Testicular cancer: Determinants of cisplatin sensitivity and novel therapeutic opportunities

de Vries, Gerda; Rosas-Plaza, Ximena; van Vugt, Marcel A. T. M.; Gietema, Jourik A.; de Jong, Steven

Published in:
CANCER TREATMENT REVIEWS

DOI:
10.1016/j.ctrv.2020.102047

Publication date:
2020

Cancer Treatment Reviews 88 (2020) 102054

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Tumour Review

Testicular cancer: Determinants of cisplatin sensitivity and novel therapeutic opportunities

Gerda de Vries[1], Ximena Rosas-Plaza[1], Marcel A.T.M. van Vugt, Jourik A. Gietema, Steven de Jong*

Department of Medical Oncology, Cancer Research Center Groningen, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

ARTICLE INFO

Keywords:
Testicular cancer
Germ cell tumors
Cisplatin
Cisplatin resistance
Targeted therapy

ABSTRACT

Testicular cancer (TC) is the most common solid tumor among men aged between 15 and 40 years. TCs are highly aneuploid and the 12p isochromosome is the most frequent chromosomal abnormality. The mutation rate is of TC is low, with recurrent mutations in *KIT* and *KRAS* observed only at low frequency in seminomas. Overall cure rates are high, even in a metastatic setting, resulting from excellent cisplatin sensitivity of TCs. Factors contributing to the observed cisplatin sensitivity include defective DNA damage repair and a hypersensitive apoptotic response to DNA damage. Nonetheless, around 10–20% of TC patients with metastatic disease cannot be cured by cisplatin-based chemotherapy. Resistance mechanisms include downregulation of OCT4 and failure to induce PUMA and NOXA, elevated levels of MDM2, and hyperactivity of the PI3K/AKT/mTOR pathway. Several pre-clinical approaches have proven successful in overcoming cisplatin resistance, including specific targeting of PARP, MDM2 or AKT/mTOR combined with cisplatin. Finally, patient-derived xenograft models hold potential for mechanistic studies and pre-clinical validation of novel therapeutic strategies in TC. While clinical trials investigating targeted drugs have been disappointing, pre-clinical successes with chemotherapy and targeted drug combinations fuel the need for further investigation in clinical setting.

Clinical and pathological characteristics of TC

Testicular cancer (TC) accounts for approximately 1% of all cancers in men worldwide, while it is the most common solid tumor among young men (15–40 years) [1]. Incidence of TC varies widely across different regions, with highest incidence in Northern Europe and very low incidence in Central Africa [1]. Incidence rates of TC have increased since the 1960s in Northern European countries, affecting age groups older than 15 years with no proven causative mechanisms identified so far [2].

Germ cell tumor (GCT) is the predominant histology of TC patients (95%), divided into seminomas and non-seminomas, of which seminomas are slightly more common than non-seminomas [3]. GCTs arise from gonocytes that fail to differentiate into spermatogonias, and the pluripotency of these cells allows them to develop into highly diverse histological tumors. Seminomas are blocked in the earliest differentiation state whereas non-seminomas consist of diverse histological subtypes with a varying degree of differentiation: embryonal carcinoma (EC) (undifferentiated), choriocarcinoma (CC), yolk sac carcinoma (YSC) or teratoma (mature and immature) [4]. Mixed GCTs are common and may appear in any form.

Diagnosis of TC is performed by clinical evaluation, ultrasound examination of the testes, orchiectomy of the involved testicle and determination of serum tumor markers. These tumor biomarkers include LDH (lactate dehydrogenase), AFP (alpha-fetoprotein) and ß-HCG (ß-human chorionic gonadotropin), and assist in the diagnosis [seminoma vs non-seminoma], staging, risk stratification and follow-up after treatment of TC patients [5]. The international clinical staging system recommended for TC is the Tumor, Node, Metastasis (TNM) classification of the International Union Against Cancer (UICC) [6]. Current risk stratification was established in 1997 by the International Germ Cell Consensus Classification (IGCCC) and constitutes a prognostic staging system for patients with disseminated disease [7].

Overall cure rate of TC is very good, also in a metastatic setting, which is mostly resulting from the high sensitivity of TCs to the chemotherapeutic drug cisplatin. The 5-year relative survival rates for seminoma and non-seminoma patients are above 90% [2]. For patients with stage I disease the survival rate is even somewhat higher exceeding

* Corresponding author at: Department of Medical Oncology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
E-mail address: s.de.jong@umcg.nl (S. de Jong).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ctrv.2020.102054
Received 2 April 2020; Received in revised form 3 June 2020; Accepted 4 June 2020

Table 1
Risk stratification for metastatic patients [7].

	Non-seminoma		Seminoma	
Risk group	Spread and tumor markers	Survival	Spread and tumor markers	Survival
Good prognosis	• Testis or retroperitoneal primary • No metastasis to organs other than the lungs and/or lymph nodes • Tumor markers level normal or at least 1 tumor marker above normal: • LDH < 1.5x ULN • beta-hCG < 5000 mlU/mL • AFP < 1000 ng/mL	56% of patients 5-year PFS: 89% 5-year OS: 92%	• Any primary site • No metastasis to organs other than the lungs and/or lymph nodes • Normal AFP • Any beta-hCG and LDH	90% of patients 5-year PFS: 82% 5-year OS: 86%
Intermediate prognosis	• Testis or retroperitoneal primary • No metastasis to organs other than the lungs and/or lymph nodes • At least 1 tumor marker substantially above normal: • LDH 1.5 − 10x ULN • beta-hCG ≥ 5000 ≤ 50000 mlU/mL • AFP ≥ 1000 ≤ 10000 ng/mL	28% of patients 5-year PFS: 75% 5-year OS: 80%	• Any primary site • Metastasis to organs other than the lungs and/or lymph nodes • Normal AFP • Any beta-hCG and LDH	10% of patients 5-year PFS: 67% 5-year OS: 72%
Poor prognosis	• Mediastinal primary with or without metastases • Metastasis to organs other than the lungs and/or lymph nodes • At least 1 or more tumor maker levels highly elevated: • LDH > 10x ULN • beta-hCG > 50000 mlU/mL • AFP > 10000 ng/mL	16% of patients 5-year PFS: 41% 5-year OS: 48%	No poor prognosis patients	No poor prognosis patients

LDH: lactate dehydrogenase, hCG: human chorionic gonadotropin, AFP: alpha-fetoprotein, ULN: upper limit of normal, PFS: progression free survival, OS: overall survival.

95% regardless of tumor type. However, patients with metastatic disease have poorer survival outcomes depending on the tumor type and extent of disease and prognosis group. Seminoma and non-seminoma tumors have different treatment sensitivity and invasive capabilities. At moment of diagnosis 25% of seminoma patients have metastatic disease, while 60% of non-seminoma patients have metastatic lesions [8]. The IGCCC classification stratifies patients into good, intermediate and poor prognosis groups which are determined based on clinical features, including the location of the metastases and the levels of different tumor biomarkers [7]. According to the IGCCC there are no advanced seminoma patients within the poor prognosis group, related to their better overall response to chemotherapy. Staging, risk stratification and 5-year survival rates for both seminoma and non-seminoma patients are summarized in Table 1.

Late relapses (LR) occur in 1% to 6% of TC patients and are considered to be more difficult to treat than early relapses [9]. A pooled analysis of several studies has shown that late relapses occur more often in non-seminomas (3.2%) than in seminomas (1.4%), with teratoma as the most frequent histological component (60%), followed by YSC (47%) [9,10]. Because of the high rate of teratoma and teratoma with malignant transformation to non-germ cell malignancies (20%), LR are less likely to respond to chemotherapy [9]. Additionally, YSC has been associated with poor prognosis, this could partly explain the worse clinical outcome associated with late relapse of this histological subtype [11].

Standard treatment of TC

Treatment of TC depends on stage and tumor type, i.e. seminoma or non-seminoma. Stage I or localized disease, either seminoma or non-seminoma, is treated with orchiectomy followed by active surveillance or with adjuvant treatment based on risk factors and patient preference. Patients with stage IIA/B seminoma are treated with orchiectomy followed by either radiotherapy or chemotherapy, and bulky stage IIC and III seminoma patients receive chemotherapy after orchiectomy. Patients with disseminated non-seminoma (intermediate or poor risk IGCCC) are treated with four courses of the BEP (bleomycin, etoposide and cisplatin) or VIP (etoposide, ifosfamide and cisplatin) regimen, after surgical removal of the affected testicle. In case of residual disease after completion of chemotherapy patients undergo a surgical removal of affected lymph nodes and/or metastases that had not completely disappeared after chemotherapy. Approximately 10–15% of patients with disseminated disease will need second-line treatment as a consequence of relapse or refractory disease [12]. Various effective salvage strategies are currently available, including standard-dose and high-dose chemotherapy regimens. The choice of standard-dose salvage treatment depends on which drugs were initially used in combination with cisplatin. Some common and effective standard-dose salvage treatments have been reported with long-term remissions ranging from 23 to 54% using VIP (cisplatin, ifosfamide and etoposide) [13,14], 63% using TIP (paclitaxel, ifosfamide and cisplatin) [15], 24% using VeIP (vinblastine, ifosfamide and cisplatin) [16] and 51% using GIP (gemcitabine, ifosfamide and cisplatin) [17]. High-dose chemotherapy with carboplatin and etoposide with autologous bone marrow transplantation was initially studied in patients with a predicted poor outcome to standard-dose salvage treatment or patients who had already failed a prior standard-dose salvage treatment. Initial results were exceptional with a complete response rate of 26% [18]. More recently, high-dose chemotherapy with autologous peripheral stem cell transplantation has shown long-term remissions in 63% of patients, including patients who received this as third-line treatment [19]. As demonstrated by the data presented above, standard-dose and high-dose salvage chemotherapy are both able to overcome cisplatin resistance. A large retrospective study suggested that high-dose chemotherapy is superior to standard-dose chemotherapy as first salvage treatment [20]. To date, it remains unclear whether standard-dose or high-dose salvage chemotherapy provides better outcomes when used as initial salvage treatment. To address this question, a prospective global trial (the TIGER trial) is currently performed in TC patients with relapsed or refractory disease. In this trial, patients are being randomized between 4 courses of TIP vs. TI-CE [with 3 courses of high dose carboplatin-etoposide with an autologous peripheral stem-cell transplantation] (NCT02375204).

Genetic alterations in TC patients

TCs are highly aneuploid and frequently show large scale copy number gains and losses [21-23]. The most common anomaly is the presence of a 12p isochromosome (i(12p)) affecting more than 80% of TCs [24]. This region of the small arm of chromosome 12 contains the *KRAS* proto-oncogene, as well as some stem cell-related genes including *NANOG* and *STELLAR*. Of note, pre-malignant lesions do not contain i (12p) [25], suggesting that this genetic event is not an early event in the development of TC. *KIT* gene amplifications at 4q12 were identified in 21% of seminomas and 9% of non-seminomas [26]. Copy number gains of the *KIT* gene were associated with an increased expression of the KIT protein, although other mechanisms besides copy number gain can also result in increased KIT expression [26].

Despite the high levels of aneuploidy of TCs, the somatic mutation rate is low. Whole exome sequencing revealed that, on average, TC tumors have a mutation rate of 0.51 somatic mutations per Mb, while lung cancers carry 8.0 mutations/Mb, and melanomas display 11.0 mutations/Mb [27]. The most frequently mutated gene in TC is *KIT*, and has been observed predominantly in seminomas [27,28]. The proportion of *KIT* hotspot mutations in seminomas ranged from 18% to 31% [27,29]. Besides *KIT*, mutations in *KRAS* and *NRAS* have been reported, albeit with a lower frequency [27,30]. Other mutations that were recently described include: *CDC27*, a potential tumor suppressor gene [27,31]; *RPL5*, a ribosomal protein [30]; and *RAC1*, a member of the Rho family of GTPases [32].

Studies looking specifically at mutations in cisplatin-resistant patients have also been performed. Results showed a higher incidence of activating mutations in *RAS, PIK3CA* and *AKT1* in chemoresistant TCs [33]. Mutations in *XRCC2* have been reported in 5 cases of refractory TCs [27,34]. XRCC2 belongs to the RAD51 protein family and participates in DNA repair through homologous recombination. XRCC2 interacts with other RAD51 protein family members and facilitates the assembly of RAD51 onto DNA double-stranded breaks. Interestingly, *XRCC2* variants were shown to confer resistance to cisplatin-induced DNA damage and have been associated with breast cancer risk and survival [35,36].

One of the characteristics of TC is the presence of wild type (WT) *TP53* in the majority of patients, although mutations in *TP53* have been observed in a few cases [37]. Activity of p53 is regulated by the E3 ubiquitin ligase MDM2, which prevents transcription of target genes by p53 through direct binding to the transactivation domain of p53 and through targeting p53 for proteasomal degradation by ubiquitination [38]. MDM2 itself is a transcriptional target of p53, which provides an autoregulatory negative feedback to maintain p53 levels low under physiological conditions. Bagrodia *et al.* showed that the incidence of p53 pathway mutations was higher in chemoresistant TC compared to chemosensitive TC, with *TP53* mutations mainly occurring in non-seminoma [32]. *MDM2* and *MYCN* amplifications, both affecting p53 signaling, have also been described in chemoresistant and refractory patients [32,34]. As *TP53* mutations are rare, *MDM2* amplification is likely a selection mechanism to prevent cell cycle arrest and DNA repair during the progression of disease.

Cisplatin sensitivity

With the introduction of cisplatin-based chemotherapy in the 1970s, cure rates of metastatic TC have improved drastically [39]. Even though cisplatin has been used in the clinic for a long time, the mechanisms behind the excellent sensitivity of these tumor types remain elusive. Two features of TC tumors that have been related to cisplatin sensitivity of TC are: insufficient DNA repair of cisplatin-induced DNA damage, and a hypersensitive apoptotic response, both discussed below and summarized in Figs. 1 and 2.

Cisplatin interacts with DNA bases generating different forms of DNA adducts that are predominantly intrastrand cross-links (> 90%)

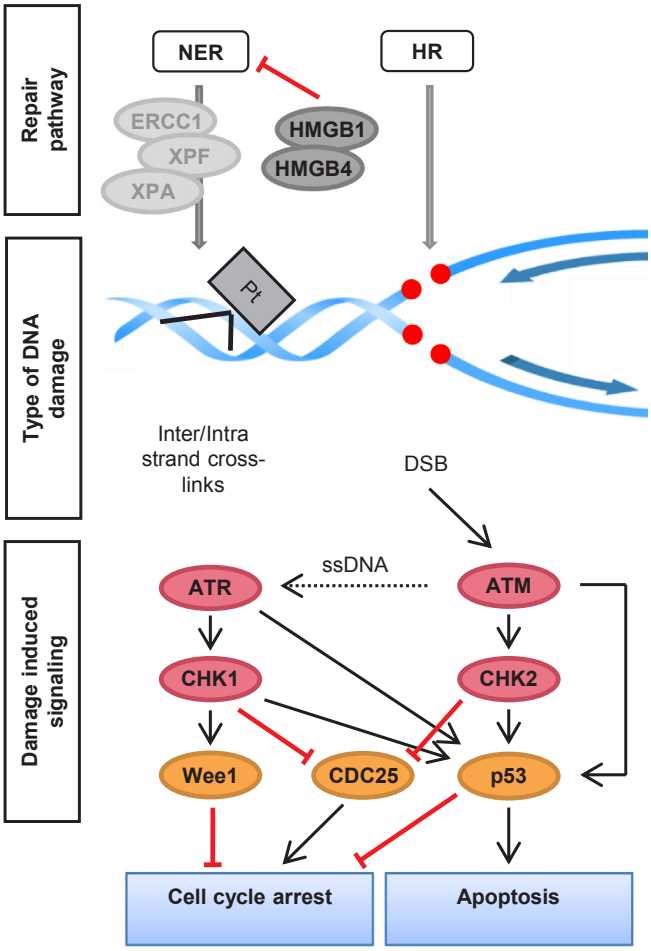

Fig. 1. Overview of cisplatin induced DNA damage and repair. Cisplatin treatment mainly induces intra- and interstrand DNA cross-links. These lesions are repaired by NER. Low expression of NER related proteins (e.g. ERCC1, XPF and XPA) are a rate limiting factor for cisplatin-DNA cross-link repair. High expression of HMGB1/4 can inhibit NER. Due to less functional NER, unrepaired lesions may further develop into DSB which require HR for repair. ATM is recruited to sites of DSBs and leads to a DNA damage induced signaling cascade that activates a p53-dependent transcriptional program leading to cell-cycle arrest or apoptosis. HR requires DNA-end resection that produces tracts of single-stranded DNA (ssDNA). ATR is recruited to tracts of ssDNA, where it phosphorylates and activates CHK1 leading to activation of Wee1 and proteasomal degradation of CDC25, thereby regulating cell cycle arrest.

but also interstrand cross-links, protein-DNA cross-links and DNA mono-adducts [40,41]. Cisplatin-induced DNA cross-links disrupt the structure of the DNA which can be recognized by DNA repair proteins. Cisplatin-induced intrastrand crosslinks are mostly repaired by the nucleotide excision repair (NER) pathway [42]. However, it has been shown that various high mobility group (HMG) proteins bind to DNA adducts inhibiting NER [43,44]. In addition, some essential proteins involved in NER including ERCC1, XPF and XPA were shown to have low expression levels in TC [45,46]. Low levels of ERCC1 and XPF, while still sufficient to perform NER, are rate-limiting for the repair of cisplatin-DNA cross-links in TC cells, contributing to the excellent sensitivity to cisplatin treatment [47]. Furthermore, cytotoxicity of cisplatin can be partially explained by defective double strand break (DSB) repair by homologous recombination. If DNA crosslinks are not repaired they can lead to collapsed replication forks, which results in the formation of DSBs. Interestingly, in a panel of TC cell lines, all models were highly sensitive to the combination of cisplatin with PARP inhibitors, providing a potential therapeutic approach for resistant TC

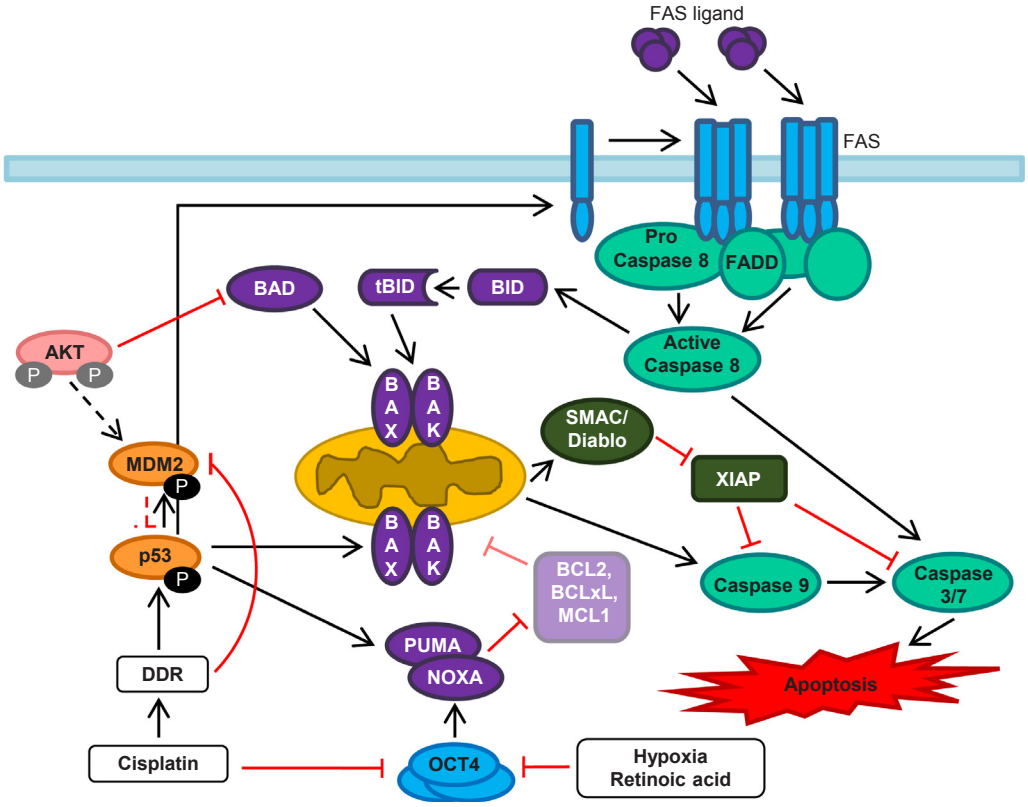

Fig. 2. Apoptotic signaling in cisplatin sensitive TC. Apoptosis is regulated at several levels with a key role for p53. Cisplatin treatment activates the DDR leading to p53 activation. P53 transcriptionally regulates proteins involved in both the extrinsic and intrinsic apoptosis pathway, including FAS death receptor, BAX, PUMA and NOXA. Activated p53 enhances expression of the FAS death receptor. Binding of FAS ligand to FAS death receptor leads to formation of the death-inducing signaling complex (DISC) including FADD and procaspase-8, cleavage of procaspase-8 and subsequently activation of a caspase cascade leading to apoptosis. The intrinsic apoptosis pathway is regulated by pro- and anti-apoptotic proteins. Pro-apoptotic proteins either directly facilitate BAX and BAK oligomerization in the mitochondrial outer membrane, or indirectly by inhibiting anti-apoptotic proteins. Mitochondrial outer membrane permeabilization leads to release of SMAC/Diablo and activation of a caspase cascade. SMAC/Diablo represses the caspase inhibitor XIAP. Crosstalk between the extrinsic and intrinsic apoptosis pathways exists in form of caspase-8 mediated cleavage of BID. High levels of OCT are correlated with high NOXA protein expression.

tumors [48]. The DNA damage response (DDR) machinery is activated in response to DSBs and involves recruitment of ATM to DSBs and activation of ATM signaling. ATM activation leads to phosphorylation of ~700 substrates, including MDM2 and p53 [49]. As a consequence, p53 is no longer ubiquitinated by MDM2, leading to its stabilization and full activation [50] (Fig. 1). Transactivation of the p53 target gene *CDKN1A,* encoding the CDK inhibitor p21, is crucial for the induction of cell cycle arrest in G1 phase. Apoptosis on the other hand, can be triggered by the p53-mediated transcription of pro-apoptotic BCL-2 family members including *BBC3* (PUMA) and *PMAIP1* (NOXA) [51].

Activation of the DDR by cisplatin leads to a rapid induction of apoptosis with a major role for WT p53 in TC. Mutations in *TP53* are rarely observed in TC. Nevertheless, *TP53* alterations have been detected in a small subset (~15%) of cisplatin-resistant or relapsed TC patients [32,37]. Of note, no correlation has been found between p53 protein expression levels and chemosensitivity [52]. In fact, chemoresistant tumors exhibited a trend towards higher positivity for both p53 and MDM2 compared to cisplatin-sensitive tumors, suggesting increased importance of MDM2-mediated inhibition of p53 activity. This hypothesis is supported by functional studies using cisplatin-sensitive and -resistant TC cell lines indicating that the interaction between p53 and MDM2 required higher doses of cisplatin to be disrupted in resistant cell lines [53].

Cisplatin treatment results in enhanced expression of the FAS death receptor, a transcriptional target of p53, and subsequent activation of the extrinsic apoptosis pathway via the interaction between FAS and FAS ligand [53,54]. Other studies showed activation of the intrinsic apoptosis pathway, where cisplatin led to increased expression of pro-apoptotic proteins PUMA and NOXA which are involved in the regulation of the intrinsic apoptosis pathway [55–57]. In addition, high protein levels of the pluripotency factor OCT4, typical for the EC subtype, strongly correlated with a high expression of NOXA, while *OCT4* knockdown resulted in a strong reduction of NOXA [57]. Interestingly, high NOXA expression has been correlated with good prognosis in TC tumors with EC histology [58]. Crosstalk between the extrinsic and intrinsic apoptosis pathways, where FAS receptor activation results in caspase-8-mediated cleavage of BID, may further strengthen the apoptotic response (Fig. 2). A recent study proposed that TC tumors with WT *TP53* have an intrinsically heightened apoptotic potential caused by their increased mitochondrial priming [30].

The difference in cisplatin-sensitivity between seminoma and non-seminoma tumors could be explained by a lower level of DNA methylation in seminomas [29,59,60]. An interesting hypothesis postulated in the study by Shen *et al.*, is that activating *KIT* mutations, predominantly observed in seminomas, induce a primordial quiescent state in seminoma cells that suppresses the expression of genes involved in methylation, including *UHRF1* and *DNMT1* [29]. Consistently, methylation levels have been linked to the differentiation status of germ cell tumors as well as to cisplatin-sensitivity in vitro [61].

Cisplatin resistance in TC and novel pre-clinical targets

While TC is a highly curable disease, partly caused by mechanisms outlined above, approximately 10–15% of patients develop a tumor relapse after initial treatment or have refractory disease [12]. Several mechanisms underlying cisplatin resistance have been identified, suggesting no uniform cause of resistance. Some of the most prominent resistance mechanisms that potentially have therapeutic value, will be discussed here (Fig. 3).

As described above, the excellent sensitivity of TC tumors to cisplatin treatment can be attributed to diminished capacity to repair cisplatin-induced DNA cross-links. Consequently, cisplatin resistance may arise from an enhanced ability to resolve DNA damage, or by increased coping mechanisms towards unrepaired DNA lesions. Increased expression of ERCC1 in ovarian cancer cell lines has been associated with cisplatin resistance [62]. However, there are currently no data that

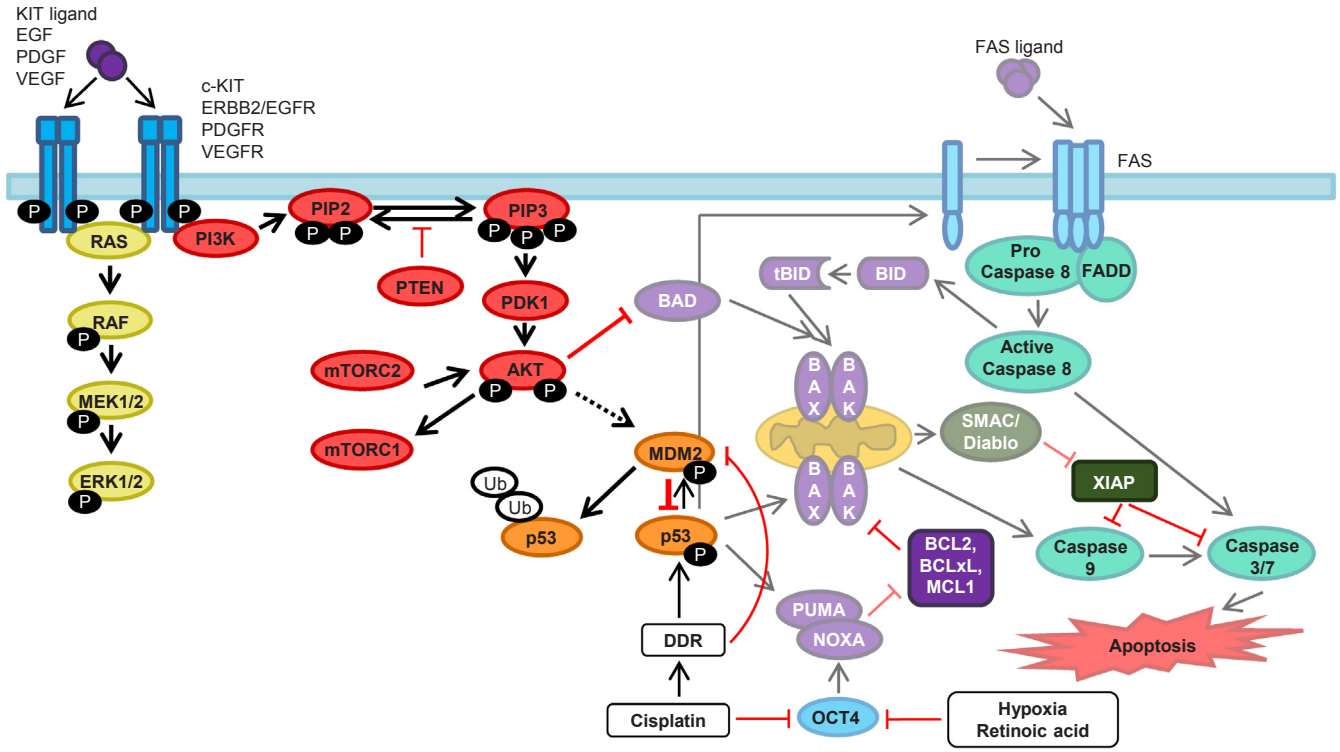

Fig. 3. Activation of cell survival signaling and lower apoptosis induction factors of cisplatin resistance in TC. Growth factor binding to different receptor tyrosine kinases (RTK) leads to activation of the MAPK and PI3K/AKT/mTOR signaling pathways that promote cell survival. PI3K downstream signaling involves conversion of PIP2 to PIP3, which recruits AKT and PDK1 to the plasma membrane. PDK1 and mTORC2 phosphorylate AKT, resulting in full activation of AKT. PTEN negatively regulates AKT through dephosphorylation of PIP3. AKT activity also results in anti-apoptotic signaling by phosphorylating pro-apoptotic protein BAD. Cisplatin-induced apoptosis is diminished by MDM2 binding to p53, which promotes p53 ubiquitination and degradation, resulting in lower p53 mediated transcriptional regulation of pro-apoptotic proteins.

provide evidence for altered levels of ERCC1 or other NER-related proteins to be involved in cisplatin resistance of TC. As a reduced ability to perform HR has been shown to determine cisplatin sensitivity in many cancers, changes in HR efficacy could determine cisplatin resistance in TC as well. In a study where cisplatin sensitive and resistant TC cell lines were compared, it was shown that cisplatin sensitivity correlates with the ability of cells to repair interstrand cross-links [48]. Despite differences in HR proficiency between cisplatin sensitive and resistant cells, all TC cell lines were shown to be more defective in HR when compared to a non-TC cisplatin resistant cell line [48]. Furthermore, an investigation into the presence of activated ATM has demonstrated that TC tumors show more activated ATM, as judged by serine 1981 phosphorylation, compared to normal tissue. However, the extend of ATM activation was less prominent compared to other tumor types [63]. These data further support the idea that TC tumors are characterized by a lower proficiency to activate the DDR machinery, but that there is no support of its involvement in cisplatin resistance.

Embryonal carcinoma cell lines and tumors, like embryonic stem cells, are characterized by high expression of the pluripotency markers OCT4 and NANOG [64,65]. Several studies have demonstrated a link between loss of OCT4 expression and lower NOXA levels, which correlated with chemoresistance in vitro and in vivo [57,66–68]. More recently, a study showed chemotherapy induced depletion of OCT4 in a mouse model of TC including teratoma and EC components [69]. Similarly, loss of pluripotency markers OCT4 and NANOG was observed in a clinical cohort of TC with emerging chemotherapy resistance [30]. In addition, cisplatin treatment itself induced chemoresistance, as demonstrated in a study by Abada & Howell, where they showed that cisplatin treatment induced differentiation of EC cells in vitro, associated with a loss of pluripotency markers OCT4 and NANOG [70]. In vitro differentiation of EC cells by retinoic acid also led to repression of OCT4, downregulation of PUMA and NOXA, and concomitantly a decreased sensitivity to cisplatin [56,71,72]. Combined, these data suggest that cisplatin-treated EC tumors which downregulate OCT4 expression become chemoresistant, either via subsequent lower NOXA levels or via differentiation of TC cells. Important to mention is that other TC subtypes, like YSC and CC, do not express OCT4, while these TC subtypes are very sensitive to cisplatin as well. Also clinically, no correlation has been demonstrated between OCT4 expression and cisplatin sensitivity [73].

As mentioned above, genomic alterations affecting the MDM2/p53 axis have been described in resistant TC. A large study examined 180 TC tumors using whole exome sequencing and described that most *MDM2/TP53* alterations were found in cisplatin resistant tumors [32]. The majority of *MDM2* amplifications were observed in post-treatment specimens suggesting that tumor cells are highly selected during treatment, a finding that is further supported by a recent study showing amplification of 12q15 containing *MDM2* in a refractory TC patient [32,34]. In TC cells it was shown that p53 function is hampered by the interaction with MDM2, especially in cisplatin-resistant cells, where a higher cisplatin concentration was needed to interfere with the MDM2/p53 interaction [53]. Therefore, MDM2 appears an interesting therapeutic target to activate WT p53. Indeed, small molecule inhibitors of MDM2 (e.g. nutlin-3a) targeting the MDM2-p53 interaction were shown to sensitize TC cells to cisplatin treatment [53,74]. Even though most TC tumors retain WT *TP53*, posttranscriptional modifications are able to repress the pro-apoptotic activity of p53. For example, lysine methylation at the carboxyl terminus of p53 repressed its transcriptional activity, as demonstrated by reduced expression of PUMA and p21 in TC cells [75]. Furthermore, the expression of miR-372 and miR-373 was

reported to repress p53 signaling, and elevated expression levels of these miRs have been found in cisplatin-resistant TC cell lines [76–78]. Deacetylation of p53 by SIRT1 might be another posttranslational modification potentially repressing p53 activity in TC. SIRT1 is highly expressed by cells in the seminiferous ducts of the testis and plays an important role in spermatogenesis and germ cell function. Reduced counts of spermatozoa and spermatogenic stem cells were observed in *SIRT1*$^{-/-}$ mice, as well as increased numbers of abnormal spermatozoa, spermatozoa with elevated levels of DNA damage, and small and abnormal seminiferous tubules [79]. Besides its role in germ cell biology, the ability of SIRT1 to deacetylate p53 suggests that SIRT1 functions as an oncogene. Upregulation of SIRT1 has been demonstrated in various cancer types [80]. Other studies, however, have suggested that SIRT1 acts as a tumor suppressor [80]. Thus, the exact role of SIRT1 repressing p53 activity in the context of TC has yet to be determined.

Recently, the PI3K/AKT/mTOR pathway has gained more attention in relation to cisplatin resistance of TC. Besides mutational deregulation, hyperactivation of the PI3K/AKT/mTOR pathway has been described. A central player in the PI3K/AKT/mTOR pathway is AKT, which functions as a pro-survival factor by promoting cell survival and inhibiting apoptosis (Fig. 4). Phosphorylated AKT has several ways to negatively regulate apoptosis. Firstly, AKT phosphorylates and thereby inhibits the pro-apoptotic protein BAD [81]. In addition, through direct inhibition of FOXO3a, which is responsible for the transcription of several pro-apoptotic proteins including PUMA and FAS ligand, AKT indirectly negatively influences apoptosis [82]. Secondly, AKT activates the transcription factor NF-κB, which promotes the transcription of several anti-apoptotic genes including BCL-2 and BCL-XL [83,84]. Thirdly, AKT phosphorylates and activates mTORC1 which is a key regulator of protein translation, cell proliferation and autophagy [85]. Finally, it has been described that AKT-mediated phosphorylation of MDM2 promotes its nuclear localization, where it interacts with p53 and thereby targets p53 for degradation [86]. Altogether, these studies emphasize the involvement of AKT in diverse tumorigenic activities, summarized in Fig. 4. The first proof of PI3K/AKT/mTOR pathway hyperactivity in TC was provided by Di Vizio *et al.* who discovered that the majority of TC tumors are characterized by loss of PTEN, a tumor suppressor that negatively regulates PI3K signaling [87]. Other reports have followed since, all showing that TC models have a highly active PI3K/AKT/mTOR pathway [88–92]. These data indicate that targeting the PI3K/AKT/mTOR pathway may have potential as a therapeutic approach. In TC cells, activation of the receptor tyrosine kinases PDGFRβ and IGF1R have been shown to signal through PI3K/AKT. Addition of the multi-kinase inhibitor pazopanib (targeting PDGFR, VEGFR and c-KIT) or the IGF1R/INSR inhibitor NVP-AEW541 resulted in a reduction of AKT phosphorylation levels. Downregulation of PDGFRβ or IGF1R sensitized TC cell lines to cisplatin treatment, and pazopanib treatment reduced tumor growth in cisplatin-sensitive and -resistant TC patient-derived xenograft (PDX) models of the YSC and CC subtype [89–91]. Likewise, several PI3K, AKT and mTOR inhibitors have been tested in combination with cisplatin in cisplatin-resistant TC cell lines and mouse models, showing increased apoptosis and tumor growth inhibition compared to cisplatin treatment alone [68,92]. Interestingly, the PDGFRβ ligand PDGF-B was produced and secreted by TC cell lines suggesting autocrine signaling [89]. Surprisingly, no differences in PDGFRβ tumor expression was observed in a heterogeneous group of cisplatin-treated and -untreated patients or between cisplatin-sensitive and -resistant patients. However, when tumors were divided based on TC subtype, choriocarcinomas from cisplatin-resistant patients had higher PDGFRβ tumor expression than choriocarcinomas from cisplatin-sensitive patients [89].

Several important pro-apoptotic members and their role in apoptosis signaling have been discussed above, including the FAS death receptor and PUMA. Less known in the context of TC are the X-linked inhibitor of apoptosis (XIAP) and the IAP antagonistic protein SMAC/Diablo. XIAP is an important determinant of the apoptotic process and functions by inhibiting the initiator caspase-9 and effector caspases-3 and -7 [93]. SMAC/Diablo is released from the mitochondria during the apoptotic process, and promotes caspase activation by binding to and inhibiting XIAP [94,95]. Clearly, a shift in balance between XIAP and SMAC/Diablo may mediate apoptotic resistance, either by overexpression of XIAP, or by downregulation of SMAC/Diablo. One study examining the mRNA expression of XIAP and SMAC/Diablo in normal testis tissue and TC showed that XIAP levels were similar between normal and TC tissue, while the levels of SMAC/Diablo were decreased in TC compared to normal tissue [96]. These data further stress that the balance between pro- and anti-apoptotic proteins in TC is key in

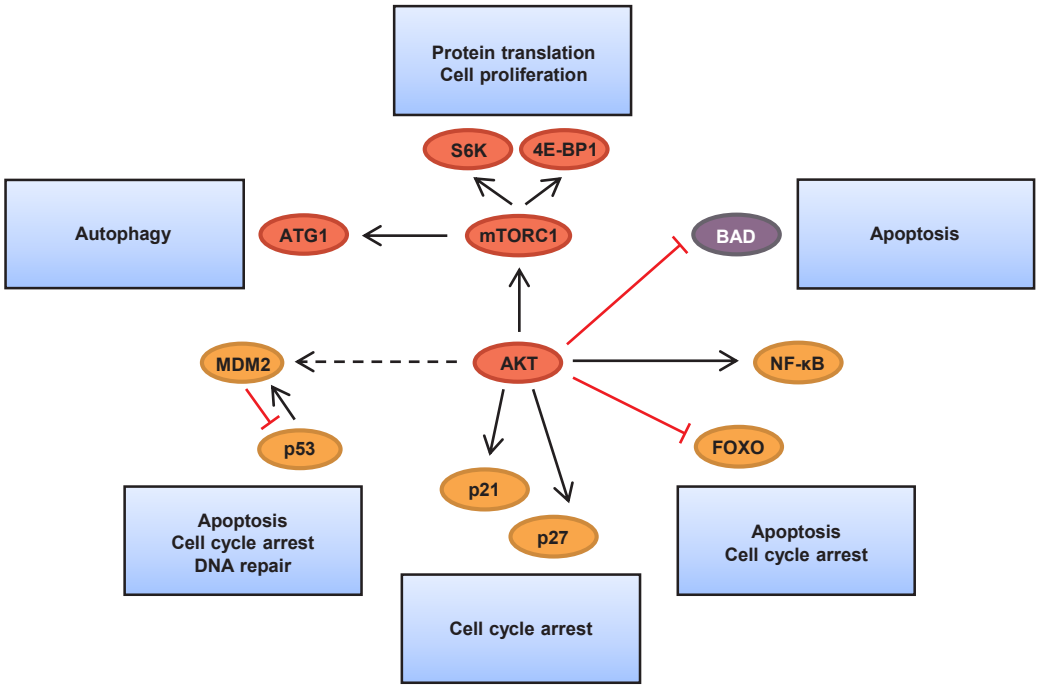

Fig. 4. AKT mediated signaling promotes cell survival. Activation of AKT leads to: (i) Phosphorylation and activation of mTORC1, which in response activates S6K, 4E-BP1 and ATG1 thereby regulating protein translation, cell proliferation and autophagy. (ii) Phosphorylation and thereby inhibition of pro-apoptotic protein BAD. (iii) Phosphorylation/ activation of transcription factor NF-κB, leading to the upregulation of anti-apoptotic proteins BCL-2 and BCL-xL and phosphorylation/inhibition of transcription factor FOXO, which upregulates the expression of pro-apoptotic proteins BIM and NOXA. (iv) Prevention of cell cycle arrest by phosphorylating and inhibiting cyclin dependent kinase inhibitors p21 and p27. (v) Phosphorylation of MDM2, resulting in p53 degradation.

controlling the apoptotic response. Interfering in this balance by blocking anti-apoptotic proteins might be a way to shift the balance in favor of apoptosis. For instance, BH3 mimetics block multiple or specific anti-apoptotic BCL-2 family members thereby inhibiting the activity of these pro-survival proteins and favoring apoptosis. Similarly, it would be interesting to test SMAC mimetics in TC models.

Novel preclinical models in TC

Despite the fact that TC is a complex and heterogeneous disease, TC models, including cell lines and xenografts, are scarce. Only ~20 human and mouse cell lines and less than 10 xenograft models have been reported [97–106]. Available cell lines and xenografts almost exclusively cover the EC subtype, highlighting the obvious need for more models representing other histological TC subtypes.

Patient-derived xenograft models are increasingly used in current cancer research due to several advantages over cell line-based xenografts, including maintenance of tumor heterogeneity, high similarity to human tumors [107,108] and increased predictive power of drug response [109,110]. So far, only 14 orthotopically established TC PDX models and 14 subcutaneous TC PDX models have been reported [92,111,112], with limited data available of their establishment [113]. One study described the establishment of 14 non-seminoma PDX models, specifically from the CC, EC and YSC subtypes, as well as mixed tumors with YSC, teratoma and EC components [113]. In this study, orthotopic implantation of the tumor pieces was more successful than subcutaneous implantation. These PDX models were used to study cisplatin resistance and to test novel combinatorial strategies using a glucosylceramide synthase (GCS) inhibitor, DL-threo-PDMP, and a multi-targeted receptor tyrosine kinase inhibitor, sunitinib, in combination with cisplatin [113,114]. Recently, we established and characterized three subcutaneous TC PDX models. Two models were obtained from a cisplatin-sensitive patient, and one model was obtained from a cisplatin-resistant patient. These PDX tumors and matched patient tumors showed retention of histological subtypes, were molecularly stable over several passages, and their chemosensitivity corresponded with patients' response to chemotherapy (de Vries *et al.*, in preparation). A broad panel of TC PDX models representing all histological subtypes, including tumors with a mixed histology, would be valuable to gain more understanding of the differential molecular make-up of histological subtypes. Differential expression of certain drug targets between histological subtypes, as demonstrated for CD30 and PDGFRβ [89,115], might drive the development of treatments tailored to specific TC subtypes.

Novel targeted therapies in TC patients

First line cisplatin-based chemotherapy will fail in 10–15% of patients with metastatic disease, including refractory and relapsed patients [12]. These patients will receive salvage treatment, as described above, with varying success rates around 50% [5]. No alternative treatment options are available for TC patients not being cured by salvage regimens, highlighting the need for clinical trials investigating novel therapies. However, initiation and design of large clinical trials are difficult due to the low incidence and high cure rates of TC. Nonetheless, some clinical trials investigating targeted therapies have been performed and will be discussed below, as well as ongoing (pre-) clinical developments (Table 2).

One pre-clinical target that has shown promising results in several TC cell lines, is MDM2. Blocking the interaction between MDM2 and p53 by nutlin-3, a small molecule inhibitor of MDM2, induced apoptosis [53,74]. In addition, synergistic effects on cell survival were observed in TC cell lines after combined nutlin-3 and cisplatin treatment [53]. Several MDM2 inhibitors, including RG7388, AMG-232 and ALRN-6924, are currently under investigation in phase I-I/II trials. No clinical studies specifically investigating the potential of MDM2 inhibitors in refractory or relapsed TC patients have been initiated to date.

Another pathway that is currently being investigated as a clinical target for treatment of TC is the DDR pathway. As TC tumors are characterized by defective repair of DSBs, targeting DDR components provides a therapeutic opportunity. This option has gained more attention, since HR-deficient tumors were shown to be highly sensitive to PARP1 inhibitors [116–118]. In vitro data has shown that treatment of TC cells with the PARP inhibitor olaparib sensitized these cells to cisplatin treatment, in particular cisplatin-resistant cells [48]. Expression of PARP1 has also been investigated in TC tumors, showing high expression in tumor tissue but not in normal testis tissue [119]. Currently, two phase II trials are evaluating the potential of PARP inhibitors in relapsed or refractory TC, either as single agent (NCT02533765, currently active) or combined with gemcitabine and carboplatin (NCT02860819, currently recruiting).

Another clinical target that has previously been investigated is CD30, a member of the TNF receptor family, which is expressed by the EC subtype of TC [115]. Interestingly, TC patients with CD30-expressing tumors had worse progression free survival and overall survival compared to patients with CD30-negative tumors [120]. Brentuximab-vedotin is an anti-CD30 antibody linked to the antimitotic agent monomethyl auristatin E. This drug is FDA-approved for treatment of Hodgkin lymphoma, anaplastic large cell lymphoma and cutaneous T-cell lymphoma. A phase II clinical trial has investigated the therapeutic potential of brentuximab-vedotin in TC. This study included five patients with non-seminoma TC tumors of which one patient achieved a complete response and one patient a partial response [121]. However, another phase II trial in 18 relapsed and refractory non-seminoma TC patients, investigating the activity of brentuximab-vedotin, was recently terminated due to lack of efficacy (NCT02689219). This study had two arms, patients with CD30 negative/unknown tumors and patients with CD30 positive tumors. None of the patients achieved a partial or complete response, and the majority of patients showed disease progression. The lack of efficacy might be caused by acquired or intrinsic resistance to brentuximab-vedotin, such as loss of CD30 expression as described previously [121].

Recently it was shown that cisplatin resistant TC cell lines express higher mRNA and protein levels of the aldehyde dehydrogenase (ALDH) isoform ALDH1A3 compared to their cisplatin sensitive parental cells. Treatment with the ALDH inhibitor disulfiram sensitized TC cells to cisplatin treatment in vitro and in vivo. In addition, a significantly higher expression of the ALDH isoform ALDH1A3 was detected in TC tumors compared to healthy testicular tissue [122]. Based on these data, a phase II clinical trial will commence and evaluate the potential of disulfiram in combination with cisplatin in relapsed or refractory TC patients (NCT03950830, currently recruiting).

Claudin 6 (CLDN6) is a tight junction membrane protein, which was found to be aberrantly expressed in TC tissue while little expression was observed in normal tissue [123]. The tumor specific expression makes CLDN6 an attractive drug target. For that purpose the anti-CLDN6 monoclonal antibody ASP1650, also known as IMAB027, was developed. Pre-clinical data in TC cell lines showed that ASP1650 induced cell death as a single agent, and that pretreatment with chemotherapeutic agents upregulated CLDN6 expression in heterogeneously expressing cell lines [123]. A first-in-human trial was performed in advanced ovarian cancer patients, demonstrating that ASP1650 was safe and well tolerated [124]. A phase II clinical trial will now investigate the safety and efficacy of ASP1650 in refractory TC patients (NCT03760081, currently recruiting).

Research into the epigenetics of TC has suggested that increased DNA methylation is associated with cisplatin resistance. Cisplatin-sensitive seminomas were shown to be hypomethylated, while EC tumors that have higher incidence of cisplatin resistance showed intermediate DNA methylation. Treatment-resistant tumors including YSC, CC and teratomas showed higher levels of DNA methylation when compared to other solid tumors [125]. In line with this notion, TC cell lines were

Table 2
Finished and ongoing clinical trials in TC patients.

Target	Drug	Trial phase	NCT identifier	Status
PARP	Olaparib	Phase II	NCT02533765	Active
PARP + DNMT	Veliparib + Gemcitabine + Carboplatin	Phase II	NCT02860819	Active and recruiting
CD30	Brentuximab-vedotin	Phase II		Completed [121]
CD30	Brentuximab-vedotin	Phase II	NCT02689219	Terminated, lack of efficacy
ALDH	Disulfiram + cisplatin	Phase II	NCT03950830	Recruiting
CLDN6	ASP1650	Phase II	NCT03760081	Recruiting
DNMT	Guadecitabine (SGI-110) + cisplatin	Phase I	NCT02429466	Active
PDGFR + VEGFR + KIT	Sunitinib	Phase II		Completed [127]
		Phase II		Completed [128]
PDGFR + VEGFR + KIT	Pazopanib	Phase II		Completed [129]
KIT + PDGFR + BCR-ABL	Imatinib	Phase II		Terminated, lack of efficacy [130]
		Phase II		Terminated, lack of efficacy [131]
mTORC1	Everolimus	Phase II		Completed [132]
		Phase II		Completed [133]
mTORC1 + EGFR	Sirolimus + Erlotinib	Phase II	NCT01962896	Terminated, low accrual
CDK4/6	Palbociclib	Phase II		Completed [134]
CDK4/6	Ribociclib	Phase II		Completed [136]
PD-1	Pembrolizumab	Phase II		Terminated, lack of efficacy [142]
PD-1 + CTLA-4	Nivolumab + Ipilimumab	Phase II	NCT03333616	Recruiting
PD-L1	Avelumab	Phase II		Completed [143]
PD-L1 + CTLA-4	Durvalumab + Tremelimumab	Phase II	NCT03158064	Recruiting
PD-L1 + CTLA-4	Durvalumab +/- Tremelimumab	Phase II	NCT03081923	Recruiting

PARP: poly(ADP) ribose polymerase; DNMT: DNA methyl transferase; ALDH: aldehyde dehydrogenase; CLDN6: claudin 6; PDGFR: platelet-derived growth factor receptor; VEGFR: vascular endothelial growth factor receptor; KIT: stem cell growth factor receptor; mTORC1; mammalian target of rapamycin complex 1; EGFR: epidermal growth factor receptor; CDK4/6: cyclin-dependent kinase 4/6; PD-1: programmed death receptor 1; PD-L1: programmed death ligand 1; CTLA-4: Cytotoxic T-Lymphocyte Associated Protein 4.

proven to be hypersensitive to the second generation DNA methylation inhibitor guadecitabine (SGI-110), both in vitro and in vivo. In addition, pretreatment of cisplatin resistant TC cells with this inhibitor re-sensitized cells to cisplatin [98]. A phase I clinical trial will evaluate the safety and efficacy of guadecitabine in combination with cisplatin in refractory TC patients (NCT02429466, currently active).

Several receptor tyrosine kinases (RTK) including KIT, ERBB2, PDGFR and VEGFR are involved in activation of the MAPK and PI3K/AKT/mTOR pathway in TC [126]. Sunitinib, pazopanib and imatinib are multi-targeted inhibitors of RTKs including VEGFR, PDGFR and KIT that showed promising activity in vitro and in vivo using orthotopic PDX models of the YSC and CC histological subtypes [90,114,127]. Results of clinical trials, however, have been disappointing, with sunitinib only showing modest activity in one study, which was not confirmed in a second trial [127,128]. The trial investigating pazopanib showed no objective responses but achieved progression free survival of at least 3 months in 13% of patients [129]. Two other small phase II clinical trials tested the efficacy of imatinib in heavily treated TC patients. Six patients with mutated *KIT* were treated with imatinib showing poor results with 5 out of 6 patients developed progressive disease [130]. In the other clinical trial, none of the patients with KIT overexpression responded [131].

In vitro and in vivo efficacy of downstream targeting of the PI3K/AKT/mTOR pathway has been demonstrated in TC models, showing increased apoptosis and tumor growth inhibition [68,92]. Two phase II clinical trials have tested the mTORC1 inhibitor everolimus in refractory or relapsed TC patients. Efficacy of everolimus was limited in both trials reporting no objective responses, but one study reported 12-week progression free survival in 40% of patients [132,133]. One other small phase II clinical trial tested the mTORC1 inhibitor sirolimus in combination with EGFR inhibitor erlotinib but closed prematurely due to lack of efficacy (NCT01962896). Summarizing, major clinical outcomes in response to sunitinib, pazopanib, imatinib and everolimus were limited to case reports and small phase II studies and can therefore not be recommended as single agent treatment [127–133]. These clinical studies used single drug treatment to test efficacy. However, several pre-clinical studies have shown that combining targeted agents with cisplatin enhances therapeutic efficacy in TC models. For example, in a recent study we showed that mTORC1/2 inhibitors sensitized TC cell lines to cisplatin treatment more strongly than everolimus, an mTORC1 inhibitor. Everolimus treatment prompted an upregulation of AKT phosphorylation levels, resulting from a positive feedback loop between mTORC2 and AKT. This feedback loop might explain the reduced efficacy of everolimus in clinical setting. Importantly, synergistic effects were observed between mTORC1/2 inhibitor AZD8055 and cisplatin in TC cell lines. Furthermore, AZD8055 potentiated the efficacy of cisplatin in two TC PDX models, one pure YSC and one mixed tumor consisting of YSC and immature teratoma components [92]. Concluding, combined targeted treatment with cisplatin hold potential for future clinical development in TC patients.

Patients with teratoma usually undergo surgery to remove residual metastatic lesions. However, there are no alternative treatments for patients with teratomas that are unresectable or progressive. Teratomas express high levels of the tumor suppressor retinoblastoma protein (Rb), unlike less differentiated TC tumors i.e. seminomas and EC [134]. Rb protein is an important regulator of the G1-S checkpoint, blocking entry into S-phase and promoting cell differentiation [135]. Cyclin-dependent kinases 4 and 6 (CDK4/6) in association with cyclin D phosphorylates Rb, leading to cell cycle progression. The inhibition of CDK4/6 is therefore an attractive therapeutic target in tumors expressing high levels of Rb. Palbociclib is a CDK4/6 inhibitor that has been evaluated in a phase II study in refractory TC patients with Rb-expressing tumors. Of the 29 patients included, eight achieved progression free survival of 24 weeks. In addition, patients with teratoma or teratoma with malignant transformation had significantly better progression free survival compared to patients with other histological TC tumor types [134]. Recently, a randomized placebo controlled phase II trial investigating the CDK4/6 inhibitor ribociclib was reported in which TC patients with unresectable and progressive teratoma without malignant transformation were included. Due to slow accrual this trials was prematurely closed. Eight patients were included in the ribociclib group which showed a progression free survival rate of 71% at 24 months, indicating some anti-tumor activity of this compound for this indication [136].

While immunotherapy has proven beneficial in many tumor types [137], limited research has been performed in TC which might be due

to the fact that testes are regarded as immune privileged. Two studies have looked into the expression of PD-L1 in TC tissue, and both reported a higher expression of PD-L1 in tumor compared to normal testis tissue [138,139]. Percentages of tumors positive for PD-L1 ranged between 73–76% for seminomas and 64–89% for non-seminomas. PD-L1 expression on tumor cells can serve as a predictive marker for response to anti-PD-1 or anti-PD-L1 immunotherapy in several tumor types [137,140,141]. Indeed, low PD-L1 expression in TC tumors was associated with a significantly better progression-free survival [139]. Two small phase II studies have evaluated immune checkpoint inhibition in refractory and relapsed TC patients. No objective responses were observed with single agent pembrolizumab or avelumab indicating lack of efficacy in unselected patient groups [142,143]. A noteworthy observation from the pembrolizumab study was the fact that only two of the 12 patients had PD-L1 positive tumors. Another possible explanation for the lack of clinical efficacy is the low mutational burden in TC tumors [27] and possibly low number of neoantigens. In addition, whereas extensive immune infiltration has been shown for seminomas, this was considerably lower for non-seminomas [29]. Regardless, several clinical studies will commence and investigate the efficacy of immunotherapy in TC. Two phase II trials will investigate the combination of anti-PD-L1 inhibitor durvalumab with anti-CTLA-4 inhibitor tremelimumab in refractory TC patients (NCT03081923, NCT03158064, both currently recruiting). The safety and efficacy of another immunotherapy combination, anti-PD-1 inhibitor nivolumab combined with anti-CTLA-4 inhibitor ipilimumab, will be investigated in a phase II trial in refractory TC patients (NCT03333616, currently recruiting).

Overall, results of clinical trials in TC patients investigating targeted drugs have been disappointing. Some limitations in these studies that might have influenced the clinical outcomes are: (1) patients were heavily pretreated, which has to do with the fact that there are several salvage treatment options available with excellent outcomes, (2) inclusion of small patient numbers and (3) the large heterogeneity of TC subtypes included. Unfortunately, these limitations are not easily solved for future clinical trials in TC patients. Nevertheless, clinical trials investigating novel targeted agents for TC are underway, including trials investigating a targeted agent combined with chemotherapy (NCT02860819, NCT03950830, NCT02429466).

Concluding remarks

Excellent sensitivity of TC tumors to cisplatin treatment has made TC a highly curable disease. Two features of TC tumors contribute to cisplatin sensitivity, and are described here: insufficient DNA repair in response to cisplatin-induced DNA damage, and a hypersensitive apoptotic response. However, even though TC is a highly curable disease there are some therapeutic challenges left.

One important issue affecting survival of TC patients is cisplatin resistance. Unfortunately, there are no alternative treatment options for patients who relapse and do not sufficiently respond to salvage treatment or those that are refractory. Some mechanisms contributing to cisplatin resistance have been identified including hyperactivity of the PI3K/AKT/mTOR pathway and elevated levels of MDM2. Following, several strategies to overcome cisplatin resistance have shown efficacy in vitro and in vivo. These strategies include combinations of cisplatin with DNA methylation inhibitors, PARP inhibitors, MDM2 inhibitors, and PI3K/AKT/mTOR inhibitors. However, despite the current knowledge on the molecular mechanisms underlying TC biology and the identification of successful pre-clinical targeted approaches, no targeted drugs have hitherto shown robust clinical benefit. Following pre-clinical results, it seems logical that future clinical trials should focus on combining targeted drugs with cisplatin in an effort to overcome cisplatin resistance. In addition, histological subtypes should be taken into account, as demonstrated for the CDK4/6 inhibitor trials in teratoma. Excitingly, three clinical trials are currently recruiting or active that will investigate the combination of chemotherapy with a targeted drug, i.e. veliparib, guadecitabine and disulfiram. Furthermore, given the hypersensitive apoptotic response of TC cells associated with high mitochondrial priming, one could speculate that the break on apoptosis present in cisplatin-resistant cells can be removed by the addition of BH3 mimetics or SMAC mimetics. Several BH3 mimetics and SMAC mimetics are in clinical development and warrant further investigation using in vivo TC models.

Author contributions

All authors conceived the study. G.V. and X.R. performed the literature search and wrote the manuscript. M.A.T.M.V., J.A.G. and S.J. supervised, reviewed and edited the manuscript. All authors discussed the results and commented on the manuscript.

Funding

This work was supported by a grant from the Dutch Cancer Society (RUG 2014–6691) to S. de Jong, M.A.T.M. van Vugt, and J.A. Gietema, and by a grant from Conacyt (381543) to X. Rosas-Plaza.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86. https://doi.org/10.1002/ijc.29210.
[2] Ylönen O, Jyrkkiö S, Pukkala E, Syvänen K, Boström PJ. Time trends and occupational variation in the incidence of testicular cancer in the Nordic countries. BJU Int 2018;122:384–93. https://doi.org/10.1111/bju.14148.
[3] Richiardi L, Bellocco R, Adami H-O, Torrång A, Barlow L, Hakulinen T, et al. Testicular cancer incidence in eight northern European countries: secular and recent trends. Cancer Epidemiol Biomarkers Prev 2004;13:2157–66.
[4] Hanna NH, Einhorn LH. Testicular cancer—discoveries and updates. N Engl J Med 2014;371:2005–16. https://doi.org/10.1056/NEJMra1407550.
[5] Albers P, Albrecht W, Algaba F, Bokemeyer C, Cohn-Cedermark G, Fizazi K, et al. Guidelines on testicular cancer: 2015 update. Eur Urol 2015;68:1054–68. https://doi.org/10.1016/j.eururo.2015.07.044.
[6] Brierley J, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours. 8th ed. Hoboken, NJ: Wiley-Blackwell; 2017.
[7] Mead GM, Stenning SP. The international germ cell consensus classification: A new prognostic factor-based staging classification for metastatic germ cell tumours. Clin Oncol 1997;9:207–9. https://doi.org/10.1016/S0936-6555(97)80001-5.
[8] Di Pietro A, De Vries EGE, Gietema JA, Spierings DCJ, De Jong S. Testicular germ cell tumours: The paradigm of chemo-sensitive solid tumours. Int J Biochem Cell Biol 2005;37:2437–56. https://doi.org/10.1016/j.biocel.2005.06.014.
[9] O'Shaughnessy MJ, Feldman DR, Carver BS, Sheinfeld J. Late relapse of testicular germ cell tumors. Urol Clin North Am 2015;42:359–68. https://doi.org/10.1016/j.ucl.2015.04.010.
[10] Michael H, Lucia J, Foster RS, Ulbright TM. The pathology of late recurrence of testicular germ cell tumors. Am J Surg Pathol 2000;24:257–73. https://doi.org/10.1097/00000478-200002000-00012.
[11] Tu S-M, Bilen MA, Hess KR, Broaddus RR, Kopetz S, Wei C, et al. Intratumoral heterogeneity: Role of differentiation in a potentially lethal phenotype of testicular cancer. Cancer 2016;122:1836–43. https://doi.org/10.1002/cncr.29996.
[12] Adra N, Einhorn LH. Testicular cancer update. Clin Adv Hematol Oncol 2017;15:386–96.
[13] Miller KD, Loehrer PJ, Gonin R, Einhorn LH. Salvage chemotherapy with vinblastine, ifosfamide, and cisplatin in recurrent seminoma. J Clin Oncol 1997;15:1427–31. https://doi.org/10.1200/JCO.1997.15.4.1427.
[14] McCaffrey JA, Mazumdar M, Bajorin DF, Bosl GJ, Vlamis V, Motzer RJ. Ifosfamide- and cisplatin-containing chemotherapy as first-line salvage therapy in germ cell tumors: response and survival. J Clin Oncol 1997;15:2559–63. https://doi.org/10.1200/JCO.1997.15.7.2559.
[15] Kondagunta GV, Bacik J, Donadio A, Bajorin D, Marion S, Sheinfeld J, 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 2005;23:6549–55. https://doi.org/10.1200/JCO.2005.19.638.
[16] Loehrer PJ, Gonin R, Nichols CR, Weathers T, Einhorn LH. Vinblastine plus ifosfamide plus cisplatin as initial salvage therapy in recurrent germ cell tumor. J Clin Oncol 1998;16:2500–4. https://doi.org/10.1200/JCO.1998.16.7.2500.
[17] Fizazi K, Gravis G, Flechon A, Geoffrois L, Chevreau C, Laguerre B, et al. Combining gemcitabine, cisplatin, and ifosfamide (GIP) is active in patients with

relapsed metastatic germ-cell tumors (GCT): a prospective multicenter GETUG phase II trial. Ann Oncol 2014;25:987–91. https://doi.org/10.1093/annonc/mdu099.
[18] Nichols CR, Tricot G, Williams SD, Van Besien K, Loehrer PJ, Roth BJ, et al. Dose-intensive chemotherapy in refractory germ cell cancer - A phase I/II trial of high-dose carboplatin and etoposide with autologous bone marrow transplantation. J Clin Oncol 1989;7:932–9. https://doi.org/10.1200/JCO.1989.7.7.932.
[19] Einhorn LH, Williams SD, Chamness A, Brames MJ, Perkins SM, Abonour R. High-dose chemotherapy and stem-cell rescue for metastatic germ-cell tumors. N Engl J Med 2007;357:340–8. https://doi.org/10.1056/NEJMoa067749.
[20] Lorch A, Bascoul-Mollevi C, Kramar A, Einhorn L, Necchi A, Massard C, 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;29:2178–84. https://doi.org/10.1200/JCO.2010.32.6678.
[21] Summersgill B, Osin P, Lu Y-J, Huddart R, Shipley J. Chromosomal imbalances associated with carcinoma in situ and associated testicular germ cell tumours of adolescents and adults. Br J Cancer 2001;85:213–9. https://doi.org/10.1054/bjoc.2001.1889.
[22] Summersgill B, Goker H, Weber-Hall S, Huddart R, Horwich A, Shipley J. Molecular cytogenetic analysis of adult testicular germ cell tumours and identification of regions of consensus copy number change. Br J Cancer 1998;77:305–13. https://doi.org/10.1038/bjc.1998.47.
[23] Sandberg AA, Meloni AM. Suijkerbuijk RF. Reviews of chromosome studies in urological tumors. III. Cytogenetics and genes in testicular tumors. J Urol 1996;155:1531–56. https://doi.org/10.1016/S0022-5347(01)66124-X.
[24] Litchfield K, Levy M, Huddart RA, Shipley J, Turnbull C. The genomic landscape of testicular germ cell tumours: from susceptibility to treatment. Nat Rev Urol 2016;13:409–19. https://doi.org/10.1038/nrurol.2016.107.
[25] Ottesen AM, Skakkebaek NE, Lundsteen C, Leffers H, Larsen J, Rajpert-De Meyts E. High-resolution comparative genomic hybridization detects extra chromosome arm 12p material in most cases of carcinoma in situ adjacent to overt germ cell tumors, but not before the invasive tumor development. Genes, Chromosom Cancer 2003;38:117–25. https://doi.org/10.1002/gcc.10244.
[26] McIntyre A, Summersgill B, Grygalewicz B, Gillis AJM, Stoop J, van Gurp RJHLM, et al. Amplification and overexpression of the KIT gene is associated with progression in the seminoma subtype of testicular germ cell tumors of adolescents and adults. Cancer Res 2005;65:8085–9. https://doi.org/10.1158/0008-5472.CAN-05-0471.
[27] Litchfield K, Summersgill B, Yost S, Sultana R, Labreche K, Dudakia D, et al. Whole-exome sequencing reveals the mutational spectrum of testicular germ cell tumours. Nat Commun 2015;6:5973. https://doi.org/10.1038/ncomms6973.
[28] Kemmer K, Corless CL, Fletcher JA, McGreevey L, Haley A, Griffith D, et al. KIT mutations are common in testicular seminomas. Am J Pathol 2004;164:305–13. https://doi.org/10.1016/S0002-9440(10)63120-3.
[29] Shen H, Shih J, Hollern DP, Wang L, Bowlby R, Tickoo SK, et al. Integrated molecular characterization of testicular germ cell tumors. Cell Rep 2018;23:3392–406. https://doi.org/10.1016/j.celrep.2018.05.039.
[30] Taylor-Weiner A, Zack T, O'Donnell E, Guerriero JL, Bernard B, Reddy A, et al. Genomic evolution and chemoresistance in germ-cell tumours. Nature 2016;540:114–8. https://doi.org/10.1038/nature20596.
[31] Pawar SA, Sarkar TR, Balamurugan K, Sharan S, Wang J, Zhang Y, et al. C/EBPδ targets cyclin D1 for proteasome-mediated degradation via induction of CDC27/APC3 expression. Proc Natl Acad Sci U S A 2010;107:9210–5. https://doi.org/10.1073/pnas.0913813107.
[32] Bagrodia A, Lee BH, Lee W, Cha EK, Sfakianos JP, Iyer G, et al. Genetic determinants of cisplatin resistance in patients with advanced germ cell tumors. J Clin Oncol 2016;34:4000–7. https://doi.org/10.1200/JCO.2016.68.7798.
[33] Feldman DR, Iyer G, Van Alstine L, Patil S, Al-Ahmadie H, Reuter VE, et al. Presence of somatic mutations within PIK3CA, AKT, RAS, and FGFR3 but not BRAF in cisplatin-resistant germ cell tumors. Clin Cancer Res 2014;20:3712–20. https://doi.org/10.1158/1078-0432.CCR-13-2868.
[34] Barrett MT, Lenkiewicz E, Malasi S, Stanton M, Slack J, Andrews P, et al. Clonal analyses of refractory testicular germ cell tumors. PLoS ONE 2019;14:e0213815. https://doi.org/10.1371/journal.pone.0213815.
[35] Danoy P, Sonoda E, Lathrop M, Takeda S, Matsuda F. A naturally occurring genetic variant of human XRCC2 (R188H) confers increased resistance to cisplatin-induced DNA damage. Biochem Biophys Res Commun 2007;352:763–8. https://doi.org/10.1016/J.BBRC.2006.11.083.
[36] Lin WY, Camp NJ, Cannon-Albright LA, Allen-Brady K, Balasubramanian S, Reed MWR, et al. A role for XRCC2 gene polymorphisms in breast cancer risk and survival. J Med Genet 2011;48:477–84. https://doi.org/10.1136/jmedgenet-2011-100018.
[37] Houldsworth J, Xiao H, Murty VVVS, Chen W, Ray B, Reuter VE, et al. Human male germ cell tumor resistance to cisplatin is linked to TP53 gene mutation. Oncogene 1998;16:2345–9. https://doi.org/10.1038/sj.onc.1201770.
[38] Vousden KH, Lu X. Live or let die: the cell's response to p53. Nat Rev Cancer 2002;2:594–604. https://doi.org/10.1038/nrc864.
[39] Einhorn LH. Treatment of testicular cancer: a new and improved model. J Clin Oncol 1990;8:1777–81. https://doi.org/10.1200/JCO.1990.8.11.1777.
[40] Eastman A. Characterization of the adducts produced in DNA by cis-diammine-dichloroplatinum(II) and cis-dichloro(ethylenediamine)platinum(II). Biochemistry 1983;22:3927–33. https://doi.org/10.1021/bi00285a031.
[41] Yang D, Wang AH-J. Structural studies of interactions between anticancer platinum drugs and DNA. Prog Biophys Mol Biol 1996;66:81–111. https://doi.org/10.1016/S0079-6107(96)00017-X.
[42] Jordan P, Carmo-Fonseca M. Molecular mechanisms involved in cisplatin cytotoxicity. Cell Mol Life Sci 2000;57:1229–35. https://doi.org/10.1007/PL00000762.
[43] Zamble DB, Mu D, Reardon JT, Sancar A, Lippard SJ. Repair of cisplatin-DNA adducts by the mammalian excision nuclease. Biochemistry 1996;35:10004–13. https://doi.org/10.1021/bi960453+.
[44] Awuah SG, Riddell IA, Lippard SJ. Repair shielding of platinum-DNA lesions in testicular germ cell tumors by high-mobility group box protein 4 imparts cisplatin hypersensitivity. Proc Natl Acad Sci U S A 2017;114:950–5. https://doi.org/10.1073/pnas.1615327114.
[45] Köberle B, Masters JRW, Hartley JA, Wood RD. Defective repair of cisplatin-induced DNA damage caused by reduced XPA protein in testicular germ cell tumours. Curr Biol 1999;9:273–8. https://doi.org/10.1016/S0960-9822(99)80118-3.
[46] Welsh C, Day R, McGurk C, Masters JRW, Wood RD, Köberle B. Reduced levels of XPA, ERCC1 and XPF DNA repair proteins in testis tumor cell lines. Int J Cancer 2004;110:352–61. https://doi.org/10.1002/ijc.20134.
[47] Usanova S, Piée-Staffa A, Sied U, Thomale J, Schneider A, Kaina B, et al. Cisplatin sensitivity of testis tumour cells is due to deficiency in interstrand-crosslink repair and low ERCC1-XPF expression. Mol Cancer 2010;9:248. https://doi.org/10.1186/1476-4598-9-248.
[48] Cavallo F, Graziani G, Antinozzi C, Feldman DR, Houldsworth J, Bosl GJ, et al. Reduced proficiency in homologous recombination underlies the high sensitivity of embryonal carcinoma testicular germ cell tumors to cisplatin and poly (ADP-ribose) polymerase inhibition. PLoS ONE 2012;7:e51563. https://doi.org/10.1371/journal.pone.0051563.
[49] Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER, Hurov KE, Luo J, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science 2007;316:1160–6. https://doi.org/10.1126/science.1140321.
[50] Cheng Q, Chen J. Mechanism of p53 stabilization by ATM after DNA damage. Cell Cycle 2010;9:472–8. https://doi.org/10.4161/cc.9.3.10556.
[51] Kastenhuber ER, Lowe SW. Putting p53 in context. Cell 2017;170:1062–78. https://doi.org/10.1016/J.CELL.2017.08.028.
[52] Kersemaekers A-MF, Mayer F, Molier M, van Weeren PC, Oosterhuis JW, Bokemeyer C, et al. Role of P53 and MDM2 in treatment response of human germ cell tumors. J Clin Oncol 2002;20:1551–61. https://doi.org/10.1200/JCO.2002.20.6.1551.
[53] Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, 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:e148https://doi.org/10.1038/cddis.2011.33.
[54] Spierings D, De Vries E, Vellenga E, De Jong S. Loss of drug-induced activation of the CD95 apoptotic pathway in a cisplatin-resistant testicular germ cell tumor cell line. Cell Death Differ 2003;10:808–22. https://doi.org/10.1038/.
[55] Mueller T, Voigt W, Simon H, Fruehauf A, Bulankin A, Grothey A, et al. Failure of activation of caspase-9 induces a higher threshold for apoptosis and cisplatin resistance in testicular cancer. Cancer Res 2003;63:513–21.
[56] Gutekunst M, Oren M, Weilbacher A, Dengler MA, Markwardt C, Thomale J, et al. p53 hypersensitivity is the predominant mechanism of the unique responsiveness of testicular germ cell tumor (TGCT) cells to cisplatin. PLoS ONE 2011;6:e19198. https://doi.org/10.1371/journal.pone.0019198.
[57] Gutekunst M, Mueller T, Weilbacher A, Dengler MA, Bedke J, Kruck S, et al. Cisplatin hypersensitivity of testicular germ cell tumors is determined by high constitutive Noxa levels mediated by Oct-4. Cancer Res 2013;73:1460–9. https://doi.org/10.1158/0008-5472.CAN-12-2876.
[58] Grande L, Bretones G, Rosa-Garrido M, Garrido-Martin EM, Hernandez T, Fraile S, 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;287:26495–505. https://doi.org/10.1074/jbc.M112.376319.
[59] Netto GJ, Nakai Y, Nakayama M, Jadallah S, Toubaji A, Nonomura N, et al. Global DNA hypomethylation in intratubular germ cell neoplasia and seminoma, but not in nonseminomatous male germ cell tumors. Mod Pathol 2008;21:1337–44. https://doi.org/10.1038/modpathol.2008.127.
[60] Brait M, Maldonado L, Begum S, Loyo M, Wehle D, Tavora FF, et al. DNA methylation profiles delineate epigenetic heterogeneity in seminoma and non-seminoma. Br J Cancer 2012;106:414–23. https://doi.org/10.1038/bjc.2011.468.
[61] Wermann H, Stoop H, Gillis AJ, Honecker F, van Gurp RJ, Ammerpohl O, et al. Global DNA methylation in fetal human germ cells and germ cell tumours: association with differentiation and cisplatin resistance. J Pathol 2010;221:433–42. https://doi.org/10.1002/path.2725.
[62] Ferry KV, Hamilton TC, Johnson SW. Increased nucleotide excision repair in cisplatin-resistant ovarian cancer cells: Role of ERCC1-XPF. Biochem Pharmacol 2000;60:1305–13. https://doi.org/10.1016/S0006-2952(00)00441-X.
[63] Bartkova J, Bakkenist CJ, Rajpert-De Meyts E, Skakkebæk NE, Sehested M, Lukas J, et al. ATM activation in normal human tissues and testicular cancer. Cell Cycle 2005;4:838–45. https://doi.org/10.4161/cc.4.6.1742.
[64] de Jong J, Stoop H, Dohle GR, Bangma CH, Kliffen M, van Esser JW, et al. Diagnostic value of OCT3/4 for pre-invasive and invasive testicular germ cell tumours. J Pathol 2005;206:242–9. https://doi.org/10.1002/path.1766.
[65] Hart AH, Hartley L, Parker K, Ibrahim M, Looijenga LHJ, Pauchnik M, et al. The pluripotency homeobox gene NANOG is expressed in human germ cell tumors. Cancer 2005;104:2092–8. https://doi.org/10.1002/cncr.21435.
[66] Mueller T, Mueller LP, Luetzkendorf J, Voigt W, Simon H, Schmoll H-J. Loss of Oct-3/4 expression in embryonal carcinoma cells is associated with induction of cisplatin resistance. Tumor Biol 2006;27:71–83. https://doi.org/10.1159/

000092324.
[67] Wu YC, Ling TY, Lu SH, Kuo HC, Ho HN, Yeh SD, et al. Chemotherapeutic sensitivity of testicular germ cell tumors under hypoxic conditions is negatively regulated by SENP1-controlled sumoylation of OCT4. Cancer Res 2012;72:4963–73. https://doi.org/10.1158/0008-5472.CAN-12-0673.
[68] Koster R, di Pietro A, Timmer-Bosscha H, Gibcus JH, van den Berg A, Suurmeijer AJ, et al. Cytoplasmic p21 expression levels determine cisplatin resistance in human testicular cancer. J Clin Invest 2010;120:3594–605. https://doi.org/10.1172/JCI41939.
[69] Pierpont TM, Lyndaker AM, Anderson CM, Jin Q, Moore ES, Roden JL, et al. Chemotherapy-induced depletion of OCT4-positive cancer stem cells in a mouse model of malignant testicular cancer. Cell Rep 2017;21:1896–909. https://doi.org/10.1016/J.CELREP.2017.10.078.
[70] Abada PB, Howell SB. Cisplatin induces resistance by triggering differentiation of testicular embryonal carcinoma cells. PLoS ONE 2014;9:e87444. https://doi.org/10.1371/journal.pone.0087444.
[71] Timmer-Bosscha H, de Vries EGE, Meijer C, Oosterhuis JW, Mulder NH. Differential effects of all-trans -retinoic acid, docosahexaenoic acid, and hexadecylphosphocholine on cisplatin-induced cytotoxicity and apoptosis in a cisplatin-sensitive and resistant human embryonal carcinoma cell line. Cancer Chemother Pharmacol 1998;41:469–76. https://doi.org/10.1007/s002800050769.
[72] Giuliano CJ, Kerley-Hamilton JS, Bee T, Freemantle SJ, Manickaratnam R, Dmitrovsky E, et al. Retinoic acid represses a cassette of candidate pluripotency chromosome 12p genes during induced loss of human embryonal carcinoma tumorigenicity. Biochim Biophys Acta 2005;1731:48–56. https://doi.org/10.1016/J.BBAEXP.2005.08.006.
[73] Looijenga LHJ, Stoop H, de Leeuw HPJC, de Gouveia Brazao CA, Gillis AJM, van Roozendaal KEP, et al. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res 2003;63:2244–50. https://doi.org/10.1158/0008-5472.can-12-0673.
[74] Bauer S, Mühlenberg T, Leahy M, Hoiczyk M, Gauler T, Schuler M, et al. Therapeutic potential of Mdm2 inhibition in malignant germ cell tumours. Eur Urol 2010;57:679–87. https://doi.org/10.1016/J.EURURO.2009.06.014.
[75] Zhu J, Dou Z, Sammons MA, Levine AJ, Berger SL. Lysine methylation represses p53 activity in teratocarcinoma cancer cells. Proc Natl Acad Sci U S A 2016;113:9822–7. https://doi.org/10.1073/pnas.1610387113.
[76] Voorhoeve PM, le Sage C, Schrier M, Gillis AJM, Stoop H, Nagel R, et al. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell 2006;124:1169–81. https://doi.org/10.1016/J.CELL.2006.02.037.
[77] Duale N, Lindeman B, Komada M, Olsen A-K, Andreassen A, Soderlund EJ, et al. Molecular portrait of cisplatin induced response in human testis cancer cell lines based on gene expression profiles. Mol Cancer 2007;6:53. https://doi.org/10.1186/1476-4598-6-53.
[78] Port M, Glaesener S, Ruf C, Riecke A, Bokemeyer C, Meineke V, et al. Micro-RNA expression in cisplatin resistant germ cell tumor cell lines. Mol Cancer 2011;10:52. https://doi.org/10.1186/1476-4598-10-52.
[79] Coussens M, Maresh JG, Yanagimachi R, Maeda G, Allsopp R. Sirt1 deficiency attenuates spermatogenesis and germ cell function. PLoS ONE 2008;3:e1571. https://doi.org/10.1371/journal.pone.0001571.
[80] Yi J, Luo J. SIRT1 and p53, effect on cancer, senescence and beyond. Biochim Biophys Acta – Proteins Proteom 2010;1804:1684–9. https://doi.org/10.1016/j.bbapap.2010.05.002.
[81] Datta SR, Dudek H, Xu T, Masters S, Haian F, Gotoh Y, et al. Akt phosphorylation of BAD couples survival signals to the cell- intrinsic death machinery. Cell 1997;91:231–41. https://doi.org/10.1016/S0092-8674(00)80405-5.
[82] Zhang X, Tang N, Hadden TJ, Rishi AK. Akt, FoxO and regulation of apoptosis. Biochim Biophys Acta 2011;1813:1978–86. https://doi.org/10.1016/j.bbamcr.2011.03.010.
[83] Xia L, Tan S, Zhou Y, Lin J, Wang H, Oyang L, et al. Role of the NFκB-signaling pathway in cancer. Onco Targets Ther 2018;11:2063–73. https://doi.org/10.2147/OTT.S161109.
[84] Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF-κB activation by tumour necrosis factor requires the Akt serine- threonine kinase. Nature 1999;401:82–5. https://doi.org/10.1038/43466.
[85] Dibble CC, Cantley LC. Regulation of mTORC1 by PI3K signaling. Trends Cell Biol 2015;25:545–55. https://doi.org/10.1016/j.tcb.2015.06.002.
[86] Mayo LD, Donner DB. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proc Natl Acad Sci U S A 2001;98:11598–603. https://doi.org/10.1073/pnas.181181198.
[87] Di Vizio D, Cito L, Boccia A, Chieffi P, Insabato L, Pettinato G, 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;24:1882–94. https://doi.org/10.1038/sj.onc.1208368.
[88] Chen KS, Fustino NJ, Shukla AA, Stroup EK, Budhipramono A, Ateek C, et al. EGF receptor and mTORC1 are novel therapeutic targets in nonseminomatous germ cell tumors. Mol Cancer Ther 2018;17:1079–89. https://doi.org/10.1158/1535-7163.MCT-17-0137.
[89] Juliachs M, Muñoz C, Moutinho CA, Vidal A, Condom E, Esteller M, et al. The PDGFRβ–AKT pathway contributes to CDDP-acquired resistance in testicular germ cell tumors. Clin Cancer Res 2014;20:658–67. https://doi.org/10.1158/1078-0432.CCR-13-1131.
[90] Juliachs M, Vidal A, del Muro XG, Piulats JM, Condom E, Casanovas O, et al. Effectivity of pazopanib treatment in orthotopic models of human testicular germ cell tumors. BMC Cancer 2013;13:382. https://doi.org/10.1186/1471-2407-13-382.
[91] Selfe J, Goddard NC, McIntyre A, Taylor KR, Renshaw J, Popov SD, et al. IGF1R signalling in testicular germ cell tumour cells impacts on cell survival and acquired cisplatin resistance. J Pathol 2018;244:242–53. https://doi.org/10.1002/path.5008.
[92] Rosas-Plaza X, de Vries G, Meersma GJ, Suurmeijer AJH, Gietema JA, van Vugt MATM, et al. Dual mTORC1/2 inhibition sensitizes testicular cancer models to cisplatin treatment. Mol Cancer Ther 2020;19:590–601. https://doi.org/10.1158/1535-7163.mct-19-0449.
[93] Deveraux QL. Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases. EMBO J 1999;18:5242–51. https://doi.org/10.1093/emboj/18.19.5242.
[94] Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 2000;102:33–42. https://doi.org/10.1016/S0092-8674(00)00008-8.
[95] Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, et al. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 2000;102:43–53. https://doi.org/10.1016/S0092-8674(00)00009-X.
[96] Kempkensteffen C, Jäger T, Bub J, Weikert S, Hinz S, Christoph F, et al. The equilibrium of XIAP and Smac/DIABLO expression is gradually deranged during the development and progression of testicular germ cell tumours. Int J Androl 2007;30:476–83. https://doi.org/10.1111/j.1365-2605.2006.00742.x.
[97] Harstrick A, Casper J, Guba R, Wilke H, Poliwoda H, Schmoll HJ. Comparison of the antitumor activity of cisplatin, carboplatin, and iproplatin against established human testicular cancer cell lines in vivo and in vitro. Cancer 1989;63:1079–83. https://doi.org/10.1002/1097-0142(19890315)63:6<1079::AID-CNCR2820630607>3.0.CO;2-J.
[98] Albany C, Hever-Jardine MP, von Herrmann KM, Yim CY, Tam J, Warzecha JM, et al. Refractory testicular germ cell tumors are highly sensitive to the second generation DNA methylation inhibitor guadecitabine. Oncotarget 2017;8:2949–59. https://doi.org/10.18632/oncotarget.13811.
[99] Aide N, Poulain L, Briand M, Dutoit S, Allouche S, Labiche A, et al. Early evaluation of the effects of chemotherapy with longitudinal FDG small-animal PET in human testicular cancer xenografts: early flare response does not reflect refractory disease. Eur J Nucl Med Mol Imaging 2009;36:396–405. https://doi.org/10.1007/s00259-008-0984-x.
[100] Douglas ML, Richardson MM, Nicol DL. Testicular germ cell tumors exhibit evidence of hormone dependence. Int J Cancer 2006;118:98–102. https://doi.org/10.1002/ijc.21330.
[101] Shirakawa T, Gotoh A, Zhang Z, Kao C, Chung LW, Gardner TA. Development of human chorionic gonadotropin subunit-beta promoter-based toxic gene therapy for testicular cancer. Urology 2004;63:613–8. https://doi.org/10.1016/J.UROLOGY.2003.09.054.
[102] Abraham D, Abri S, Hofmann M, Hotl W, Aharinejad S. Low dose carboplatin combined with angiostatic agents prevents metastasis in human testicular germ cell tumor xenografts. J Urol 2003;170:1388–93. https://doi.org/10.1097/01.ju.0000075917.98929.57.
[103] Dunn TA, Schmoll HJ, Grüunwald V, Casper J, Bokemeyer C. Pre-clinical activity of taxol in non-seminomatous germ cell tumor cell lines and nude mouse xenografts. Invest New Drugs 1997;15:91–8. https://doi.org/10.1023/A:1005852521656.
[104] Andrews PW, Bronson DL, Benham F, Strickland S, Knowles BB. A comparative study of eight cell lines derived from human testicular teratocarcinoma. Int J Cancer 1980;26:269–80. https://doi.org/10.1002/ijc.2910260304.
[105] Rahman NA, Huhtaniemi IT. Testicular cell lines. Mol Cell Endocrinol 2004;228:53–65. https://doi.org/10.1016/J.MCE.2003.05.001.
[106] Nakagawa H, Ueda T, Ito S, Shiraishi T, Taniguchi H, Kayukawa N, et al. Androgen suppresses testicular cancer cell growth in vitro and in vivo. Oncotarget 2016;7:35224–32. https://doi.org/10.18632/oncotarget.9109.
[107] Cassidy JW, Caldas C, Bruna A. Maintaining tumor heterogeneity in patient-derived tumor xenografts. Cancer Res 2015;75:2963–8. https://doi.org/10.1158/0008-5472.CAN-15-0727.
[108] Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med 2015;21:1318–25. https://doi.org/10.1038/nm.3954.
[109] Pompili L, Porru M, Caruso C, Biroccio A, Leonetti C. Patient-derived xenografts: a relevant preclinical model for drug development. J Exp Clin Cancer Res 2016;35:189. https://doi.org/10.1186/s13046-016-0462-4.
[110] Ricci F, Bizzaro F, Cesca M, Guffanti F, Ganzinelli M, Decio A, et al. Patient-derived ovarian tumor xenografts recapitulate human clinicopathology and genetic alterations. Cancer Res 2014;74:6980–90. https://doi.org/10.1158/0008-5472.CAN-14-0274.
[111] Byrne AT, Alférez DG, Amant F, Annibali D, Arribas J, Biankin AV, et al. Interrogating open issues in cancer precision medicine with patient-derived xenografts. Nat Rev Cancer 2017;17:254–68. https://doi.org/10.1038/nrc.2016.140.
[112] Burger AM, Fiebig HH. Screening using animal systems. In: Baguley BC, Kerr DJ, editors. Anticancer Drug Dev Elsevier; 2001. p. 285–99. https://doi.org/10.1016/b978-012072651-6/50017-6.
[113] Piulats JM, Vidal A, García-Rodríguez FJ, Muñoz C, Nadal M, Moutinho C, et al. Orthoxenografts of testicular germ cell tumors demonstrate genomic changes associated with cisplatin resistance and identify PDMP as a resensitizing agent. Clin Cancer Res 2018;24:3755–66. https://doi.org/10.1158/1078-0432.CCR-17-1898.
[114] Castillo-Ávila W, Piulats JM, Garcia Del Muro X, Vidal A, Condom E, Casanovas O, et al. Sunitinib inhibits tumor growth and synergizes with cisplatin in orthotopic models of cisplatin-sensitive and cisplatin-resistant human testicular germ cell

tumors. Clin Cancer Res 2009;15:3384–95. https://doi.org/10.1158/1078-0432.CCR-08-2170.
[115] Pallesen G, Hamilton-Dutoit SJ. Ki-1 (CD30) antigen is regularly expressed by tumor cells of embryonal carcinoma. Am J Pathol 1988;133:446–50.
[116] Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434:917–21. https://doi.org/10.1038/nature03445.
[117] Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 2005;434:913–7. https://doi.org/10.1038/nature03443.
[118] McCabe N, Turner NC, Lord CJ, Kluzek K, Białkowska A, Swift S, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly (ADP-ribose) polymerase inhibition. Cancer Res 2006;66:8109–15. https://doi.org/10.1158/0008-5472.CAN-06-0140.
[119] Mego M, Cierna Z, Svetlovska D, Macak D, Machalekova K, Miskovska V, et al. PARP expression in germ cell tumours. J Clin Pathol 2013;66:607–12. https://doi.org/10.1136/jclinpath-2012-201088.
[120] Giannatempo P, Paolini B, Miceli R, Raggi D, Nicolai N, Faré E, et al. Persistent CD30 expression by embryonal carcinoma in the treatment time course: Prognostic significance of a worthwhile target for personalized treatment. J Urol 2013;190:1919–24. https://doi.org/10.1016/j.juro.2013.04.057.
[121] Albany C, Einhorn L, Garbo L, Boyd T, Josephson N, Feldman DR. Treatment of CD30-expressing germ cell tumors and sex cord stromal tumors with brentuximab vedotin: identification and report of seven cases. Oncologist 2018;23:316–23. https://doi.org/10.1634/theoncologist.2017-0544.
[122] Schmidtova S, Kalavska K, Gercakova K, Cierna Z, Miklikova S, Smolkova B, et al. Disulfiram overcomes cisplatin resistance in human embryonal carcinoma cells. Cancers (Basel) 2019;11:1224. https://doi.org/10.3390/cancers11091224.
[123] Türeci Ö, Kreuzberg M, Walter K, Wöll S, Schmitt R, Mitnacht-Kraus R, et al. The anti-claudin 6 antibody, IMAB027, induces antibody-dependent cellular and complement-dependent cytotoxicity in claudin 6-expressing cancer cells. Cancer Res 2018;78:882. https://doi.org/10.1158/1538-7445.am2018-882.
[124] Sahin U, Jaeger D, Marme F, Mavratzas A, Krauss J, De Greve J, et al. First-in-human phase I/II dose-escalation study of IMAB027 in patients with recurrent advanced ovarian cancer (OVAR): Preliminary data of phase I part. J Clin Oncol 2015;33:5537. https://doi.org/10.1200/jco.2015.33.15_suppl.5537.
[125] Singh R, Fazal Z, Freemantle SJ, Spinella MJ. Mechanisms of cisplatin sensitivity and resistance in testicular germ cell tumors. Cancer Drug Resist 2019;2:580–94. https://doi.org/10.20517/cdr.2019.19.
[126] Koster R, van Vugt MATM, Timmer-Bosscha H, Gietema JA, de Jong S. Unravelling mechanisms of cisplatin sensitivity and resistance in testicular cancer. Expert Rev Mol Med 2013;15:e12. https://doi.org/10.1017/erm.2013.13.
[127] Oechsle K, Honecker F, Cheng T, Mayer F, Czaykowski P, Winquist E, et al. Preclinical and clinical activity of sunitinib in patients with cisplatin-refractory or multiply relapsed germ cell tumors: a Canadian urologic oncology group/German testicular cancer study group cooperative study. Ann Oncol 2011;22:2654–60. https://doi.org/10.1093/annonc/mdr026.
[128] Feldman DR, Turkula S, Ginsberg MS, Ishill N, Patil S, Carousso M, et al. Phase II trial of sunitinib in patients with relapsed or refractory germ cell tumors. Invest New Drugs 2010;28:523–8. https://doi.org/10.1007/s10637-009-9280-2.
[129] Necchi A, Lo Vullo S, Giannatempo P, Raggi D, Calareso G, Togliardi E, et al. Pazopanib (PZP) in germ cell tumors (GCT) after chemotherapy (CT) failure: Final results of the open label, single-group, phase 2 Pazotest trial. Eur Urol Suppl 2017;16:e1910–1. https://doi.org/10.1016/s1569-9056(17)31146-6.
[130] Einhorn LH, Brames MJ, Heinrich MC, Corless CL, Madani A. Phase II study of imatinib mesylate in chemotherapy refractory germ cell tumors expressing KIT. Am J Clin Oncol 2006;29:12–3. https://doi.org/10.1097/01.coc.0000195086.47548.ef.
[131] Piulats J, Garcia del Muro X, Huddart R, Aparicio J, Paz-Ares L, Sanchez M, et al. Phase II multicenter study of imatinib in patients with chemorefractory germ cell tumors that express c-kit. Cancer Res 2007;67:2648.
[132] Fenner M, Oing C, Dieing A, Gauler T, Oechsle K, Lorch A, et al. Everolimus in patients with multiply relapsed or cisplatin refractory germ cell tumors: results of a phase II, single-arm, open-label multicenter trial (RADIT) of the German testicular cancer study group. J Cancer Res Clin Oncol 2019;145:717–23. https://doi.org/10.1007/s00432-018-2752-z.
[133] Mego M, Svetlovska D, Miskovska V, Obertova J, Palacka P, Rajec J, et al. Phase II study of everolimus in refractory testicular germ cell tumors. Urol Oncol Semin Orig Investig 2016;34:122.e17–22. https://doi.org/10.1016/J.UROLONC.2015.10.010.
[134] Vaughn DJ, Hwang WT, Lal P, Rosen MA, Gallagher M, O'Dwyer PJ. Phase 2 trial of the cyclin-dependent kinase 4/6 inhibitor palbociclib in patients with retinoblastoma protein-expressing germ cell tumors. Cancer 2015;121:1463–8. https://doi.org/10.1002/cncr.29213.
[135] Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995;81:323–30. https://doi.org/10.1016/0092-8674(95)90385-2.
[136] Castellano DE, Quinn DI, Feldman DR, Fizazi K, Garcia del Muro X, Gietema JA, et al. A phase II study of ribociclib in men with unresectable, incurable teratoma with recent progression. J Clin Oncol 2019;37:517. https://doi.org/10.1200/jco.2019.37.7_suppl.517.
[137] Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science 2018;359:1350–5. https://doi.org/10.1126/science.aar4060.
[138] Fankhauser CD, Curioni-Fontecedro A, Allmann V, Beyer J, Tischler V, Sulser T, et al. Frequent PD-L1 expression in testicular germ cell tumors. Br J Cancer 2015;113:411–3. https://doi.org/10.1038/bjc.2015.244.
[139] Cierna Z, Mego M, Miskovska V, Machalekova K, Chovanec M, Svetlovska D, 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;27:300–5. https://doi.org/10.1093/annonc/mdv574.
[140] Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti–PD-1 antibody in cancer. N Engl J Med 2012;366:2443–54. https://doi.org/10.1056/NEJMoa1200690.
[141] Herbst RS, Soria J-C, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014;515:563–7. https://doi.org/10.1038/nature14011.
[142] Adra N, Einhorn LH, Althouse SK, Ammakkanavar NR, Musapatika D, Albany C, et al. Phase II trial of pembrolizumab in patients with platinum refractory germ-cell tumors: a Hoosier Cancer Research Network Study GU14-206. Ann Oncol 2018;29:209–14. https://doi.org/10.1093/annonc/mdx680.
[143] Mego M, Svetlovska D, Chovanec M, Rečkova M, Rejlekova K, Obertova J, et al. Phase II study of avelumab in multiple relapsed/refractory germ cell cancer. Invest New Drugs 2019;37:748–54. https://doi.org/10.1007/s10637-019-00805-4.