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Targeted treatment approaches in refractory germ cell tumors

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ABSTRACT

Germ cell tumors (GCTs) are the most common type of solid tumor amongst patients between 15 and 35 years of age. They are also one of the types of tumor with the highest cure rate, due to their high sensitivity to cisplatin based chemotherapy. Nonetheless, around 15–20% of metastatic patients will not have curative options after a relapse on the first and second line. This proves that new therapeutic options for these refractory GCTs patients need to be developed. This article offers a bibliographic review of all studies using targeted treatment or immunotherapy for refractory GCTs patients.

1. Introduction

Germ cell tumors (GCTs) are the most common type of solid tumor amongst adolescents and young adults (15–35 y.o.). They are also one of the malignant tumor types with the hightest cure rate, as only 3–5% of patients die due to the tumor (Sturgeon et al., 2011; Tandstad et al., 2009; Gandaglia et al., 2014). The cure rate of disseminated testicular carcinoma after a cisplatin based first line treatment is 70–75%, while, after a relapse, the second line (first salvage treatment) of conventional-dose chemotherapy (CDQT) may achieve a complete remission on 40–50% of patients (Einhorn, 1997; Loehrer et al., 1998; Motzer et al., 1992). With high-dose chemotherapy combined with a stem cell transplant (HDCT-ASCT) the cure rate may reach 60% (Einhorn et al., 2007; Feldman et al., 2010a). A randomized clinical trial is currently taking place in order to establish the best out of these two salvage therapies (Standard-Dose Combination Chemotherapy or High-Dose Combination Chemotherapy and Stem Cell Transplant in Treating Patients with Relapsed or Refractory Germ Cell Tumors, 2019).

Patients who relapse after the second line treatment show very poor results, without a curative option when surgical resection is not possible (Murphy et al., 1993). Despite the high response rate to first and second line treatment, 15–20% of all metastatic patients do not receive therapeutic curative options. Hence the importance of the development of targeted treatments for this group of patients. So far, the best therapeutic option is the combination of gemcitabine, oxaliplatin and paclitaxel (GOP), which achieves an objective response rate of 51% and combined with the resection of residual mass may achieve around 11% of progression free survival (PFS) for two years (Bokemeyer et al., 2007). The response rate to other lines of treatment ranges between 20 and 40%, with an overall median survival rate (OS) of 6–8 months.

Targeted treatment therapies mean a paradigm shift for the treatment of multiple solid tumors, allowing for the selection of the population who may benefit from these agents. For GCTs, this aim is harder to achieve, due the low number of patients who relapse and the diversity of the population, including several histological subtypes; therefore the cooperation between multiple healthcare centers is essential. So far, the available data reveal the possible results of some targeted treatments in highly selected refractory GCT patients (Sánchez-Muñoz et al., 2012). Here is an updated review containing all targeted treatments/immunotherapy.

2. EGFR family

The epidermal growth factor receptor family (EGFR) consists of four members: HER1 or EGFR, HER2, HER3 and HER4. The EGFR family proteins consist of three domains: a tyrosine kinase intracellular domain, a transmembrane domain and an extracellular domain where the ligand binds to become active (with the exception of HER2, which has no known ligand and becomes active when it forms a heterodimer with other EGFR family members). The activation of these receptors eventually leads to the activation of a cascade of intracellular pathways with genes involved in cell proliferation, differentiation, angiogenesis, migration and survival. The immunohistochemical (IHC) expression varies between 28 and 65% in GCTs, whereas the gene amplification is around 5% (Wang et al., 2009; Durán et al., 2010; Madani et al., 2003; Moroni et al., 2001).

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The EGFR expression in GCTs is still controversial, as its contribution to the tumor development, differentiation and progression is not completely known. This expression is higher in NSGCTs (non-seminomatous germ cell tumors) with positive human chorionic gonadotropin (HCG), as shown by an EGFR study with positive results for 16 out of 18 patients, from which 25% expressed HER2 as well. Moreover, patients with seminoma, one patient with Leydig cell tumor and those with negative non-seminomatous HCG did not express EGFR in this study (Moroni et al., 2001).

A different study showed 71% of EGFR expression in teratoma, 100% in choriocarcinoma, 29% in embryonal carcinoma and 0% in seminoma and yolk sac tumor (Hechelhammer et al., 2003). In addition, the EGFR expression does not seem to change in platinum-sensitive and refractory patients, which states that this expression does probably not affect the resistance development (Kollmannsberger et al., 2002a).

The pre-clinical trial carried out by Juliachs et al. (2013) showed that the inhibition of EGFR with gefitinib (small anti-EGFR molecule) or cetuximab (anti-EGFR antibody) was ineffective for the reduction of tumor size, whereas the use of lapatinib (dual inhibitor of ErbB1 and ErbB2) did reduce the tumor growth by 50%, reduced positive Ki67 cells by 80% and increased apoptosis by 3.5 times (Juliachs et al., 2013a). The NCT01962896 clinical trial, which researches the combination of erlotinib + sirolimus (anti-EGFR + m-TOR inhibitor) is currently being carried out and awaiting results (A Phase II Study of Sirolimus and Erlotinib in Recurrent/Refractory Germ Cell Tumors, 2019).

Regarding the positivity for HER2, and in contrast to breast cancer, in GCTs there is a lack of equivalence between determination by IHC and immunofluorescence (FISH). This lack of equivalence in GCTs is made evident in a study where 22 out of 96 patients expressed HER2 by IHC (essentially differentiated choriocarcinomas and teratomas), but only three of them were HER2 positive by FISH (Soule et al., 2002).

There is very little experience with antiHER2 drugs in refractory GCTs. A pre-clinical trial using mice with refractory and platinum-sensitive GCTs showed that the combination of pazopanib and lapatinib reduced the tumor volume to a larger extent than when administered as a monotherapy in both cohorts (platinum-sensitive and platinum-refractory) (Juliachs et al., 2013b). Regarding the treatment with trastuzumab, there are only data of isolated clinical cases, with a poor response (Kollmannsberger et al., 1999).

3. PI3K/AKT/mTOR

In testicles, the PTEN tumor suppressor gene is highly expressed in normal germ cells, whereas it is absent in an important percentage of seminomas, embryonal carcinomas and in nearly all teratomas. The unclassified intratubular germ cell neoplasia (IGCNU) expresses high levels of PTEN, suggesting that the loss of the PTEN expression is linked to the transition to invasive GCT and tumor progression. PTEN inactivation is linked to the deregulation of the PI3K/AKT pathway and the increase of mammalian target of rapamycin (mTOR). mTOR is a protein present in mammalian animal cells with important functions. The TOR protein family is involved in the control of the start of the mRNA transcription, the organization of the actine cell cytoskeleton, the membrane traffic, ribosome formation and regulation of cell growth, proliferation and death. Everolimus is an mTOR inhibitor, and it also sensibilizes p53 wildtype tumor cells (present in most GCTs) to cis-platinum induced apoptosis (Di Vizio et al., 2005). Table 1 displays the main studies with everolimus in GTCs.

Mego et al. (2016) published the results of a phase II clinical trial, where 15 patients with refractory GCTs (at least two platinum-based previous lines) were treated with everolimus 10 mg/day until there was progression, unacceptable toxicity or complete response. This research failed on its primary objective, which was the objective response rate, with no complete nor partial response. However, one patient maintained stability of the disease during 22.2 months. PFS after 3 months was 40%, with a PFS median of 1.7 months (95% CI: 1.1.-4) and median OS of 3.6 months (95% CI: 2–11) (Mego et al., 2016).

The RADIT phase II clinical trial, which treated 22 patients with refractory GCTs (76% had received 5 or more previous treatment lines) were treated with everolimus 10 mg/day. The primary objective was the progression-free survival at week 12. It was not achieved, therefore the result was 0%. No objective response was achieved either. Only one patient showed stability of the disease after 6 weeks of treatment. The PFS and OS medians were 7.4 weeks (80% CI: 4.9–7.6 weeks) and 8.3 weeks (80% CI: 7.1–9.1 weeks), respectively (Fenner et al., 2019).

4. c-KIT/stem cell factor pathway

The c-KIT proto-oncogene (also known as stem cell growth factor receptor (SCF), CD117) encodes a type III tyrosine-kinase protein family member receptor, which includes the platelet derived growth factor receptors, a macrophage and monocyte colony-stimulating factor. The c-KIT/SCF pathway is crucial for the embryonal development of testicles and spermatogenesis (Oosterhuis and Looijenga, 2005).

c-KIT is expressed in a high proportion of refractory germ tumors, 48% of cases (11 out of 23 positive patients) according to research. However, the expression of the c-KIT proto-oncogene is not present evenly in all histological subtypes, being predominantly expressed in seminomas (80–100%). It is expressed by 7–48% in nonseminomas, and its expression is absent or almost absent in choriocarcinomas and teratorcarcinomas (Durán et al., 2010; Madani et al., 2003; Izquierdo et al., 1995; Strohmeyer et al., 1995; Bokemeyer et al., 1996; Nikolaou et al., 2007).

The high expression of c-KIT opens the possibility of response to imatinib in germ tumors. Imatnib was initially approved in chronic myelogenous leukemia, to inhibit the activation of BCR-ABL, and it is the treatment of choice for metastatic gastrointestinal stromal tumor.

A phase II clinical trial studied the expression of c-KIT through immunohistochemistry in 18 patients with refractory germ cell tumors,

Table 1
Main studies with everolimus in GCTs.

Drug	Study	Design	Efficiency
Everolimus	(Mego et al., 2016)	Phase II 15 pt Refractory GCTs	OR 0%. 1 pt SD 22 months. PFS 3 months: 40%. Median PFS 1.7 months. Median OS 3.6 months.
Everolimus	RADIT (Fenner et al., 2019)	Phase II 22 pt Refractory GCTs (76 % ≥ 5 previous lines)	PFS 12 weeks: 0%. OR 0%. 1 pt SD 6 weeks. Median PFS 7.4 weeks. Median OS 8.3 weeks.

GCTs: germ cell tumors; pt: patient; OR: objective response rate; SD: tumor stability; PFS: progression-free survival; OS: overall survival.

non-subsidiary of curative treatment with chemotherapy or surgery. Six out of those 18 patients (33%) displayed a positive expression for c-KIT and received treatment with oral imatinib 600 mg/day. No objective response was obtained. Five out of those patients showed tumor progression during the first 8 weeks after starting the imatinib treatment. The sixth patient was a case of late relapse, 6 years after his only previous treatment with chemotherapy according to the BEP scheme (bleomycin, etoposide and platinum) with rescue surgery. This patient showed tumor stability with a reduction of > 50% of AFP during 3 months after progression (Einhorn et al., 2006).

A different research with imatinib included 7 positive c-KIT chemorefractory patients (1 seminoma and 6 nonseminoma). The same study was closed prematurely due to the low rate of c-KIT expression (< 10% of initially studied patients) and the absence of antitumor activity (all 7 patients presented a quick radiological and biochemical tumor progression, with a progression median of 34 days) (Piulats et al., 2007).

A likely explanation of this absence of efficiency in the two forementioned studies is the fact that the most frequent mutation encountered in seminomas is in exon 17 (where there is usually no imatinib response) and not in exon 11, which is a positive predictive factor of response (Madani et al., 2003; Kemmer et al., 2004). The mutational status of the c-KIT receptor should therefore be assessed before starting the treatment with imatinib in future clinical trials with GCTs.

There are several clinical trials in patients with c-KIT positive refractory germ cell tumors with response to imatinib. In one of those cases, a 24 year old male patient with a chemoresistant, metastatic pure seminoma at the time of diagnosis (retroperitoneal space, lung and liver) who received a third line with paclitaxel 150 mg/m2 + oxaliplatin 100 mg/m2 + gemcitabine 800 mg/m2 every two weeks together with imatinib 400 mg/day (due to the intense positive c-KIT expression). After the first month of treatment, a partial response in the lung and a full biochemical response were observed. These responses were maintained after the 6 cycles of chemotherapy. The patient went under rescue surgery and necrosis only was found in the histopathological findings. The disease free patient continued the treatment with imatinib 32 months after the surgery (Pectasides et al., 2008). In the other clinical case, a 29 year old patient was diagnosed with bulky retroperitoneal disease. After three lines of platinum based treatment (the last one with HDCT-ASCT) and lung progression, he received imatinib 400 mg/day when the positivity to c-KIT was confirmed. After three months with imatinib, a complete response was observed. 24 months after the start of imatinib treatment, the patient remains disease free (Pedersini et al., 2007).

Table 2 displays the main publications with imatinib.

Table 2
Main publications with imatinib in GCTs.

Drug	Study	Design	Efficiency
Imatinib	(Einhorn et al., 2006)	Phase II 6 pts c-KIT + Refractory GCTs	5 pts: PD at week 8. 1 pt: SD with decrease of markers for 3 months.
Imatinib	(Piulats et al., 2007)	Phase II 7 pts Refractory GCTs c-KIT +	All: fast PD. Median PD: 34 days.
Paclitaxel + oxaliplatin + gemcitabine + Imatinib	(Pectasides et al., 2008)	Clinical case Chemoresistant seminoma c-KIT +	PR = > surgical resection = > Maintenance with imatinib 32 months disease free.
Imatinib	(Pedersini et al., 2007)	Clinical case Refractory GCTs c-KIT +	CR after 3 months. On going maintenance imatinib after 24 initial months.

GCTs: germ cell tumors; pt: patient; SD: tumor stability; PR: partial response; CR: complete response; PD: tumor progression.

5. Anti-angiogenic agents

Angiogenesis is the development of new blood vessels. It rarely occurs in normal adult tissue. However, it is a key factor in tumor growth and metastasis development. The vascular endothelium growth factor (VEGF) is a crucial angiogenic factor in the immediate embryonic and postnatal development. VEGF receptors are nearly exclusively expressed in the surface of endothelial cells, and, however, they are overexpressed in GCTs. The VEGF stimulates the proliferation, migration and survival of endothelial cells, increases vascular permeability and inhibits apoptosis; these processes are highly related to tumor development and dissemination.

Microvascular density may predict the presence of ganglion metastasis in localized GCTs, and even worse prognosis (Olivarez et al., 1994). The research made by Fukuda S et al. (1999), which included 80 cases of GCTs, indicated that the VEGF expression is substantially related to microvascular density (p = 0.001). In seminomas (n = 33), both VEGF and microvascular density were significantly related to the presence of metastasis (p = 0.008 and p = 0.001, respectively). However, only the VEGF expression was significant (p = 0.006) in the multivariate analysis. In NSGCT (n = 47), four variables were related to the presence of metastasis: VEGF expression, microvascular density, venous invasion and presence of embryonal carcinoma in primary tumor. However, only the VEGF expression and microvascular density were significant in the multivariate analysis (p = 0.007 and p = 0.001, respectively). Therefore, this research seems to suggest that VEGF expression is involved in tumor development, angiogenesis and metastasis in GCTs (Fukuda et al., 1999). These data may provide a chance to create anti-VEGF targets in the treatment of GCTs. Table 3 displays the main publications using anti-angiogenic agents.

Two clinical cases which show response to bevacizumab in patients with refractory GCTs were released. In one of them, the patient received bevacizumab combined with ifosfamide in high doses, etoposide and carboplatin, obtaining an almost complete response of hepatic metastasis, but the PFS was only five months (Voigt et al., 2006). In the other clinical case, a tumor stability during 6 months with bevacizumab was obtained. After suspending the treatment, the tumor progressed (Mego et al., 2007).

Additionally, the phase II clinical trial by Jain et al. (2014), with oxaliplatin and bevacizumab in patients with refractory GCTs showed an objective response of 27.6% (8 patients out of 29), including the complete response of a patient during more than 12 months. These patients had received an average of 4 previous chemotherapy lines. The response duration median was 5 months (range: 4 to 22 months), with a survival median of 8 months for overall population and 17.5 months for responding population. However, the main objective of the research was not achieved, as there was only one patient free from progression after 12 months. This combination was well tolerated, with manageable adverse effects. As published by Voigt et al. (2006), the contribution of bevacizumab to the effectiveness of the treatment cannot be determined (Jain et al., 2014). Moreover, this rate of response does not differ from the one presented by oxaliplatin in monotherapy, in patients with refractory GCTs (19–25%) and is inferior to the response shown by the combination of oxaliplatin and gemcitabine, or without paclitaxel (46%) (Kollmannsberger et al., 2002b; Oechsle et al., 2011a).

A different study treated 43 patients with GCTs (14% of which were platinum-sensitive) with high doses of bevacizumab associated chemotherapy. They received a first cycle of gemcitabine, docetaxel, carboplatin and bevacizumab. An objective response of 89% was achieved (32% CR, 35% minor PR, 22% major PR). The diversity of the population and the high rate of adverse effects, with 4 treatment-related deaths, must be taken into consideration (Nieto et al., 2015).

Table 3
Main publications using anti-angiogenic agents in GCTs.

Therapy	Study	Design	Efficiency
Bevacizumab + ifosfamide high doses + carboplatin + Etoposide	(Voigt et al., 2006)	Clinical case Refractory GCT	PR. PFS 5 months.
Bevacizumab	(Mego et al., 2007)	Clinical case Teratocarcinoma = > BEP x4 = > surgery with AP teratoma = > bevacizumab maintenance.	SD 6 months.
Oxaliplatin +	(Jain et al., 2014)	Phase II	OR 27.6%, including one CR > 12 months.
bevacizumab		29 pts Refractory GCTs	Survival in responding population: 17.5 months. PFS 12 months: 3.4%.
HDCT + bevacizumab	(Nieto et al., 2015)	Phase II 43 pts (14% platinum-sensitive)	OR 89% (32% CR).
Thalidomide	(Rick et al., 2006)	Phase II 15 pts Refractory GCTs	OR 0%. Reduction TM 33%.
Lenalidomide	(Oechsle et al., 2010)	Phase II 4 pts Refractory GCTs	PD during first cycle. Median survival 8 weeks.

GCTs: germ cell tumors; pt: patient; OR: objective response rate; SD: tumor stability; PR: partial response; CR: complete response; PFS: progression free survival; PD: tumor progression; TM: tumor markers.

Apart from the use of bevacizumab in patients with refractory GCTs, there are also data about the use of thalidomide in these patients (drug with anti-angiogenic properties as it works as an inhibitor of the VEGF receptor). A phase II clinical trial treated 15 patients with refractory GCTs with thalidomide, obtaining a reduction of tumor markers in 5 patients (33%). However, no objective response was obtained. This biochemical response occurred in patients with a low tumor burden, slowly progressive disease and high AFP levels (Rick et al., 2006). The other research treated 4 patients with refractory GCTs, with an average of 7 previous lines (4 of them previous to cisplatin) with lenalidomide 25 mg/day on days 1–21 every 28 days. This therapy, among other anti-tumor mechanisms, inhibits angiogenesis through the blockage of endothelial cell migration and adhesion and the inhibition of micro-vessel formation. Despite the acceptable toxicity profile, all patients progressed both biochemically and radiologically during the first cycle, presenting a survival median of 8 weeks (Oechsle et al., 2010).

6. Multiple tyrosine-kinase inhibitors

Several studies have found a substantially higher expression of VEGF and the platelet derived growth factor (PDGF) in patients with GCTs compared with normal tissue, which indicates that they may play an important role in tumor angiogenesis, progression and metastasis (Fukuda et al., 1999; Viglietto et al., 1996). Table 4 gathers the main studies with different therapies which inhibit both factors.

Sunitinib is an oral, small-molecule and powerful tyrosine kinase inhibitor of VEGF, PDGF receptors, the stem cell growth factor receptor (c-KIT) and the colony-stimulating factor 1 (SCF-1) receptor. In a research carried out on mice with sensitive GCTs and CDDP resistant, mice were separated into four groups: controlled without treatment, treated with sunitinib, treated with CDDP and treated with a combination of CDDP and sunitinib. Better results were observed in animals treated with the combination of drugs, with a survival median of 13, 33, 36 and 47 days in the controlled group, CDDP group, sunitinib group and the combination group, respectively. Moreover, a decrease of Ki67 and CD31 was observed in the treated population, indicating a reduction of cell proliferation and tumor vasculature. There was, in the cohort of CDDP resistant mice, response only in the sunitinib-treated group and the CDDP-sunitinib-treated group, although there was no difference between both groups, as expected (Castillo-Avila et al., 2009).

Table 4
Main studies with different therapies which inhibit PDGFR and VEGF in GCTs.

Therapy	Estudio	Design	Efficiency
Sunitinib	(Oechsle et al., 2011b)	Phase II 32 evaluable pts	PR 9%. Median PFS 2 months. PFS 3 months: 11%. PFS 6 months: 36.4%.
Sunitinib	(Subbiah et al., 2014)	Phase II 5 pts 1 st line refractory GCTs	PFS 12 weeks: 20% (1/5).
Sunitinib	(Feldman et al., 2010b)	Phase II 10 pts Refractory GCTs	All of them PD in 3 first cycles.
Pazopanib	(Necchi et al., 2017)	Phase II 43 pts Refractory GCTs	PR 4.7%. PFS 3 months: 12.8%. OS 24 months: 14.2%.
Sorafenib	(Skoneczna et al., 2014)	Phase II 18 pts Refractory NSGCTs	OR 0%. SD 3/18 patients > 1 year.

GCTs: germ cell tumors; pt: patient; OR: objective response rate; SD: tumor stability; PR: partial response; PFS: progression free survival; OS: overall survival; PD: tumor progression; NSGCTs: nonseminoma germ cell tumors.

A research by Oechsle et al. (2011) treated patients with refractory GCTs with sunitinib 50 mg/day during 4 weeks in cycles of 6 weeks. Out of 32 evaluable patients, three showed a partial response (9%) and the progression free survival rate on these three patients was 5, 6.4 and 12.2 months. The progression free survival median was 2 months (95% CI: 1.4–2.6), with a PFS at 6 months of 11%. The overall survival median was 3.8 months (95% CI: 3–6.6), with an OS rate at 6 months of 36.4% (Oechsle et al., 2011b).

Another phase II clinical trial treated five patients with seminoma and nonseminoma refractory GCTs at the first line of treatment with sunitinib 50 mg/day during 4 weeks in cycles of 6 weeks. Only one patient was progression-free at week 12 (20%), maintaining the response during 17 months with sunitinib (Subbiah et al., 2014).

In a different study on ten patients with refractory GCTs, sunitinib only achieved a stabilization of tumor markers as the best response. Not even the change of the schedule of administration from intermittent (50 mg/day during 4 weeks in cycles of 6 weeks) to continuous

(37.5 mg/day continuous) achieved better results. All patients progressed during the first three cycles (Feldman et al., 2010b).

Pazopanib is an inhibitor of multiple tyrosine-kinase receptors, including VEGFR, PDGFR and c-KIT. It is used for multiple types of carcinoma, mostly kidney carcinoma and some types of soft tissue sarcoma. Due to its mechanism of action, it may also have an effect in refractory GCTs. In one study, a reduction of markers was obtained in 70.3%, and a partial response in two patients (4.7%). However, the primary objective, which was the PFS after 3 months, was not achieved. It reached 12.8%. The rate of PFS after 6 months was only 2.6% (95% CI: 0.4–178), while the rate of OS after 24 motnhs was 14.2% (95% CI: 6–33.7) (Necchi et al., 2017). As previously mentioned, the study published by Juliachs et al. (2013), in which mice with refractory or CDDP-sensitive GCTs received pazopanib as monotherapy or combined with lapatinib, the effect was stronger in animals treated with the combination (Juliachs et al., 2013b).

Regarding sorafenib (inhibitor of multiple tyrosin-kinase receptors used mostly in advanced kidney carcinoma, hepatocellular carcinoma and papillary carcinoma of the thyroid) a phase II study where 18 polytreated NSGCTs patients (with a median of 3 previous lines) were treated with sorafenib 400 mg twice a day until progression or unacceptable toxicity. A reduction of tumor markers was obtained in 8 patients, with radiological stability in three of them during more than 350 days, although no objective response was obtained (Skoneczna et al., 2014).

7. Anti-PD1

The programmed death receptor 1 (PD-1, CD279) is expressed in the surface of activated T cells, B cells and macrophages. Its ligand, PDL1 (B7-H1, CD274), is expressed in tumor cells, macrophages, T cells and other types of tissue (Keir et al., 2008). The interaction of these two molecules regulates the immune response in a negative manner. Therefore the PDL1 expression is an important way for tumor cells to suppress the anti-tumor immune activity in the tumor micro-environment.

There are two main studies which suggest that the inhibition of immune checkpoints may play a relevant role in the treatment of GCTs. One of them is the research by Frankhaouser et al. (2015), who reported a higher expression of PDL1 in GCTs (mostly in teratomas) than in normal testicular tissue (73% in seminomas, 64% in nonseminomas, and no expression in its precursor (IGCNU) nor in normal testicular tissue). This research also adds a possible prognostic value to the PDL1 expression in such patients, linking a high PDL1 expression to worse clinical features and results in survival (Fankhauser et al., 2015). Another research is the Cierna et al. (2016) research, who reported 76% of PDL1 expression in seminomas and 89% in nonseminomas, also linking worse prognostic features to the PDL1 expression (including ≥ 3 metastatic locations, high tumor markers and/or non-pulmonary visceral metastasis). In the latter study, patients with low PDL1 (score < 10 (based on the extension and intensity of the staining)) obtained better results in PFS (HR 0.4, 95% CI 0.16–1.01, p = 0.0081) and OS (HR 0.43, 95% CI 0.15–1.23, p = 0.0397) than those patients with a higher PDL1 (score ≥ 10). In the multivariate analysis, the PDL1 expression was only independently linked to PFS (regardless of the IGCCCG (International Germ Cell Consensus Cancer Group) classification). In this research, unlike the Fankhauser study, the subpopulation with higher PDL1 levels was choriocarcinomas (Cierna et al., 2016).

Zschäbitz et al. (2017) revised, in retrospect, patients with refractory GCTs treated with anti-PD1 (nivolumad or pembrolizumab). Out of 7 patients, 4 patients progressed quickly after the first cycle and 3 patients received at least 6 months of anti-PD1 treatment. Out of those three responding patients, one patient (after 4 cycles of HDCT-ASCT, and GOG) showed a pseudo-progression after two cycles of pembrolizumab, so they continued with nivolumab up to a number of ten cycles, when progression was confirmed. At the moment of publishing, this patient was still alive (19 months after initiating the anti-PD1 treatment). It cannot be settled whether this patient's slow evolution was the result of the efficiency of the anti-PD1 treatment received, or the result of a slow basal tumor evolution. In the case of the other two responding patients, there was a large tumor response. One of them, with PDL1 60% and after several previous relapses (including HDCT-ASCT), received 7.6 months of nivolumab and was still receiving the treatment at the moment of publishing. The other patient, with PDL1 70% and not a candidate to HDCT-ASCT treatment, after several previous treatments (including GOP and sunitinib) received pembrolizumab combined with etoposide; after three months, etoposide was suspended and the treatment continued with pembrolizumab in monotherapy. This latest patient, after cycle number 15, still maintained an almost complete response. In month 16, it was decided to suspend the treatment and to observe. Four months after the interruption, the patient progressed and the treatment with pembrolizumab was restarted (Zschäbitz et al., 2017).

There are also negative results of anti-PD1, like a study with 12 patients with refractory NSGCTs, from which only two patients were PDL1 positive. They were treated with pembrolizumab and none of the patients achieved an objective response rate. Only two patients obtained disease stability, in weeks 28 and 19 (both PDL1 negative), although with a progressive increase of tumor markers (Adra et al., 2018).

These results make us consider the immune checkpoint inhibitors as potential effective treatments for selected GCTs patients. To make these results more evident, there is a phase II clinical trial using pembrolizumab (NCT02499952) for patients with refractory GCTs and the clinical trial of the combination of ipilimumab with nivolumab in patients with rare tumors (NCT02834013) in process.

Table 5 gathers the main studies with anti-PD1 therapies.

Table 5
Main studies with anti-PD1 therapies in GCTs.

Therapy	Study	Design	Efficiency
Anti-PD1(nivolumab or pembrolizumab)	(Zschäbitz et al., 2017)	Retrospective 7 pts treated with anti-PD1	− 4/7 progression after 1 st cycle. − 1 pt: pembrolizumab x2 = > nivolumab x 8 cycles. − 1 pt (PDL1 60%): nivolumab > 7.6 months. − 1 pt (PDL1 70%): pembrolizumab + etoposide x 3 cycles = > pembrolizumab > 16 months.
Pembrolizumab	(Adra et al., 2018)	Phase II 12 pts (2 pts PDL1 +) Refractory NSGCTs	OR 0%.

GCTs: germ cell tumors; pt: patient; OR: objective response rate; NSGCTs: nonseminoma germ cell tumors.

8. Cyclin inhibitors

The phosphorylation of the retinoblastoma protein (pRb) through cyclin-dependent kinases (CDKs) 4 and 6 (together with cyclin D) changes phase G1 of the cell cycle to phase S, stimulating tumor growth (Harbour et al., 1999). Although undifferentiated GCTs (like embryonic carcinomas) hardly express any pRb, more differentiated GCTs like teratomas have shown high levels of pRb expression (Strohmeyer et al., 1991; Bartkova et al., 2003). Therefore, selective inhibition of CDK 4/6 may play a role in the inhibition of growing teratomas.

The results of Vaughn et al.'s phase I and II clinical trials (2009, 2015) with cyclin inhibitors are available, as displayed in Table 6. In clinical trial phase I, three patients with unresectable growing teratomas received palbociclib. The first patient received 150 mg/day during 21 days in cycles of 28 days, achieving tumor stability during 18 months. The second patient, who received 200 mg/day during 14 days in cycles of 21 days, obtained a partial response for more than 22 months. The third patient, who was treated with 125 mg/day during 21 days in cycles of 28 days, obtained disease stability during more than 24 months (Vaughn et al., 2009). These data express that the inhibition of cyclins 4/6 probably plays a role in those germ cell tumors with pRb expression.

A different phase II clinical trial treated 29 patients with incurable refractory GCTs with pRb expression by IHC, with oral palbociclib 125 mg/day during 21 days in cycles of 28 days. The primary objective was PFS at 24 weeks, which was reached with a rate of 28% (90% CI: 15%–44%). Out of the 11 patients with teratomas, 5 were progression-free at week 24 (one of them with affected CNS and spinal cord). At week 80, three patients with teratomas were still progression-free. Out of the 10 patients with malignant transformation teratomas, two were progression-free at week 24 and out of the 8 patients with non-teratoma GCTs, only one patient achieved PFS at week 24. The median of PFS in the group of patients with teratomas, malignant transformation teratomas and non-teratoma GCTs was 23, 18 and 5 weeks, respectively. Despite these promising results in population with teratomas with or without malignant transformation, no objective response rate was obtained according the RECIST criteria in overall population. Toxicity was mostly haematological, essentially neutropenia (43% grade 3/4), although only one patient showed febrile neutropenia and it was uncomplicated (Vaughn et al., 2015).

9. PARP inhibitors

The PARP (poly ADP-ribose polymerase) pathway includes a family of enzymes involved in DNA damage repair. PARP-1 is the most frequent member of the PARP family. It plays an important role in DNA repair, genomic stability, energy metabolism, transcriptional regulation and cell inflammation and death. DNA repair is a complex and multifaceted process which is crucial for cell survival.

When the PARP pathway is inhibited, damage is accumulated in only one strand of DNA, which may lead to later damage of both strands and finally to selective cell death. This one strand damage is normally repaired by homologous recombination of double DNA strand, where BRCA1 and BRCA2, which participate in the PARP pathway, are fundamental. This overactivated pathway may be one of the mechanisms used by tumor cells to avoid apoptosis after DNA damage. Hence the PARP-1 pathway inhibition being a powerful target for tumor treatment, especially platinum-sensitive ones like GCTs.

A translational research studied the expression of PARP pathway in 124 patients with GCTs. There was a high expression rate, the greatest one in IGCNU (100%), followed by seminomas with 52.6% expression, embryonic carcinomas with 47%, yolk sac tumor with 33.3%, teratomas with 26.7% and choriocarcinomas with 25%. The rate in normal testicular tissue was however only 1.9%. There were no clinical differences between the population with PARP overexpression and those without it (Mego et al., 2013).

There are still no clinical trial results about the inhibition of this pathway in GCTs, only the preclinical data of the Guggenheim et al. (2008) study, where a cell population with testicle carcinoma (NTera2) showed great sensitivity to PARP-1 inhibition (Guggenheim et al., 2008).

A currently in process clinical trial treats patients with refractory GCTs with olaparib (NCT02533765). Its results will allow for a better understanding of the role of this pathway in these patients.

10. c-MET/HGF

The tyrosine-kinase MET receptor and its ligand, the hepatocyte growth factor, are expressed in human testicular tissue and are involved in testicle development and spermatogenesis (Lail-Trecker et al., 1998). The proto-oncogene MET is involved in the regulation of cell survival, adhesion, migration and angiogenesis through the AKT/MAPK pathways (Beviglia et al., 1997). Likewise, the MET overexpression is present in a variety of human tumors (Cecchi et al., 2010).

The expression of MET has been detected by IHC in 67% of GCTs. From this data, there arose the idea of the possible anti-tumor effect of an anti-MET target. The results of the phase II clinical trial with tivantinib (tyrosin-kinase MET anti-receptor) are available, as displayed in Table 7. 27 patients with refractory GCTs (93% non-seminoma) were treated with tivantinib 360 mg twice a day. Out of 25 evaluable patients, no objective response rate was obtained. The best response was tumor stability in 5 patients. The PFS median was one month. The PFS rate at week 12 was 21%, and the OS median was 6 months. The therapy was well tolerated, with the exception of one grade 3 pneumonia and one grade 3 syncope. In this study, however only 6 patients expressed MET and only in a very weak way (+1) (none of them had a high MET expression (+3)) (Feldman et al., 2013). Therefore, for upcoming studies, the MET expression as an inclusion criterion should be taken into consideration.

Table 6
Main studies with cyclin inhibitors in GCTs.

Therapy	Study	Design	Efficiency
Palbociclib	(Vaughn et al., 2009)	Phase I 3 pts	1 pt SD 18 months. 1 pt PR > 22 months. 1 pt PR > 24 months.
Palbociclib	(Vaughn et al., 2015)	Phase II 29 pts Refractory GCTs	PFS 24 weeks: 28%. OR 0%. Promising results in population with teratoma +/- malignant transformation.

GCTs: germ cell tumors; pt: patient; OR: objective response rate; SD: tumor stability; PR: partial response;
PFS: progression-free survival.

Table 7
Main study with anti-MET target in GCTs.

Therapy	Study	Design	Efficiency
Tivantinib	(Feldman et al., 2013)	Phase II 27 pts (25 evaluable) Refractory GCTs 6/27 pts MET + (+1 all)	OR 0%. Median PFS 1 month. PFS 12 weeks: 21%. Median OS 6 months.

GCTs: germ cell tumors; pt: patient; OR: objective response rate; PFS: progression-free survival; OS: overall survival.

11. CD30

Hodgkin's lymphomas, anaplastic large cell lymphomas and embryonal carcinomas are histopathologically characterized by the expression of CD30. Brentuximab is a conjugated antibody formed by a monoclonal antibody directed against CD30 (recombinant chimeric immunoglobulin G1 [IgG1], produced by recombinant DNA technology in Chinese hamster ovary cells) which is covalently bound to the antimicrotubular agent monomethyl auristatin E (MMAE)). This drug was approved in 2011 for the treatment of relapsing Hodgkin's lymphoma. The phase II clinical trial (NCT01851200) by Necchi et al. (2016) currently studies the data of 9 patients with refractory GCTs CD30+ treated with brentuximab 1.8 mg/kg iv tri-weekly. From these patients, 3 had previously received three lines and the rest had received at least three previous lines. The objective response rate was 22.2% (1 CR and 1 PR (> 80%)), a 44% decrease of tumor markers after the second cycle. However, the PFS after three months was only 11% (95% CI: 0.6–38.8) and OS after 6 months was 85.7% (95% CI: 33.4–97.9). There were no discontinuations for the treatment due to toxicity. In addition to antitumor activity, the study observed an immunomodulatory effect with reduction of activated T cells, granulocytes and mature dendritic cells, and an increase of immature dendritic cells (Necchi et al., 2016).

Table 8
Main studies with anti-CD30 target in GCTs.

Therapy	Study	Design	Efficiency
Brentuximab	(Necchi et al., 2016)	Phase II 9 pts Refractory GCTs CD30+	OR 22.2% (1 CR). PFS 3 months: 11%. OS 6 months: 85.7%.
Brentuximab	(Albany et al., 2013)	Phase II 3 pts Poly-treated EC CD30+	2 pts PR after two cycles. 1 pt SD after two cycles.

GCTs: germ cell tumors; pt: patient; OR: objective response rate; SD: tumor stability; PR: partial response; CR: complete response; PFS: progression-free survival; OS: overall survival; CE: embryonal carcinoma.

Also available are the results of phase II clinical trial by Albany et al. (2013), which treated three poly-treated patients with embryonal carcinoma CD30 positive (two patients as fourth line treatment and one as third line treatment) with brentuximab. One patient achieved a partial biochemical (via BHCG) and radiological response after two cycles, with posterior progression. Another patient achieved, after two cycles, a partial pulmonary radiological and mediastinal response (maintaining negative markers from the beginning). The third patient achieved a biochemical response with radiological stability after two cycles (Albany et al., 2013).

Both studies are summarized in Table 8.

12. Conclusions

GCTs have a high rate of cure with chemotherapy, due to their high sensitivity to cisplatin. However, 3–5% of these patients will die due to the tumor (15%–20% of metastatic patients). Thus far, no targeted treatment in GCTs has proved a reasonable efficiency. This makes us wonder whether GCTs are really a type of tumor untreatable with targeted drugs. The reasons for this lack of results may be due, firstly, to the diversity of the subtypes of germ cell tumors; secondly, to the difficulty to recruit a sufficient number of patients in clinical trials due to the low rate of refractory GCTs; thirdly, to the fact that these patients are highly pre-treated and have a bad prognosis. This means that the results of pre-clinical models have rarely been translated into a benefit in clinical practice. The future of the research lies in the appropriate selection of patients with a certain molecular profile and in finding predictive response and targeted drugs resistance factors. The low rate of impact and mortality of germ cell tumors highlights the challenge of clinical trials development and the need of international cooperation. Table 9 gathers the main clinical trials in process or awaiting results with targeted therapies for GCTs.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Table 9
Ongoing or recently completed clinical trials with targeted therapies for GCTs.

Protocol ID and reference	Drug	Status	Sponsor
NCT01851200 (Brentuximab Vedotin (SGN-35) as Salvage Treatment for CD30-positive Germ Cell Tumors, 2019)	Brentuximab	Completed	Fondazione Michelangelo
NCT01743482 (Pazopanib in Advanced and Cisplatin-resistant Germ Cell Tumors, 2019)	Pazopanib	Unknown	Fondazione IRCCS Istituto Nazionale dei Tumori, Milano
NCT03726281 (Nivolumab in Platinum Recurrent or Refractory Metastatic Germ Cell Tumors, 2019)	Nivolumab	Recruiting	Hospital Beatriz Ângelo
NCT02721732 (Pembrolizumab in Treating Patients With Rare Tumors That Cannot Be Removed by Surgery or Are Metastatic, 2019)	Pembrolizumab	Recruiting	M.D. Anderson Cancer Center
NCT02458638 (A Study of Atezolizumab in Advanced Solid Tumors, 2019)	Atezolizumab (MPDL3280A)	Active, not recruiting	Hoffmann-La Roche
NCT02834013 (Nivolumab and Ipilimumab in Treating Patients with Rare Tumors, 2019)	Ipilimumab + nivolumab	Recruiting	National Cancer Institute (NCI)
NCT02034110 (Efficacy and Safety of the Combination Therapy of Dabrafenib and Trametinib in Subjects With BRAF V600E- Mutated Rare Cancers, 2019)	Dabrafenib + Trametinib	Active, not recruiting	Novartis Pharmaceuticals
NCT02187783 (LEE011 for Patients with CDK4/6 Pathway Activated Tumors (SIGNATURE), 2019)	LEE011	Completed	Novartis Pharmaceuticals
NCT02533765 (Olaparib as Salvage Treatment for Cisplatin-resistant Germ Cell Tumor, 2019)	Olaparib	Active, not recruiting	Istituto Scientifico Romagnolo per lo Studio e la cura dei Tumori
NCT01962896 (A Phase II Study of Sirolimus and Erlotinib in Recurrent/Refractory Germ Cell Tumors, 2019)	Sirolimus + Erlotinib	Terminated	Theodore Laetsch

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