Treat. Options Urol. Cancer* **2008**, *24*, 279–292.
13. Andrews, P.W. Teratocarcinomas and human embryology: Pluripotent human EC cell lines. Review Article. *APMIS* **1998**, *106*, 158–168. [CrossRef] [PubMed]
14. Zalel, Y.; Piura, B.; Elchalal, U.; Czernobilsky, B.; Antebi, S.; Dgani, R. Diagnosis and management of malignant germ cell ovarian tumors in young females. *Int. J. Gynecol. Obstet.* **1996**, *55*, 1–10. [CrossRef]
15. Norris, H.J.; Jensen, R.D. Relative frequency of ovarian neoplasms in children and adolescents. *Cancer* **1972**, *30*, 713–719. [CrossRef]
16. Brown, J.; Friedlander, M.; Backes, F.J.; Harter, P.; O'Connor, D.M.; De La Motte Rouge, T.; Lorusso, D.; Maenpaa, J.; Kim, J.W.; Tenney, M.E.; et al. Gynecologic cancer intergroup (GCIG) consensus review for ovarian germ cell tumors. *Int. J. Gynecol. Cancer* **2014**, *24*, S48–S54. [CrossRef] [PubMed]
17. Mangili, G.; Sigismondi, C.; Lorusso, D.; Cormio, G.; Candiani, M.; Scarfone, G.; Mascilini, F.; Gadducci, A.; Mosconi, A.M.; Scollo, P.; et al. The role of staging and adjuvant chemotherapy in stage I malignant ovarian germ cell tumors (MOGTs): The MITO-9 study. *Ann. Oncol.* **2017**, *28*, 333–338. [CrossRef] [PubMed]
18. Gershenson, D.M. Current advances in the management of malignant germ cell and sex cord-stromal tumors of the ovary. *Gynecol. Oncol.* **2012**, *125*, 515–517. [CrossRef]
19. Ray-Coquard, I.; Morice, P.; Lorusso, D.; Prat, J.; Oaknin, A.; Pautier, P.; Colombo, N. Non-epithelial ovarian cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **2018**, *29*, iv1–iv18. [CrossRef]
20. Segelov, E.; Campbell, J.; Ng, M.; Tattersall, M.; Rome, R.; Free, K.; Hacker, N.; Friedlander, M.L. Cisplatin-based chemotherapy for ovarian germ cell malignancies: The Australian experience. *J. Clin. Oncol.* **1994**, *12*, 378–384. [CrossRef]
21. Williams, S.D.; Birch, R.; Einhorn, L.H.; Irwin, L.; Greco, F.A.; Loehrer, P.J. Treatment of Disseminated Germ-Cell Tumors with Cisplatin, Bleomycin, and either Vinblastine or Etoposide. *N. Engl. J. Med.* **1987**, *316*, 1435–1440. [CrossRef]
22. Dimopoulos, M.A.; Papadopoulou, M.; Andreopoulou, E.; Papadimitriou, C.; Pavlidis, N.; Aravantinos, G.; Aspropotamitis, A.; Anagnostopoulos, A.; Fountzilas, G.; Michalas, S.; et al. Favorable outcome of ovarian germ cell malignancies treated with cisplatin or carboplatin-based chemotherapy: A hellenic cooperative oncology group study. *Gynecol. Oncol.* **1998**, *70*, 70–74. [CrossRef]
23. Simone, C.G.; Markham, M.J.; Dizon, D.S. Chemotherapy in ovarian germ cell tumors: A systematic review. *Gynecol. Oncol.* **2016**, *141*, 602–607. [CrossRef] [PubMed]
24. Gershenson, D.M.; Morris, M.; Cangir, A.; Kavanagh, J.J.; Stringer, C.A.; Edwards, C.L.; Silva, E.G.; Wharton, J.T. Treatment of malignant germ cell tumors of the ovary with bleomycin, etoposide, and cisplatin. *J. Clin. Oncol.* **1990**, *8*, 715–720. [CrossRef] [PubMed]
25. Ammakkanavar, N.R.; Matei, D.; Abonour, R.; Einhorn, L.H. High-Dose Chemotherapy for Recurrent Ovarian Germ Cell Tumors. *J. Clin. Oncol.* **2015**, *33*, 226–227. [CrossRef] [PubMed]
26. Mead, G.M.; Stenning, S.P. The international germ cell consensus classification: A new prognostic factor-based staging classification for metastatic germ cell tumours. *Clin Oncol.* **1997**, *9*, 207–209. [CrossRef]
27. Bower, M.; Fife, K.; Holden, L.; Paradinas, F.J.; Rustin, G.J.S.; Newlands, E.S. Chemotherapy for Ovarian Germ Cell Tumours. *Eur. J. Cancer* **1996**, *32*, 593–597. [CrossRef]
28. Murugaesu, N.; Schmid, P.; Dancey, G.; Agarwal, R.; Holden, L.; McNeish, I.; Savage, P.M.; Newlands, E.S.; Rustin, G.J.S.; Seckl, M.J. Malignant ovarian germ cell tumors: Identification of novel prognostic markers and long-term outcome after multimodality treatment. *J. Clin. Oncol.* **2006**, *24*, 4862–4866. [CrossRef]
29. Mangili, G.; Sigismondi, C.; Gadducci, A.; Cormio, G.; Scollo, P.; Tateo, S.; Ferrandina, G.; Greggi, S.; Candiani, M.; Lorusso, D. Outcome and risk factors for recurrence in malignant ovarian germ cell tumors: A MITO-9 retrospective study. *Int. J. Gynecol. Cancer* **2011**, *21*, 1414–1421. [CrossRef]
30. Meisel, J.L.; Woo, K.M.; Sudarsan, N.; Eng, J.; Patil, S.; Jacobsen, E.P.; Murali, R.; Gardner, G.J.; Bosl, G.J.; Aghajanian, C.; et al. Development of a risk stratification system to guide treatment for female germ cell tumors. *Gynecol. Oncol.* **2015**, *138*, 566–572. [CrossRef]
31. Elashry, R.; Hemida, R.; Goda, H.; Abdel-Hady, E.-S. Prognostic factors of germ cell and sex cord-stromal ovarian tumors in pediatric age: 5 years experience. *J. Exp. Ther. Oncol.* **2013**, *10*, 181–187.

32. Nasioudis, D.; Chapman-Davis, E.; Frey, M.K.; Caputo, T.A.; Witkin, S.S.; Holcomb, K. Prognostic significance of residual disease in advanced stage malignant ovarian germ cell tumors. *Int. J. Gynecol. Cancer* **2019**, *29*, 554–559. [CrossRef]
33. Fizazi, K.; Oldenburg, J.; Dunant, A.; Chen, I.; Salvioni, R.; Hartmann, J.T.; De Santis, M.; Daugaard, G.; Flechon, A.; De Giorgi, U.; et al. Assessing prognosis and optimizing treatment in patients with postchemotherapy viable nonseminomatous germ-cell tumors (NSGCT): Results of the sCR2 international study. *Ann. Oncol.* **2008**, *19*, 259–264. [CrossRef] [PubMed]
34. Leary, A.F.; Quinn, M.; Fujiwara, K.; Coleman, R.L.; Kohn, E.; Sugiyama, T.; Glasspoo, R.; Ray-Coquard, I.; Colombo, N.; Bacon, M.; et al. Fifth ovarian cancer consensus conference of the Gynecologic Cancer InterGroup (GCIG): Clinical trial design for rare ovarian tumours. *Ann. Oncol.* **2017**, *28*, 718–726. [PubMed]
35. Lorch, A.; Beyer, J.; Kramar, A.; Einhorn, L.H.; Necchi, A.; Massard, C.; De Giorgi, U.; Flechon, A.; Margolin, K.A.; Lotz, J.P.; et al. Prognostic factors in patients with metastatic germ cell tumors who experienced treatment failure with cisplatin-based first-line chemotherapy. *J. Clin. Oncol.* **2010**, *28*, 4906–4911. [PubMed]
36. Koster, R.; van Vugt, M.A.T.M.; Timmer-Bosscha, H.; Gietema, J.A.; de Jong, S. Unravelling mechanisms of cisplatin sensitivity and resistance in testicular cancer. *Expert Rev. Mol. Med.* **2013**, *15*, e12. [CrossRef] [PubMed]
37. Andrews, P.A.; Howell, S.B. Cellular pharmacology of cisplatin: Perspectives on mechanisms of acquired resistance. *Cancer Cells* **1990**, *2*, 35–43.
38. Houldsworth, J.; Xiao, H.; Murty, V.V.V.S.; Chen, W.; Ray, B.; Reuter, V.E.; Bosl, G.J.; Chaganti, R.S.K. Human male germ cell tumor resistance to cisplatin is linked to TP53 gene mutation. *Oncogene* **1998**, *16*, 2345–2349. [CrossRef]
39. Gutekunst, M.; Oren, M.; Weilbacher, A.; Dengler, M.A.; Markwardt, C.; Thomale, J.; Aulitzky, W.E.; van der Kuip, H. P53 hypersensitivity is the predominant mechanism of the unique responsiveness of testicular germ cell tumor (TGCT) cells to Cisplatin. *PLoS ONE* **2011**, *6*, e19198. [CrossRef]
40. Gutekunst, M.; Mueller, T.; Weilbacher, A.; Dengler, M.A.; Bedke, J.; Kruck, S.; Oren, M.; Aulitzky, W.E.; Van Der Kuip, H. Cisplatin hypersensitivity of testicular germ cell tumors is determined by high constitutive noxa levels mediated by oct-4. *Cancer Res.* **2013**, *73*, 1460–1469. [CrossRef]
41. Koster, R.; Di Pietro, A.; Timmer-Bosscha, H.; Gibcus, J.H.; Van Den Berg, A.; Suurmeijer, A.J.; Bischoff, R.; Gietema, J.A.; De Jong, S. Cytoplasmic p21 expression levels determine cisplatin resistance in human testicular cancer. *J. Clin. Investig.* **2010**, *120*, 3594–3605. [CrossRef]
42. Wu, Y.-C.; Ling, T.-Y.; Lu, S.-H.; Kuo, H.-C.; Ho, H.-N.; Yeh, S.-D.; Shen, C.-N.; Huang, Y.-H. Chemotherapeutic sensitivity of testicular germ cell tumors under hypoxic conditions is negatively regulated by SENP1-controlled sumoylation of OCT4. *Cancer Res.* **2012**, *72*, 4963–4973. [CrossRef]
43. Grande, L.; Bretones, G.; Rosa-Garrido, M.; Garrido-Martin, E.M.; Hernandez, T.; Fraile, S.; Botella, L.; De Alava, E.; Vidal, A.; del Muro, X.G.; et al. Transcription factors Sp1 and p73 control the expression of the proapoptotic protein NOXA in the response of testicular embryonal carcinoma cells to cisplatin. *J. Biol. Chem.* **2012**, *287*, 26495–26505. [CrossRef] [PubMed]
44. Di Pietro, A.; Koster, R.; Boersma-van Eck, W.; Dam, W.A.; Mulder, N.H.; Gietema, J.A.; De Vries, E.G.E.; De Jong, S. Pro- and anti-apoptotic effects of p53 in cisplatin-treated human testicular cancer are cell context-dependent. *Cell Cycle* **2012**, *11*, 4552–4562. [CrossRef] [PubMed]
45. Li, B.; Cheng, Q.; Li, Z.; Chen, J. p53 inactivation by MDM2 and MDMX negative feedback loops in testicular germ cell tumors. *Cell Cycle* **2010**, *9*, 1411–1420. [CrossRef] [PubMed]
46. Koster, R.; Timmer-Bosscha, H.; Bischoff, R.; Gietema, J.A.; De Jong, S. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. *Cell Death Dis.* **2011**, *2*, e148. [CrossRef] [PubMed]
47. Wermann, H.; Stoop, H.; Gillis, A.J.M.; Honecker, F.; Van Gurp, R.J.H.L.M.; Ammerpohl, O.; Richter, J.; Oosterhuis, J.W.; Bokemeyer, C.; Looijenga, L.H.J. Global DNA methylation in fetal human germ cells and germ cell tumours: Association with differentiation and cisplatin resistance. *J. Pathol.* **2010**, *221*, 433–442. [CrossRef]
48. Koul, S.; McKiernan, J.M.; Narayan, G.; Houldsworth, J.; Bacik, J.; Dobrzynski, D.L.; Assaad, A.M.; Mansukhani, M.; Reuter, V.E.; Bosl, G.J.; et al. Role of promoter hypermethylation in cisplatin treatment response of male germ cell tumors. *Mol. Cancer* **2004**, *3*, 16. [CrossRef]

49. Biswal, B.K.; Beyrouthy, M.J.; Hever-Jardine, M.P.; Armstrong, D.; Tomlinson, C.R.; Christensen, B.C.; Marsit, C.J.; Spinella, M.J. Acute Hypersensitivity of Pluripotent Testicular Cancer-Derived Embryonal Carcinoma to Low-Dose 5-Aza Deoxycytidine Is Associated with Global DNA Damage-Associated p53 Activation, Anti-Pluripotency and DNA Demethylation. *PLoS ONE* **2012**, *7*, e53003. [CrossRef]
50. Beyrouthy, M.J.; Garner, K.M.; Hever, M.P.; Freemantle, S.J.; Eastman, A.; Dmitrovsky, E.; Spinella, M.J. High DNA methyltransferase 3B expression mediates 5-aza-deoxycytidine hypersensitivity in testicular germ cell tumors. *Cancer Res.* **2009**, *69*, 9360–9366. [CrossRef]
51. Albany, C.; Hever-Jardine, M.P.; von Herrmann, K.M.; Yim, C.Y.; Tam, J.; Warzecha, J.M.; Shin, L.; Bock, S.E.; Curran, B.S.; Chaudhry, A.S.; et al. Refractory testicular germ cell tumors are highly sensitive to the second generation DNA methylation inhibitor guadecitabine. *Oncotarget* **2017**, *8*, 2949–2959. [CrossRef]
52. Di Vizio, D.; Cito, L.; Boccia, A.; Chieffi, P.; Insabato, L.; Pettinato, G.; Motti, M.L.; Schepis, F.; D'Amico, W.; Fabiani, F.; et al. Loss of the tumor suppressor gene PTEN marks the transition from intratubular germ cell neoplasias (ITGCN) to invasive germ cell tumors. *Oncogene* **2005**, *24*, 1882–1894. [CrossRef]
53. Juliachs, M.; Muñoz, C.; Moutinho, C.A.; Vidal, A.; Condom, E.; Esteller, M.; Graupera, M.; Casanovas, O.; Germà, J.R.; Villanueva, A.; et al. The PDGFRβ-AKT pathway contributes to CDDP-acquired resistance in testicular germ cell tumors. *Clin. Cancer Res.* **2014**, *20*, 658–667. [CrossRef] [PubMed]
54. Mego, M.; Svetlovska, D.; Miskovska, V.; Obertova, J.; Palacka, P.; Rajec, J.; Sycova-Mila, Z.; Chovanec, M.; Rejlekova, K.; Zuzák, P.; et al. Phase II study of everolimus in refractory testicular germ cell tumors. *Urol. Oncol. Semin. Orig. Investig.* **2016**, *34*, 122.e17–122.e22. [CrossRef] [PubMed]
55. Koster, R.; De Jong, S. Lessons learned from testicular cancer: Identification of cytoplasmic p21 as an Achilles' heel of cisplatin resistance. *Cell Cycle* **2010**, *9*, 4776–4777. [CrossRef] [PubMed]
56. Looijenga, L.H.J.; Gillis, A.J.M.; Stoop, H.; Biermann, K.; Oosterhuis, J.W. Dissecting the molecular pathways of (testicular) germ cell tumour pathogenesis; from initiation to treatment-resistance. *Int. J. Androl.* **2011**, *34*, e234–e251. [CrossRef] [PubMed]
57. Gorczyca, W.; Gong, J.; Ardelt, B.; Iraganos, F.; Darzynkiewicz, Z. The Cell Cycle Related Differences in Susceptibility of HL-60 Cells to Apoptosis Induced by Various Antitumor Agents. *Cancer Res.* **1993**, *53*, 3186–3192. [PubMed]
58. Barry, M.A.; Behnke, C.A.; Eastman, A. Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins and hyperthermia. *Biochem. Pharmacol.* **1990**, *40*, 2353–2362. [CrossRef]
59. Abada, P.B.; Howell, S.B. Cisplatin induces resistance by triggering differentiation of testicular embryonal carcinoma cells. *PLoS ONE* **2014**, *9*, e87444. [CrossRef]
60. Timmer-Bosscha, H.; De Vries, E.G.E.; Meijer, C.; Oosterhuis, J.W.; Mulder, N.H. Differential effects of all-trans-retinoic acid, docosahexaenoic acid, and hexadecylphosphocholine on cisplatin-induced cytotoxicity and apoptosis in a cisplantin-sensitive and resistant human embryonal carcinoma cell line. *Cancer Chemother. Pharmacol.* **1998**, *41*, 469–476. [CrossRef]
61. De Giorgi, U.; Richard, S.; Badoglio, M.; Kanfer, E.; Bourrhis, J.H.; Nicolas-Virelizier, E.; Vettenranta, K.; Lioure, B.; Martin, S.; Dreger, P.; et al. Salvage high-dose chemotherapy in female patients with relapsed/refractory germ-cell tumors: A retrospective analysis of the European Group for Blood and Marrow Transplantation (EBMT). *Ann. Oncol.* **2017**, *28*, 1910–1916. [CrossRef]
62. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Ovarian cancer including fallopian tube cancer and primary peritoneal cancer Version 2.2019 NCCN Guidelines for Patients. 2019. Available online: https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf (accessed on 16 october 2019).
63. Kondagunta, G.V.; Bacik, J.; Donadio, A.; Bajorin, D.; Marion, S.; Sheinfeld, J.; Bosl, G.J.; Motzer, R.J. Combination of paclitaxel, ifosfamide, and cisplatin is an effective second-line therapy for patients with relapsed testicular germ cell tumors. *J. Clin. Oncol.* **2005**, *23*, 6549–6555. [CrossRef]
64. Mead, G.M.; Cullen, M.H.; Huddart, R.; Harper, P.; Rustin, G.J.S.; Cook, P.A.; Stenning, S.P.; Mason, M. A phase II trial of TIP (paclitaxel, ifosfamide and cisplatin) given as second-line (post-BEP) salvage chemotherapy for patients with metastatic germ cell cancer: A medical research council trial. *Br. J. Cancer* **2005**, *93*, 178. [CrossRef] [PubMed]
65. Zanetta, G.; Fei, F.; Mangioni, C. Chemotherapy with paclitaxel, ifosfamide, and cisplatin for the treatment of squamous cell cervical cancer: The experience of Monza. *Semin. Oncol.* **2000**, *27*, 23–27. [PubMed]

66. Pawinski, A.; Favalli, G.; Ploch, E.; Sahmoud, T.; Van Oosterom, A.T.; Pecorelli, S. PVB chemotherapy in patients with recurrent or advanced dysgerminoma: A phase II study of the EORTC Gynaecological Cancer Cooperative Group. *Clin. Oncol.* **1998**, *10*, 301–305. [CrossRef]
67. De Giorgi, U.; Rosti, G.; Aieta, M.; Testore, F.; Burattini, L.; Fornarini, G.; Naglieri, E.; Lo Re, G.; Zumaglini, F.; Marangolo, M. Phase II Study of Oxaliplatin and Gemcitabine Salvage Chemotherapy in Patients with Cisplatin-Refractory Nonseminomatous Germ Cell Tumor. *Eur. Urol.* **2006**, *50*, 1032–1039. [CrossRef]
68. Albers, P. Surgery is an Essential Part of Salvage Treatment in Refractory Germ Cell Tumors. *Eur Urol.* **2006**, *50*, 893–894. [CrossRef]
69. Lorch, A.; Bascoul-Mollevi, C.; Kramar, A.; Einhorn, L.; Necchi, A.; Massard, C.; De Giorgi, U.; Fléchon, A.; Margolin, K.; Lotz, J.P.; et al. Conventional-dose versus high-dose chemotherapy as first salvage treatment in male patients with metastatic germ cell tumors: Evidence from a large international database. *J. Clin. Oncol.* **2011**, *29*, 2178–2184. [CrossRef]
70. Beyer, J.; Albers, P.; Altena, R.; Aparicio, J.; Bokemeyer, C.; Busch, J.; Cathomas, R.; Cavallin-Stahl, E.; Clarke, N.W.; Claßen, J.; et al. Maintaining success, reducing treatment burden, focusing on survivorship: Highlights from the third European Consensus Conference on Diagnosis and Treatment of Germ-Cell Cancer. *Ann. Oncol.* **2013**, *24*, 878–888. [CrossRef]
71. Einhorn, L.H.; Williams, S.D.; Chamness, A.; Brames, M.J.; Perkins, S.M.; Abonour, R. High-dose chemotherapy and stem-cell rescue for metastatic germ-cell tumors. *N. Engl. J. Med.* **2007**, *357*, 340–348. [CrossRef]
72. Feldman, D.R.; Sheinfeld, J.; Bajorin, D.F.; Fischer, P.; Turkula, S.; Ishill, N.; Patil, S.; Bains, M.; Reich, L.M.; Bosl, G.J.; et al. TI-CE high-dose chemotherapy for patients with previously treated germ cell tumors: Results and prognostic factor analysis. *J. Clin. Oncol.* **2010**, *28*, 1706–1713. [CrossRef]
73. Oing, C.; Kollmannsberger, C.; Oechsle, K.; Bokemeyer, C. Investigational targeted therapies for the treatment of testicular germ cell tumors. *Expert Opin. Investig. Drugs* **2016**, *25*, 1033–1043. [CrossRef]
74. Bryant, H.E.; Schultz, N.; Thomas, H.D.; Parker, K.M.; Flower, D.; Lopez, E.; Kyle, S.; Meuth, M.; Curtin, N.J.; Helleday, T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **2005**, *434*, 913–917. [CrossRef] [PubMed]
75. Farmer, H.; McCabe, H.; Lord, C.J.; Tutt, A.H.J.; Johnson, D.A.; Richardson, T.B.; Santarosa, M.; Dillon, K.J.; Hickson, I.; Knights, C.; et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **2005**, *434*, 917–921. [CrossRef] [PubMed]
76. Mego, M.; Cierna, Z.; Svetlovska, D.; Macak, D.; Machalekova, K.; Miskovska, V.; Chovanec, M.; Usakova, V.; Obertova, J.; Babal, P.; et al. PARP expression in germ cell tumours. *J. Clin. Pathol.* **2013**, *66*, 607–612. [CrossRef] [PubMed]
77. Schmidt, B.A.; Rose, A.; Steinhoff, C.; Strohmeyer, T.; Hartmann, M.; Ackermann, R. Up-regulation of cyclin-dependent kinase 4/cyclin D2 expression but down-regulation of cyclin-dependent kinase 2/cyclin E in testicular germ cell tumors. *Cancer Res.* **2001**, *61*, 4214–4221.
78. Noel, E.E.; Yeste-Velasco, M.; Mao, X.; Perry, J.; Kudahetti, S.C.; Li, N.F.; Sharp, S.; Chaplin, T.; Xue, L.; McIntyre, A.; et al. The association of CCND1 overexpression and cisplatin resistance in testicular germ cell tumors and other cancers. *Am. J. Pathol.* **2010**, *176*, 2607–2615. [CrossRef]
79. Strohmeyer, T.; Reissmann, P.; Cordon-Cardo, C.; Hartmann, M.; Ackermann, R.; Slamon, D. Correlation between retinoblastoma gene expression and differentiation in human testicular tumors. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 6662–6666. [CrossRef]
80. Bartkova, J.; Lukas, C.; Sørensen, C.; Rajpert-De Meyts, E.; Skakkebæk, N.E.; Lukas, J.; Bartek, J. Deregulation of the RB pathway in human testicular germ cell tumours. *J. Pathol.* **2003**, *200*, 149–156. [CrossRef]
81. Vaughn, D.J.; Hwang, W.T.; Lal, P.; Rosen, M.A.; Gallagher, M.; O'Dwyer, P.J. Phase 2 trial of the cyclin-dependent kinase 4/6 inhibitor palbociclib in patients with retinoblastoma protein-expressing germ cell tumors. *Cancer* **2015**, *121*, 1463–1468. [CrossRef]
82. Fankhauser, C.D.; Curioni-Fontecedro, A.; Allmann, V.; Beyer, J.; Tischler, V.; Sulser, T.; Moch, H.; Bode, P.K. Frequent PD-L1 expression in testicular germ cell tumors. *Br. J. Cancer* **2015**, *113*, 411–413. [CrossRef]
83. Cierna, Z.; Mego, M.; Miskovska, V.; Machalekova, K.; Chovanec, M.; Svetlovska, D.; Hainova, K.; Rejlekova, K.; Macak, D.; Spanik, S.; et al. Prognostic value of programmed-death-1 receptor (PD-1) and its ligand 1 (PD-L1) in testicular germ cell tumors. *Ann. Oncol.* **2016**, *27*, 300–305. [CrossRef]

84. Adra, N.; Einhorn, L.H.; Althouse, S.K.; Ammakkanavar, N.R.; Musapatika, D.; Albany, C.; Vaughn, D.; Hanna, N.H. Phase II trial of pembrolizumab in patients with platinum refractory germ-cell tumors: A Hoosier Cancer Research Network Study GU14-206. *Ann. Oncol.* **2018**, *29*, 209–214. [CrossRef] [PubMed]
85. Mego, M.; Svetlovska, D.; Chovanec, M.; Rejlekova, K.; Obertova, J.; Palacka, P.; Sycova-Mila, Z.; De Giorgi, U.; Mardiak, J. Phase II study of avelumab in multiple relapsed/refractory testicular germ cell cancer. *J. Clin. Oncol.* **2019**, *37*, e16045. [CrossRef]
86. Chovanec, M.; Cierna, Z.; Miskovska, V.; Machalekova, K.; Kalavska, K.; Rejlekova, K.; Svetlovska, D.; Macak, D.; Spanik, S.; Kajo, K.; et al. Systemic immune-inflammation index in germ-cell tumours. *Br. J. Cancer* **2018**, *118*, 831–838. [CrossRef] [PubMed]
87. De Giorgi, U.; Demirer, T.; Wandt, H.; Taverna, C.; Siegert, W.; Bornhauser, M.; Kozak, T.; Papiani, G.; Ballardini, M.; Rosti, G.; et al. Second-line high-dose chemotherapy in patients with mediastinal and retroperitoneal primary non-seminomatous germ cell tumors: The EBMT experience. *Ann. Oncol.* **2005**, *16*, 146–151. [CrossRef] [PubMed]
88. Cushing, B.; Giller, R.; Cullen, J.W.; Marina, N.M.; Lauer, S.J.; Olson, T.A.; Rogers, P.C.; Colombani, P.; Rescorla, F.; Billmire, D.F.; et al. Randomized comparison of combination chemotherapy with etoposide, bleomycin, and either high-dose or standard-dose cisplatin in children and adolescents with high-risk malignant germ cell tumors: A pediatric intergroup study—Pediatric Oncology Group 904. *J. Clin. Oncol.* **2004**, *22*, 2691–2700. [CrossRef]
89. De Giorgi, U.; Rosti, G.; Slavin, S.; Yaniv, I.; Harousseau, J.L.; Ladenstein, R.; Demirer, T.; Dini, G. Salvage high-dose chemotherapy for children with extragonadal germ-cell tumours. *Br. J. Cancer* **2005**, *93*, 412–417. [CrossRef]