Review

Desmoplastic small round cell tumor: a review of main molecular abnormalities and emerging therapy

Celso Abdon Mello [1*], Fernando Augusto Batista Campos[1], Tiago Goss Santos[2,3], Maria Leticia Gobo Silva[4], Giovana Tardin Torrezan[3,5], Felipe D´Almeida Costa[6], Maria Nirvana Formiga[1]. Ulisses Nicolau[1]. Antonio Geraldo Nascimento[6], Cassia Silva[1], , Maria Paula Curado[7], Suely Nakagawa[8], Ademar Lopes[8], Samuel Aguiar Jr[8]

1 Dept Medical Oncology, A.C.Camargo Cancer Center, Sao Paulo, Brazil.
2 Laboratory of Tumor Biology and Biomarkers, International Center of Research CIPE, A.C.Camargo Cancer Center, Sao Paulo, Brazil.
3 National Institute of Science and Technology in Oncogenomics and Therapeutic Innovation, Sao Paulo, Brazil.
4 Dept of Radiation Oncology, ACCamargo Cancer Center, Sao Paulo, Brazil.
5 Genomics and Molecular Biology Group, International Center of Research CIPE, A.C.Camargo Cancer Center, Sao Paulo, Brazil.
6 Dept of Pathology, A.C.Camargo Cancer Center, Sao Paulo, Brazil.
7 Dept of Epidemiology, A.C.Camargo Cancer Center, Sao Paulo, Brazil
8 Dept of Surgery, A.C.Camargo Cancer Center, Sao Paulo, Brazil.

* Correspondence: celso.almello@gmail.com Tel.: (+5511 21892779)

Simple Summary: Desmoplastic small round cell tumor is a rare neoplasm with an extremely aggressive behavior. Despite the multimodal treatment for newly diagnosed patients with chemotherapy, cytoreductive surgery and radiation, the cure rate is still low. For relapsed or progressive disease, there is limited data regarding second and third line therapies. Novel agents have shown only modest activity. Recent molecular changes have been identified in this disease and opens opportunity to be explored in future clinical trials.

Abstract: Desmoplastic small round cell tumor (DSRCT) is an extremely rare, aggressive sarcoma affecting adolescents and young adults with male predominance. Generally, it originates from serosal surface of abdominal cavity. The hallmark characteristic of DSRCT is the EWSR1-WT1 gene fusion. This translocation up-regulates the expression of PDGFRα, VEGF and other proteins related to tumor and vascular cell proliferation. Current management of DSRCT includes a combination of chemotherapy, radiation and aggressive cytoreductive surgery plus intra-peritoneal hyperthermic chemotherapy (HIPEC). Despite advances in multimodal therapy, outcomes remain poor since the majority of patients present disease recurrence and die within 3 years. The dismal survival makes DSRCT an orphan disease with urgent need of new drugs. The treatment of advanced and recurrent disease with tyrosine kinase inhibitors, such as pazopanib, sunitinib, and mTOR inhibitors have been evaluated in small studies. Recent works using comprehensive molecular profiling of DSRCT

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

identified potential therapeutic targets. In this review, we aim to describe the current studies conducted to better understand DSRCT biology and to explore the new therapeutic strategies under investigation in preclinical models and in early phase clinical trials.

.

Keywords: desmoplastic small round cell tumor; treatment; prognosis; surgery; radiotherapy; chemotherapy; tyrosine kinase receptor; target therapy; rare disease

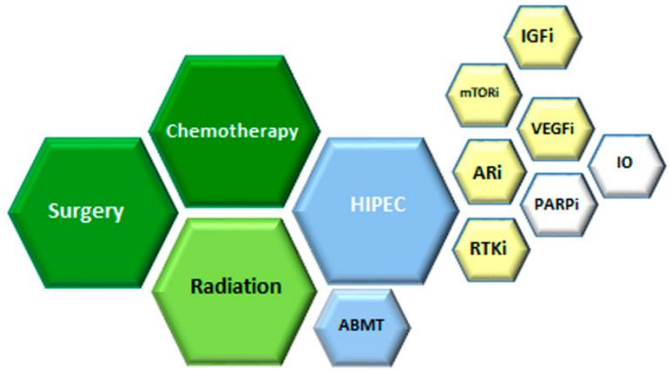

Figure 1. Therapeutic options for DSRCT. Based on multiple retrospective and few prospective studies, the benefit was observed for therapies in green, low evidence if benefit for therapies in blue. For relapsed or progressive disease, strategies in yellow had been used and in white are perspectives. HIPEC (hyperthermic intraperitoneal chemotherapy), RTKi (Receptor Tyrosine Kinase inhibition), ARi (Androgen Receptor inhibition), VEGFi (Vascular Endothelial Growth Factor inhibition), IO (immune check point inhibition), IGFi (Insulin Growth Factor inhibiotion), mTORi (mammalian Target of Rapamycin inhibition), ABMT (autologous bone marrow transplant)

Introduction

Desmoplastic small round cell tumor (DSRCT) is an extremely rare, aggressive sarcoma. It affects mainly adolescents and young adults and originates in and primarily involves the serosal surfaces of the abdominal cavity. It was first described by Gerald and Rosai in 1989 as a newly characterized clinicopathologic entity [1]. Current management includes a combination of chemotherapy, radiation, and aggressive surgical resection [2,3] as summarized in Figure 1. Despite advances in multimodal therapy, outcomes remain poor since the majority of patients develop significant disease recurrence or die within 3 years [4,5] . Due to the dismal survival, DSRCT has an urgent unmet need for more effective and innovative therapeutic options.

1.1 Demographics of DSRCT

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

Desmoplastic small round cell tumor is a very rare subtype of sarcoma. For research purpose, DSRCT cases can be searched using the histology and behavior (malignant) classification code 8806/3. There is no uniform definition of rare sarcoma, however the burden of rare cancer in our current days is great. The US Orphan Drug Act of 1983 defined rare diseases as those affecting less than 200,000 people in the United States [6]. In 2010, Greenlee et al [7] described the US burden of rare cancers according to the National Cancer Institute definition as those cancers with fewer than 15 cases per 100,000 people per year. More recently, a consortium from the European Union, Surveillance of Rare Cancer in Europe (RARECARE) [8], described a new definition of rare cancer in Europe as those with fewer than 6 cases per 100,000 people per year.

In a study published in 2014 [9], a total of 192 cases of DSRCT were identified in the SEER database between 1973 to 2007. The age-adjusted incidence rate based on this analysis was 0.3 cases/million, with a peak incidence of 0.74 in individuals 20-24 years-old. There is a predominance of DSRCT in male gender. The age-adjusted incidence rates for males and females were 0.4 and 0.1 cases/million, respectively ($p < 0.001$) [9]. There is predominance in african-american individuals and it is more common in male gender. The age-adjusted incidence rates is higher among african-americans as compared to Caucasians (0.5 x 0.2, $p = 0.037$, respectively). Out of 192 cases, the common primary sites of disease were the peritoneum or soft tissue of abdomen and pelvis (42%) and less common primary sites included the ovary/fallopian tube (6 cases), orbit (1 case), cerebellum (1 case), and cerebral ventricle (1 case) [9].

1.2 Molecular Profile of DSRCT

Cytogenetic and molecular characterization of DSRCT identified a unique chromosomal rearrangement, t(11;22)(p13;q12), associated with this tumor[10]·[11]. The EWS-WT1 is the driver to tumorigenesis of DSRCT and it acts by up-regulating the expression of several genes[12]. The chimeric product of the EWS-WT1 fusion protein acts as a dominant transcriptional activator factor that regulates the expression of several growth factor genes, including *PDGFRA*, *IGF1R*, *EGFR*, *IL2*, *IL15* and also transcriptional factors such as *MYC*, *PAX2* and *WT1* [3][13].

The up-regulation of PDGFRα is a hallmark event in the development and DSRCT. The role of PDGFRα in physiologic healing process is well described and is responsible for collagenous

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

stromal production, inflammatory cells infiltration, specially macrophage chemotaxis and neo-angiogenesis [14], induces proliferation and is a chemo-attractant to fibroblasts and endothelial cells [14] [15]. The development and growth of DSRCT is primarily dependent on this translocation product [12]. The EWS-WT1 transcription factor translocation produces a chimeric protein that induces the expression of PDGFRα that can explain the histological characteristics of DSRCT that is marked by profuse stromal proliferation and increased vascular density[16] (Figure 2).

Our group published an study with a comprehensive molecular profiling of a patient with diagnosis of DSRCT [17]. We identified genetic variants leading to protein alterations including 12 somatic and 14 germ-line events affecting genes predominantly involved in mesenchymal cell differentiation pathways. Regarding copy number alterations (CNA) few events were detected, mainly restricted to gains in chromosomes 5 and 18 and losses at 11p, 13q, and 22q. We developed a personalized test to follow up the patient and monitor disease recurrence by assessing the circulating tumor DNA (ctDNA) in the patient's plasma. The genomic breakpoint of the EWS-WT1 gene fusion was tracked for presence of minimal residual disease after surgery. This biomarker has been used in four post-treatment blood samples, 3 years after surgery, and no trace of EWS-WT1 gene fusion was detected, in accordance with imaging tests showing no evidence of disease and with the good general health status of the patient [17]. One interesting finding of our study is the fact that 7 out of 15 genes harboring somatic mutations (*CHL, MEGF10, MEIS2, MYH8, RIMS4, TBPL1*, and *ZFPM2*) are regulated by the same transcription factor, *LEF1* ($p < 0.001$), which, in turn, is regulated by *WT1* [18]. We therefore postulate that DSRCT tumors presenting increased activity of *WT1* might up-regulate the expression of several genes mediated by *LEF1*. However, the accumulation of mutations in this set of genes regulated by *LEF1* and its relationship with the EWS-WT1 fusion protein remains to be addressed.

More recently, the molecular analysis [19]of 6 patients with DSRCT revealed a total of 137 somatic mutations which were related to specific biological processes: DNA damage-response (DDR) network and mesenchymal–epithelial reverse transition/epithelial–mesenchymal transition (MErT/EMT), reinforcing the relevance of these processes in tumor heterogeneity, aggressiveness and drug resistance [19].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

There are many similarities between DSRCT and Ewing Sarcoma (ES) family tumors. Most of these tumors carry the EWS translocation. However, one important molecular aberration that distinguishes DSRCT from ES is the increased Androgen Receptor (*AR*) expression. In a comprehensive analysis of 35 patients [20] with diagnosis of DSRCT using next-generation sequencing, immunohistochemistry and gene amplification tests it was found that the most common alterations that distinguished DSRCTs from ES included higher expression of *AR*, *TUBB3*, *EGFR*, and *TOPO2A* expression. Independent analysis using RNAseq confirmed higher AR expression from an independent data set of EWS-WT1 fusion–positive DSRCTs compared with ES and a pan-cancer analysis [20].

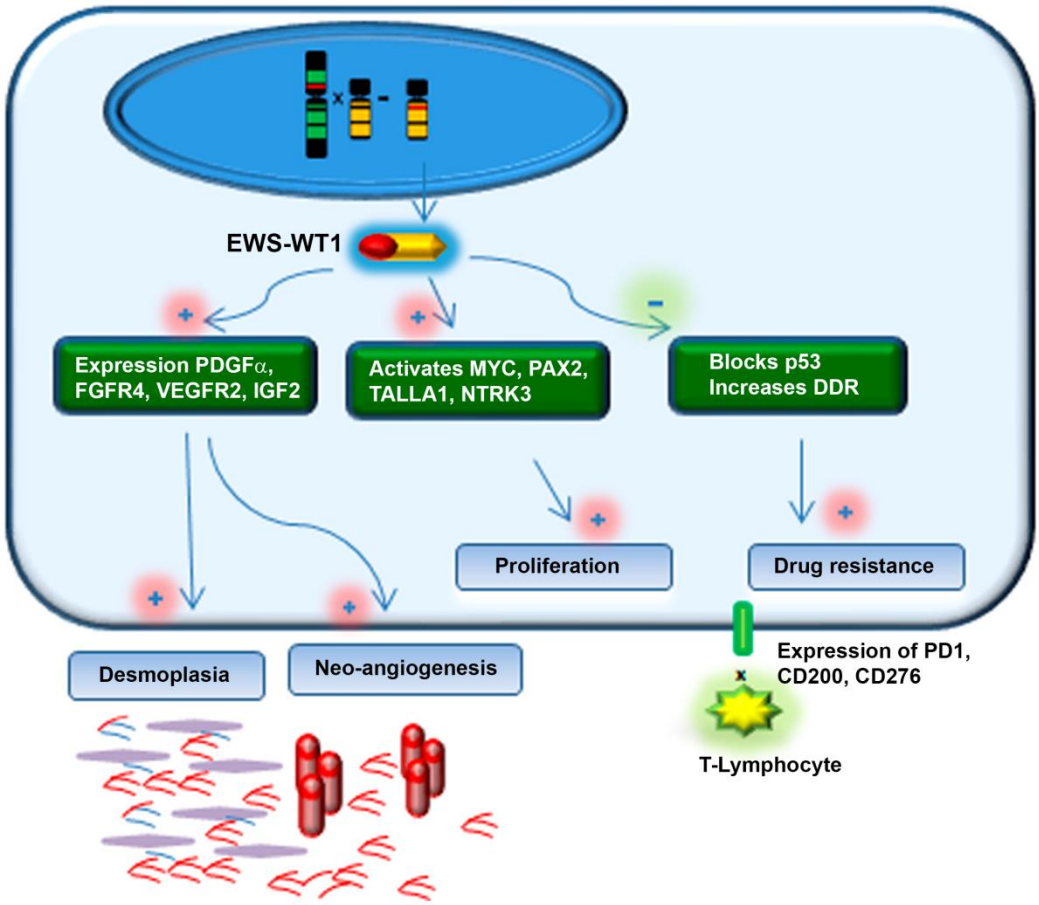

Figure 2. Schematic representation of EWS-WT1 fusion protein mechanism of action in desmoplastic small round cell tumor. Increase in Tyrosine-kinase receptor expression, modulation of DNA replication proteins, activation of DDR (DNA-Demage Repair) machinery resulting in proliferation, desmoplasia, neo-angiogenesis and drug resistance.

 doi:10.20944/preprints202012.0288.v1

2. Clinical Presentation and Diagnosis

The disease predominantly originates from the peritoneum or retro-peritoneum and can invade the omentum with multiple peritoneal implants involving the diaphragm, splenic hilum, mesentery of small and large bowel, and the pelvic peritoneum[2,21,22]. Patients can be asymptomatic for long periods of time until symptoms of pain, ascites, constipation, weight loss, distension and jaundice [2,21]. Other sites of the primary tumor are described in the literature as thoracic cavity, testicle, head and neck, intracranial, thigh, axilla/shoulder, intraosseous, uterine corpus, ovary, skull, middle ear, and others[23–27]. About half of the patients will present extra-peritoneal metastasis at the time of diagnosis[21,28–30], although this percentage was lower in the report of Stiles ZE, et al[31]. Liver and lung are the two most common sites for distant metastatic disease [2,3,22,29]

In most of the cases, patients with abdominal disease are diagnosed in an advanced stage, with large masses and/or extensive seeding in the visceral and parietal peritoneum[32] [33]. Symptoms, which are related to the tumor burden and location of the lesions, motivate investigation by image exams.

The most common imaging finding is multiple, lobulated, low-attenuated, heterogeneous peritoneal, omental and serosal soft tissue masses usually discrete, round or ovoid, without an apparent primary organ of origin[34–36]. Almost all patients will present a dominant mass, mainly in the retrovesical or recto-uterine location, peritoneal or omental [35]. MRI can be helpful in delineating the extent of the disease, if surgical resection is considered [37] and can reveal lesions with heterogeneous contrast enhancement [38]. The role for position emission tomography (PET)-CT is not well established in DSRCT imaging, although it has been used each more as part of staging evaluation together with a chest CT scan [33,38]. There is no formal staging system for DSRCT [39]. One was proposed by Hayes-Jordan *et al* using the Peritoneal Cancer Index but it has not yet been validated [40].

Histologically, the tumor consists of solid sheets, large nests, small clumps, or cords of cohesive, small, round, ovoid, or spindled cells, with inconspicuous nucleoli, and scant cytoplasm, lying in a hypocellular, desmoplastic, collagenous stroma [41]. In immunohistochemistry, there is expression of desmin, membrane antigen (EMA), cytokeratins (AE1/AE3 and CAM5.2) and neural

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

markers (neuron-specific enolase and CD57), and smaller numbers expressing chromogranin, synaptophysin, CD56, neurofilament protein and S100 protein[42]. DSRCT can be immunoreactive for antibodies selectively directed toward the carboxy terminus of the WT1 protein in more than 90% of cases [43]. It is important to note that DSRCT can show a polyphenotypic immunoprofile as well as a marked variation in morphologic appearances from tumor to tumor and within the same neoplasm[44].

3. Differential Diagnosis

The differential diagnosis of DSRCTs can be made with a spectrum of other round cell neoplasms, which includes ES, rhabdomyosarcoma, small cell carcinoma and mesothelioma [44]. Since the diagnosis of DSRCT is made by a combination of the histologic appearance and immunohistochemical staining results, it can be challenging in core biopsy specimens, once some of the distinctive features such as the prominent stromal pattern may not be easily appreciable, and atypical immunohistochemical features can be present due the limited material[44]. Nowadays, large fusion panels using RT-PCR are able to help in differentiating small round cell sarcomas from ES family and new entities are been recognized such as tumors harboring *CIC-DUX4*, *BCOR-CCNB3* and *CIC-FOX04* fusions[45].

4 Treatment

4.1 Therapeutic approach for newly diagnosed patients

There is no general consensus on the best therapeutic approach. Multimodal therapy combining multi-agent intensive chemotherapy, aggressive debulking surgery and adjuvant radiotherapy is considered the standard of care for patients presenting without extra-abdominal metastases [29,46].

The role for surgery in the management of DSRCT is well established in the literature. In a review of 12 patients treated at Mayo Clinic, Hassan *et al* showed the median survival of patients treated with surgical resection was 34 months, whereas the median survival of those who underwent biopsy alone was 14 months [47]. In the report of Wong *et al*, the median survival for patients who had resection for their abdominal or pelvic tumors was 47 months, compared to 16 months for those who did not [48]. Complete cytoreductive surgery is associated with improved survival and should be considered a cornerstone of treatment together with chemotherapy[49].

 doi:10.20944/preprints202012.0288.v1

The most effective chemotherapeutic regimen with the curative intention is still ebated, but most are based in those used in other small round cell sarcomas, a combination of an anthracycline, alkylating agent and vinca alkaloid. DSRCT is somehow sensitive to chemotherapy, although a transient response followed by disease progression is common [48]. Farhat F *et al* reported four patients with intra-abdominal disease who experienced disease stabilization lasting 4-9 months after chemotherapy including cyclophosphamide, etoposide, doxorubicin and cisplatin[50]. Kushner *et al* reported 12 patients with median survival of 19 months with the P6-protocol, which has seven courses of chemotherapy consisting of cyclophosphamide, doxorubicin, vincristine (HD-CAV), etoposide and ifosfamide. This was followed by surgery, radiotherapy, and myeloablative chemotherapy using thiotepa and carboplatin with stem cell rescue in some cases[51]. Bertuzzi *et al* published a trial that included 7 patients with DSRCT treated with induction chemotherapy consisting of ifosfamide, epirubicin and vincristine, and those who responded were then treated with high-dose chemotherapy and autologous bone marrow transplantation in conjunction with local therapy (surgery and/or radiotherapy). The authors concluded that high-dose chemotherapy probably has no role in the treatment of DSRCT[52]. More recently, Scheer M *et al* found that patients treated with the VAIA scheme (ifosfamide, vincristine, doxorubicin, actinomycin D)[53] presented longer event-free survival (29.4 months) compared to other protocols, including the P6 protocol[54]. The interval-compressed regimen of vincristine, irinotecan, temozolamide (VIT) was evaluated in 6 pediatric patients and presented tolerable profile with objective response rate of 50% to the first 2 cycles of VIT [55].

Given the propensity for morbid intra-peritoneal progression, consolidative whole abdominopelvic radiotherapy (WAP-RT) as part of multimodal treatment (chemotherapy, surgical debulking and WAP-RT) was first reported using the P6 protocol, in an attempt to improve local control [51]. Honoré *et al*[29], reported in a series of 38 patients with a median follow-up of almost 5 years, that multimodal treatment combining systemic chemotherapy, complete macroscopic resection, and postoperative WAP-RT could prolong survival in patients without extra-peritoneal disease (EPM) – median survival of 37.7 months (range 7.9 – 42.9 months). The factors predictive of 3-year overall survival were the absence of EPM, complete surgical resection, postoperative WAP-RT and postoperative chemotherapy [29].

 doi:10.20944/preprints202012.0288.v1

Atallah *et al*,[56] studied the prognostic role of WAP-RT on oncologic outcomes as part of multimodal treatment in 103 patients with abdominal DSRCT treated at 8 French centers from 1991 to 2014. Patients were retrospectively divided into three groups for evaluation: Group A treated with adjuvant RT after cytoreductive surgery, Group B without RT after cytoreductive surgery, and Group C treated with chemotherapy alone. Three-year OS was 61.2% (range 41% - 76%) in Group A, 37.6% (range 22% - 53.1%) in Group B, and 17.3% (range 6.3% - 32.8%) for Group C, respectively ($p<.001$). Peritoneal progression-free survival (PPFS) and progression-free survival (PFS) also differed significantly between the 3 groups ($p<.001$), but not distant progression. They concluded that RT seems to improve survival after cytoreductive surgery, with better PPFS, PFS and OS for the patients treated in a multimodal approach, with the limitations of a retrospective study, lack of statistical power (due to the small number of patients), and the need of randomized prospective studies to confirm these results [56].

In a more recent publication, Subbiah *et al* [49] reported the MD Anderson Cancer Center experience with the treatment of 187 patients over 2 decades with a multidisciplinary approach. The 5-year OS rate was substantially improved from 5% to 25% with newer chemotherapy agents and better surgical and RT techniques. Chemotherapy response and complete cytoreductive surgery (CCS) were associated with improved survival. Their results also supported the use of WAP-RT when the time of diagnosis was used as a reference to estimate OS (univariate analysis, $p=0.01$; HR, 0.44) [49]. However, because RT was given almost exclusively to patients who underwent CCS after chemotherapy, they removed these confounding factors and assessed the effects of WAP-RT using the date of the surgery as the start date in a time-variant analysis, and surprisingly, WAP-RT did not improve OS. This unexpected result conflicts with current clinical practice of a tri-modality therapy (chemo, surgery and radiation), and updated their treatment recommendation to consider WAP-RT in highly selected patients that are prospectively monitored in clinical trials [57]. Desai *et al* [58] reported that acute toxicities of WAP-RT were primarily gastrointestinal and hematologic, and were improved in comparisons of IMRT against 2D-RT (gastrointestinal grade 2 or higher: 33% x 77%, $p=.04$; and grade 4 hematologic: 33% x 82%, $p=.02$), with no survival differences. Late toxicity (small bowel obstruction) did not statistically differ between RT modalities [58].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

Due to the rarity of this disease and analysis based in retrospective series, the role of radiotherapy in the management of DSRCT is still controversial. Appropriate patient selection is critical, as severe toxicites can occur. Despite aggressive multidisciplinary approaches, patients have poor prognosis. Prospective randomized multicenter studies will be needed to evaluate the role of local treatments such as RT in the course of the disease

Even after chemotherapeutic cytoreduction and surgical resection of gross, visible disease, microscopic residual disease is often present[4]. Hence, hyperthermic intra-peritoneal chemotherapy (HIPEC) has been examined as an adjunctive intraoperative strategy. In a recent phase 2 trial, 14 DSRCT patients were treated with neoadjuvant chemotherapy, followed by cytoreductive surgery (CRS), which was complete (CR0) or near complete (CR1 =< 2.5 cm of tumor remaining) in all patients, with closed technique HIPEC using 100 mg/m^2 of cisplatin for 90 minutes at 41 degrees Celsius, then followed by WAP-RT[59]. The 3-year overall survival from time of diagnosis for DSRCT patients was 79%, and the estimated median recurrence-free survival (RFS) was 14.0 months. In 100% of patients without hepatic or portal metastasis, there was no peritoneal disease recurrence after CRS-HIPEC. They concluded that CRS, HIPEC and WART are effective local control therapy in DSRCT patients. Earlier, it was demonstrated that patients who had CR0 or CR1 and HIPEC had significantly longer median survival compared with patients who had HIPEC and gross residual disease greater than 2.5 cm after surgical cytoreduction (63.4 vs. 26.7 months)[60]. Patients with DSRCT and disease outside the abdomen at the time of surgery do not benefit from HIPEC[60]. Until now, there is no randomized trials designed to evaluate the relative contribution to improved outcome from complete surgical excision of intra-abdominal implants, versus with the addition of hyperthermic intraperitoneal cisplatin.

A retrospective study with 187 DSRCT patients confirmed that chemotherapy and CCS remain the cornerstone of treatment, and suggest that prospective randomized studies will be required to prove whether HIPEC or WART are important in the management of DSRCT[49].

4.2 Prognosis

Despite multimodal treatment, DSRCT has a poor prognosis, and approximately 60 - 70% of patients die due to disease progression usually within 3 years after diagnosis [5,31,59,61]. The

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

median overall survival varies between 28 – 60 months, with a median disease-free survival between 10 - 15.5 months[29,31,59,61,62]. Table 1 shows a comparison of multimodal treatment in DSRCT.

Author	Year	N	Study Design/Region	Therapy	Overall Survival	Relapse
Dave R et al [63]	2005	66	Retrospective, single center, USA	ChT, CRS, RdT	5-year 15%	NR
Forlenza C et al [64]	2015	19	Prospective, single center, USA	ChT, CRS, BMT	5-year 16%	3-year EFS 11.0%
Osborne E et al [61]	2015	32	Retrospective, Single center USA	ChT, CRS, RdT	5-year 38%	3-year EFS 9.9%
Honore C et al [65]	2017	48	Retrospective	ChT, CRS, HIPEC, RdT	5-year 19%	5-year DFS 12%
Scheer M et al [54]	2018	60	Prospective, multicenter, Germany, Poland, Austria, Sweden, Switzerland	ChT, CRS, BMT, HIPEC	3-year 30%	3-year EFS 11.0%
Stiles ZE et al [22]	2018	125	Retrospective, multicenter, USA	ChT, CRS, BMT HIPEC Rx	5-year 10%	NR
Subbiah V et al [66]	2018	165	Retrospective, single center, USA	ChT, CRS, BMT, HIPEC, RdT	5-year 25%	NR
Honore C et al [5]	2019	100	Retrospective, multicenter, France	ChT, CRS, HIPEC, RdT	5-year 5%	3-year DFS 7.0%
Campos FA et al [62]	2020	19	Retrospective, single center, Brazil	ChT, CRS, HIPEC RdT	5-year 12%	Median DFS 10 months

Table 1. Summary of studies with multimodal therapy for first line treatment of patients with Desmoplastic Small Round Cell Tumor. ChT-chemotherapy, CRS-cytoreductive surgery, BMT- autologous bone marrow transplant, HIPEC-hyperthermic intraperitoneal chemotherapy, RdT- radiation therapy, DFS – disease-free survival, EFS – event-free survival.

One study proposed that the absence of extra-peritoneal metastasis, complete surgical resection and postoperative WAP-RT are factors predictive of 3-year overall survival[29]. The multimodality treatment combining chemotherapy, cytoreductive surgery, HIPEC and WAP-RT can warrant local control of the disease, but patients will still present distant metastasis during follow up, meaning that more effective chemotherapy is necessary to improve long-term outcomes[59].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

4.3 Current and emerging therapy for relapsed or progressive disease

DSRCT is characterized by poor response to conventional chemotherapy and early relapse after radical surgery. Second line treatment is ineffective in most of cases. In our cohort, out of 19 patients treated with first line chemotherapy, 13 received second line and the progression free survival was only 3.9 months [62]. It shows the aggressiveness of this disease and the challenge in developing new therapeutic to treat these young patients. Despite the development of new regimens for ES and other soft tissue and bone sarcoma in recent years, DSRCT is underrepresented or were not included in the trials that lead to the drug approval[67]. As a result, the evidence to use second line therapy is very limited and restricted to case reports. It is paramount to develop active cooperative groups to quickly collect data and propose new strategies for treatment of DSRCT. Moreover, patients outside Europe and North America are almost never offered the opportunity in participating in clinical trials for rare disease. The SELNET (selnet-h2020.org), that is a Horizon2020/EU project, aims to create a network between European and Latin America countries to improve diagnosis and treatment of sarcomas and eventually to develop clinical trials in these centers across the Atlantic ocean.

4.3.1 The importance of pre-clinical models to drug development for rare sarcoma

The use of preclinical models is an important step in the development of new therapies for tumors in general and is crucial for rare tumors such as DSRCT. Due to the rarity of DSRCT, conducting clinical trials with new drugs is extremely difficult for many reasons [68]. First, the fact that genetic and functional comprehensive analyses of these tumors are limited. Second, the aggressive behavior and chemotherapy resistance and exclusion from target therapy clinical trials. Also, accrual of patients in clinical trials is difficult due to the limited number of individual affected yearly. As a result, the use of pre-clinical models is an important step in the developing of novel drugs since a higher number of mechanisms can be modulated and faster therapeutic targets can be explored. Modeling these tumors with experimental models allow the investigation of the molecular mechanisms that underlies tumor origin and progression. The fidelity of the model is also related to predictive capacity to anticipate eventual effects of drugs which will help to determine efficiency and efficacy of anti-tumoral drugs. In case of rare tumors, the possibility of scaling-up is crucial to translate technology from the bench to the bedside.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

In 2002, Nishio and collaborators [69] reported for the first time the development of the a DSRCT cell line, named JN-DSRCT-1, derived from pleural effusion from a 7-year-old patient with lung metastasis. JN-DSRCT-1 cells are small, round or spindle-shaped with oval nuclei and were maintained continuously in vitro for more than 190 passages for more than 40 months. The cell has tumorigenic capacity and the histology of heterotransplanted tumors in SCID mice maintains the characteristics of the original DSRCT, including the expression of immunohistochemical markers (vimentin, desmin, CD57, among others), a t(11; 22) translocation (p13; q12) and presence of EWS-WT1 fusion [69]. JN-DSRCT-1 cells are being used in a several studies that help to unveil DSRCT biology, specially the role of EWS-WT1 fusion protein and also to identify targets for therapeutic interventions[12,70]

As mentioned before, *EWS-WT1* translocation is the major driver in DSRCT and plays many roles in tumor biology that have the potential to be used as therapeutic targets. As already described in other studies, *EWS-WT1* gene underwent RNA splicing and one variant lacks three amino-acids and was named *EWS-WT1*(-KTS) due the absence of Lys-Thr-Ser residues [71]. This isoform activates a gene encoding a tetraspanin-family protein, T-cell acute lymphoblastic leukemia-associated antigen 1 (*TALLA-1*) [72]. *TALLA-1* is part of multi-protein family involved in several processes, such as cell adhesion, migration and metastasis and this gene could be a candidate for diagnostic marker and a putative target for therapy [72]. Another target for *EWS-WT1* fusion gene is *ENT4* (equilibrative nucleoside transporter 4) which encodes a pH-dependent adenosine transporter [73]. Neural genes induction is also triggered by *EWS-WT1* in JN-DSRCT-1 cells and neural reprogramming factor *ASCL1* is an important player in mediating multiple WT1-responsive elements, suggesting that neural differentiation pathway could be tested as therapeutic agents for DSRCT [74]. A recent work described the dependence of *EWS-WT1* in DSRCT survival [12]. Silencing EWS-WT1 causes proliferation loss, growth arrest and gene expression analysis indicates a repression of estrogen signaling and highlight therapeutic genetic vulnerabilities, such as *FGFR4, JAK3, mTOR, PDGF, ERG,* and TGFB1 genes [12]. Another study that evaluated potential therapeutic targets performed RNA sequencing of 12 tumor samples from pediatric patients with DSRCT found high expression of *IGF2, FGFR4, CD200* and *CD276*, the latter two molecules are candidates for immune checkpoint inhibitor therapy [75]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

In terms of therapy, the first use of JN-DSRCT-1 cells was to test de effect of rapamycin induced in inducing apoptotic death [76]. The mechanism involves the up-regulation of Bax concomitant Bcl-xL down-regulation. Rapamycin also down-regulates *EWS-WT1* and 26S p44.5 proteasome subunit, suggesting that rapamycin induces apoptosis by preventing the degradation of the Bax protein by the proteasome, and that this process is independent of mTOR inhibition. Furthermore, these results strongly support the introduction of the use of rapamycin as a cytotoxic agent for the treatment of DSRCT. JN-DSRCT-1 cells were tested to verify the effectiveness of the TRAIL receptor agonist (apoptosis inducer), called ONC201 [77]. In this study it was found that the induction of TRAIL decreases proliferation and induces apoptosis in vitro and decreases tumor growth in vivo. The potential of anti-angiogenic agents in decreasing tumor growth of the JN-DSRCT-1 cell was also investigated [78]. Animals with JN-DSRCT-1 cell xenografts were treated with bevacizumab and showed a prolongation of the time to progression and there were marked long-term regressions after treatment with the combination of irinotecan and bevacizumab compared to irinotecan alone [78]. Interestingly, there is recent evidence that indicates that the use of other anti-angiogenic agents may be effective in the treatment of DSRCT, as in the case report of a patient with advanced DSRCT, in a second line of treatment, refractory to cisplatin, was treated with apatinib (VEGFR-2 inhibitor drug) and had a positive response, with significant reduction in tumor mass [79]. JN-DSRCT-1 cells are sensitive to alkylating agent trabectedin and the mechanism of action involves the expression of genes involved with proliferation and apoptosis [80]. An alternative mechanism of action of trabectedin is the impairment of transactivation of *FUS-CHOP* fusion protein in liposarcoma [81]. This activity is also observed in *EWS-WT1* fusion protein, trabectedin reduces its binding on its target gene promoters and, thus, affects *EWS-WT1*-dependent gene expression in JN-DSRCT-1 cells [80]. Recently, the combination of PARP inhibitor olaparib with the alkylating agent temozolomide was tested in JN-DSRCT-1 cells *in vitro* and *in vivo* and the results indicates that the combination have synergistic effects upon cell viability, inducing cell cycle arrest which progress to apoptosis induction, causing tumor reduction [70].

Additionally to JN-DSRCT-1 cells, Markides and collaborators established more DSRCT cell lines, including BER lineage that also presents *EWS-WT1* fusion protein and have similar behavior of JN-DSRCT-1 [12,82]. Together, this evidence shows the importance of obtaining tumor

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

models to accelerate preclinical research, bring possibilities for investigating new therapeutic approaches for this rare but lethal malignancy.

4.3.2 Targeting angiogenesis and other TKR

As a hipervascular tumor, DSRCT is characterized by an overexpression of proteins that promote and maintain the angiogenic process necessary for continued tumor growth and proliferation. *EWS-WT1* is able to induce *PDGFA* expression [83] and activation of *IGF1R* gene [14] (figure 3). Other tyrosine kinase receptors (TKR) expression have been found to be disrupted in DSRCT and are related to proliferation and angiogensis. VEGFR-2 and VEGFA expression was found to be markedly increased in DSRCT tumor sample and in the human DSRCT cell line, JN-DSRCT [78]

The use of tyrosine kinase inhibitors (TKI) for VEGF, VEGFR, PDGFRα and other proteins involved in tumoral vascular proliferation has been explored in the clinical scenario. [79,84,85]. Pazopanib, apatinib and sunitinib inhibit angiogenesis by abrogating the VEGF-induced phosphorylation of VEGF receptors as well as other TKRs including PDGFR, FGFR, and c-KIT, affecting downstream activation of the PI3K/AKT, PKC, and other pathways that mediate cell proliferation, migration, and survival [86]

In the PALETTE study, 369 patients were randomized to receive pazopanib 800 mg/ day versus placebo [67]. Median PFS was 4.6 months (95%CI 3.7–4.8) for pazopanib compared with 1.6 months. A combined analysis of patients with diagnosis of DSRCT treated in the EORTC phase II study 62043 (3 patients), EORTC phase III 62072 (3 patients) and in a UK Pazopanib expanded access program (3 patients) was performed [84]. Data from nine patients included in this analysis revealed a median age of 30 years and all patients were males with widespread metastatic DSRCT. Four patients had one previous chemotherapy line (44%), four had 2 previous chemotherapy lines (44%) and one patient 3 (12%). The response rate was partial response (PR) in 2/9 (22%) patients, stable disease (SD) in 5/9 patients (56%) and progressive disease (PD) in 2/9 (22%) with a clinical benefit rate (PR + SD > 12 weeks) of 78%. Median PFS and OS were 9.2 (95%CI: 0–23.2) and 15.4 (95%CI: 1.5-29.3) months respectively [84]. In a relatively large, retrospective study with 29 patients treated with pazopanib, clinical benefit was observed in 62% (18/29) of patients with DSRCT (CR in 1 patient, PR in 1 patient, SD in 16 patients) and the median progression –free survival was 5,4 months [87].

 doi:10.20944/preprints202012.0288.v1

Sunitinib was one of the first generation of TKIs with great inhibition of VEGF receptor 2 among other TKRs. In vascular sarcomas, sunitinib showed early and promising activity in alveolar sarcoma, a chemo-resistant subtype of sarcoma [88]. In a retrospective analysis of patients with DSRCT, sunitinib showed clinical benefit in 8 patients evaluated [89]. Partial response was observed in 2 patients and SD in 3 patients treated in second and beyond line of therapy. Sorafenib was used in 2 patients[85], both in fifth line of therapy. The best response to sorafenib was stable disease and the median progression free survival of of 3.5 and 4 months. On the other hand, as first line treatment, apatinib was used in only one patient[79]. Apatinib is another VEGFR-2 inhibitor with demonstrated activity in gastric and other tumors [90]. Clinical benefit and tumor shrinkage was reported in one patient treated with apatinib in first line. The patient had not received chemotherapy previously. [79]. Another report showed partial response with apatinib in combination with chemotherapy as second line treatment [91] Ramucirumab, a VEGFR inhibitor is been tested in combination with cyclophosfomide and vinblastine in patients with relapsed and refractory DSRCT (NCT04145349)

Bevacizumab, a VEGF-A inhibitor was combined with irinotecan and temozolamide (ITB regimen) in the first line treatment of DSRCT. In this single arm pilot study, 14 out of 15 patients completed the planned treatment that comprised 2 cycles of ITB followed by the conventional trial P6 with VAC and IE. The response rate to ITB was 27% and no major unexpected adverse event was observed[92].

Most of the trials with novel agents are designed to treat a myriad of histologies, including DSRCT. It is difficult to identify trials accruing only patients with this disease. We provided a summary with selected ongoing trials accruing patients with, but not limited to DSRCT are it is displayed in table 2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

Phase of trial	Design	Primary outcome	ClinicalTrials.gov Identifier
Phase 1/2	Ramucirumab IV + Cyclophosphamide p.o. + Vinorelbine IV (experimental arm), *versus* Cyclophosphamide p.o. + Vinorelbine IV	1. Progression Free Survival	NCT04145349 (a)
Phase 1	2 cycles of the investigational combination irinotecan, temozolomide and bevacizumab, will be given followed by conventional chemotherapy with a modified P6 approach and surgical local control. Completion of modified P6 chemotherapy will be followed by a second-look surgery.	1. Tolerability 2. Adverse event profile	NCT01189643 (b)
Phase 2	Experimental arm A: Single dose of IP RIT administered through an IP catheter with 131 I-omburtamab at 80mCi/m2, followed by WA-IMRT approximately 2-4 weeks after completing IP RIT Experimental arm B: Single dose of IP RIT administered through an IP catheter with 131 I-omburtamab at 80mCi/m2 Experimental arm C: Single dose of IP RIT administered through an IP catheter with 131 I-omburtamab at 80mCi/m2	1. Progression Free Survival	NCT04022213 (c)
Phase 1/2	Dose Escalation/Dose Expansion Study of Prexasertib in Combination With Irinotecan 15 mg/m2 IV daily x 10 days in 21 day cycles	1. Recommended phase II does of Prexasertib 2. Response	NCT04095221 (d)
Phase 2	Nab-paclitaxel) will be administered as follows: Age ≥ 21: 125 mg/m2 days 1, 8 and 15 in cycles of 28 days Age ≥ 6 months and ≤ 20 years: 240 mg/m2 (for patients weighing > 10 kg) and 11.5 mg/kg (for patients weighing ≤ 10 kg) on days 1, 8 and 15 in cycles of 28 days	1. Overall response rate 2. Objective response rate	NCT03275818 (e)
Phase 2	Participants will receive vincristine, doxorubicin, cyclophosphamide, ifosfamide, etoposide, irinotecan, temozolomide, temsirolimus, bevacizumab, and sorafenib. Depending on the size and location of the participant's tumor, they will have surgery alone, radiation alone or surgery followed by radiation.	Participantes with DSRCT will not be included in the analysis of primary outcome	NCT01946529 (f)
Phase 2	Allogeneic Hematopoietic Stem Cell Transplantation	1. Transplant-related mortality	NCT04530487 (g)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

		2. Rate of grade III or higher organ toxicity attributable to conditioning	
Phase 1	Patients undergo cytoreduction and HIPEC over 60 minutes consisting of doxorubicin and cisplatin. Patients then receive sodium thiosulfate IV over 12 hours.	1. To assess the feasibility of HIPEC with doxorubicin and cisplatin after surgical resection. 2. To assess morbidity, hospital length of stay and peri-operative mortality outcome.	NCT04213794 (h)
Phase 1	Experimental arm A: participants will receive B7H3-specific CAR T cells only Experimental arm B: participants will receive CAR T cells directed at B7H3 and CD19	1. Safety and tolerability 2. Determine the MTD 3. Assess the DLT and describe the full toxicity profile 4. Assess the feasibility of manufacturing B7H3 and B7H3xCD19 specific CARs	NCT04483778 (i)
Phase 1	Experimental arm A: participants will receive EGFR-specific CAR T cells only. Experimental arm B: participants will receive CAR T cells directed at EGFR and CD19	1. Estimate the MTD and DLT 2. Assess the number of successfully manufactured EGFR806 and EGFR806xCD19 CAR T cell products 3. Safety	NCT03618381 (j)
Phase 2	Nivolumab 240 mg IV every 2 weeks plus Ipilimumab 1 mg/m2 IV every 6 weeks	1. Response to therapy as evaluated by RECIST 1.1	NCT02982486 (k)
Phase 2	Reduced intensity chemotherapy, haploidentical bone marrow, post-transplant cyclophosphamide and shortened duration tacrolimus	1. Safety	NCT01804634 (l)
Phase 1	CLR 131 intravenous administration	1. Number of participants with DLT	NCT03478462 (m)

Table 2 - Selected trials including desmoplastic small round cell tumor patients

4.3.3 Targeting Androgen Receptor Pathway

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

The increased prevalence of DSRCT in young males motivated the investigation of testosterone synthesis pathway in tumorigenesis of this disease. In 2007, Fine *et al* first demonstrated AR expression in DSRCT[93]. In a cohort of 27 heavily pretreated patients, 37% stained positive for AR. The functionality of the pathway was demonstrated by *in vitro* assay that showed growth of tumor cells when stimulated by di-hydro-testosterone, and inhibition of growth by flutamide[93]. Another study performing single sample gene set enrichment analysis found that majority of DSRCTs was enriched for the AR signature when compared to other sarcomas, such as ES and alveolar rhabdomyosarcoma[3,94].

In the previous reported Fine *et al* study[93], six patients with AR-positive DSRCT received combined androgen blockade (CAB) with bicalutamide and leuprorelin. Three patients had clinical tumor benefit for a period lasting 3 to 4 months. All them had normal testosterone levels at the initiation of CAB therapy, while the other three non-responders had castrate levels. In other report, a patient with strong AR expression received anti-androgen therapy with bicalutamide, presenting progressive disease 2 months later[95]. Negri *et al*, using whole genome gene expression profiling and a cancer stem cell gene array, showed that AR-positive DSRCT cells harbor characterеristics of stemness, which could explain the limited effectiveness of targeting this pathway[83].

4.3.4 Targeting PI3K/AKT/mTOR Pathway

Activation of the phosphatidylinositol-3-kinase (PI3K)–protein kinase B (Akt)–mammalian target of rapamycin (mTOR) pathway is proposed to be implicated in the development of a variety of sarcomas [96][96],[97],[98]. Also, there is emerging data indicating possible involvement of mTOR pathway in DSRCT. A single center small study attempted to evaluate the morphoproteomic profiling of the mTOR pathway in DSRCT, ES and Wilms´ tumor, and showed that the PI3K/Akt/mTOR pathway is constitutively activated in DSRCT[99]. Another study described a patient with DSRCT harboring secondary somatic mutation in the *PIK3CA* gene[100].

In vitro study demonstrated that rapamycin, a mTOR inhibitor, induced the apoptotic death of DSRCT line cells[76]. There are few data on clinical efficacy of inhibition of PI3K/AKT/mTOR pathway. In one case report, a 21-year-old man with DSRCT achieved stable disease with temsirolimus for 40 weeks[95]. Tarek *et al* reported their experience with five patients with relapsed

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

DSRCT treated with vinorelbine, cyclophosphamide and temsirolimus, all of them presenting partial response, with median time to progression of 8.5 months (range 7 – 16 months)[101]. A phase I trial evaluated the combination of cixutumumab (an IGFR antibody) with temsirolimus, which resulted in stable disease lasting longer than 5 months in two of the three patients with DSRCT of the study[102]. In a retrospective series of patients with high grade STS treated with pazopanib plus sirolimus following progression on pazopanib, one patient with DSRCT had stable disease for 11 months with the combination treatment[103]. Recently, a trial was designed to estimate the response rate to two initial courses of temsirolimus, temozolomide and irinotecan (window therapy) in previously untreated patients with high-risk ES family of tumors, including DSRCT (ClinicalTrials.gov, identifier: NCT01946529). Interim analysis determined the window therapy did not meet the anticipated response, and trial accrual was stopped.

4.3.5 Targeting DNA damage repair (DDR) proteins

Studies using next-generation sequencing characterized a subgroup of DSRCT with secondary genomic alterations in genes associated with DNA damage repair (DDR), including *ATM*, *RAD50*, *BARD1*, *BRCA1/2*, *PALB2* and *CHEK2* [19,104]. It is still unknown if those genomic alterations act as driver mutations in DSRCT tumorigenesis[104].

Since poly(ADP-ribose) polymerases (PARPs) perform an important role in DDR, specifically in the base excision repair of single-strand DNA breaks, PARP inhibitors recently emerged as new treatment for cancer based on synthetic lethality concept, particularly in *BRCA*-mutant cancers defective in homologous repair[105,106]. DSRCT has high level of PARP1, the most abundant enzyme of PARP family, and combination of olaparib and temozolamide has demonstrated enhanced antitumor effects *in vitro* and *in vivo*[70,107,108].

It was described that *EWS-FLI1* in ES and *EWS-WT1* in DSRCT might share common mechanisms of gene expression de-regulation[100]. EWS-WT1 up-regulates the expression of *ERG*, an ETS family member of FLI1. It is possible that *ERG* may drive the expression of these targets, making this tumor an ETS-like tumor [109]. Another characteristic observed *in vitro* is that the tumor modulates the DNA damage response, both suppressing p53 signaling and driving the expression of gene sets associated with the DNA damage response, suggesting a direct link to the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

resistance to chemotherapy in DSRCT[12]. Deregulation of DNA damage response is another important feature of the other FET family fusions such as *EWS-FLI1* in ES. This characteristic makes the use of PARP inhibitor an attractive therapeutic strategy in both ES and DSRCT. The combination trabectedin and PARP inhibitor is under investigation in clinical trials. Trabectedin is a intercalating DNA agent that promotes DNA damage and deregulate several repair pathways, inhibits transcriptor factors such as *FUS-CHOP* factor a exert cytotoxic activity in certain subtypes of cells such as the tumor associated macrophages (TAM), myxoid liposarcoma and ES [110,111]. The combination of trabectedin and olaparib has shown robust inhibition of tumor proliferation in sarcoma mouse models [112].The heavy damage in the single strand or double strand DNA was not repaired by olaparib in experimental model[112]. An phase Ib trial (TOMAS) conducted by the Italian Sarcoma Group was designed to explore the synergistic effect of trabectedine and olaparib in patients with advanced sarcoma and showed promising results[113]. Out of 50 accrued, 11 had diagnosis of bone tumor and of these only 4 with ES and no activity was observed in this group of patients, despite the biological rational for this combination in ES. The efficacy of this combination is under evaluation by the phase II trial TOMAS2 (NCT03838744).

In an ongoing trial, prexarsetinib, an inhibitor of checkpoint kinase 1 (chk1) in combination with irinotecan and temozolamide is currently under evaluation in an early phase trial (NCT0409522).

4.3.6 Targeting c-MET and Insulin Growth Factor pathway

c-Met (mesenchymal-epithelial transition factor) has been found to be overexpressed in a variety of solid tumors, including sarcomas[114–117], but the involvement of this receptor in DSRCT development is still scarce [100] . In the largest DSRCT comprehensive genomic profile study, no secondary mutation on *c-MET* was found[104]. There is a case report of a patient with intra-abdominal DSRCT who received anlotinib, a multi-kinase inhibitor that targets c-Met, for progressive disease after surgery and first line chemotherapy showing stable disease for 4 months[118].

The oncogenic fusion product EWSR1-WT1 in DSRCT was reported to activate the IGF-1R gene promoter, providing the basis to test the activity of anti-IGF-1R antibodies in the metastatic

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

setting[119]. Also, IGF2 has been up-regulated by the fusion product and is a potential target [75]. In a phase II study, ganitumab administered to 16 metastatic DSRCT patients determined one PR (6%) and 10 (63%) SD, with a median PFS of 15 months [120]

4.3.7 Vaccines

Although initial studies on cancer vaccines had shown disappointing results with low response rates, better understanding of interaction between tumor, microenvironment, and immune system on last decades have opened new perspectives for this therapy, including in sarcoma field[121,122]. Cancer vaccines seek to induce tumor immune responses through antigen presentation and stimulation of new T cell responses[121]. Few studies have explored vaccines in DSRCT treatment. A phase I study using a vaccine of tumor lysate-pulsed autologous dendritic cells in treatment of pediatric patients with solid tumors demonstrated feasibility for generating specific T-cell responses and regression or stabilization of metastatic disease in some patients, but failed to prevent progressive disease in the patient with DSRCT included in the trial[123]. A more recent trial evaluated efficacy of an adjuvant dendritic cell vaccine administered three to eight weeks after completion of standard treatment in pediatric patients with high-risk sarcomas[124]. Survival advantage was demonstrated for patients with ES and rhabdomyosarcoma, but no clinical benefit was seen in the two patients with DSRCT in the study[124].

4.3.8 Perspectives with novel targets (immune checkpoint and NTRK inhibitors)

In general, sarcomas are not considered good candidates for immune therapy the way this therapy has currently been applied for other tumors [125]. Around one third of sarcomas are characterized by single gene translocation that acts as a driver mutation. Moreover, sarcomas are amongst the neoplasms with lowest tumor mutational burden, a recognized predictor of response to immune check point inhibitors (IO)[126].

Initial data using the combination of anti-PD1 +/- anti-CTLA-4 have shown promising results, particularly in alveolar soft part sarcoma (ASPS), undifferentiated pleomorphic sarcoma (UPS) and dedifferentiated lipossarcoma [127,128]. The ALLIANCE trial demonstrated that nivolumab monotherapy is not effective with only 5% of objective response rate against 16% for the combination of nivolumab and ipilimumab [128]. In SARC28 trial, pembrolizumab induced objective

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 December 2020 doi:10.20944/preprints202012.0288.v1

response rate in 5 UPS patients [127] and a *post hoc* analysis of this trial demonstrated that patients with a specific sarcoma immunological classification (SIC) based on gene expression analysis derived the greatest benefit from pembrolizumab. This immune "high or hot" signature was associated with the structure of T lymphocyte, follicular dendritic cells and enriched B cells [129]. The combination of immune check point inhibitor and TKIs was evaluated and showed safe profile and promising activity in a phase II trial with predominant ASPS patients [130]. Out of 11 patients with ASPS, 6 achieved a partial response (54·5%, 95% CI 24·6–81·9), and two (18%) of 11 achieving stable disease with a median PFS of 12 months[130].

It is difficult to predict that IO will be active in DSRCT based on preliminary data about immune regulation and biomarkers expression in this rare disease. Gene expression analysis revealed that DSRCT is characterized by a signature of immunological ignorance[131]. However, a study with samples of paraffin embed tumor sample showed elevated PD1 expression by tumor cells [132]. The expression of PD1, PDL-1, and CD8 was analyzed in a cohort of 11 patients with DSRCT and it was observed a high rate of PD1 (81%), CD8 (64%) and low PDL-1 (18%) expression[132]. Additionally, *in vitro* assay using the JN-DSRCT-1 cells culture to test the activity of nivolumab showed no effect in decreasing tumor cell proliferation. It is in line with the most recent data that demonstrated the single agent nivolumab was ineffective for most sarcoma subtype[128]. On the other hand, the combination of an anti-PD1 and anti-CTLA4 (ipilimumab) resulted in better outcome[133]. However, preclinical data using gene expression analysis showed that DSRCT overexpress immune regulatory proteins such as CD200 and CD276 (B7H3), which is not regulated by the EWS-WT1 fusion protein[75]. Currently, enoblituzumab is being tested in many solid tumors (NCT02982941), including pediatric patients with DRSCR, the results of this trial is pending. It has previously described that DSRCT shows high expression of CD276 (B7H3) [75] and it was the basis for a phase I trial designed to evaluate the safety, pharmacokinetics and bio-distribution of intra-peritoneal radio-immunotherapy with a monoclonal antibody anti-B7H3 ^{131}I-omburtamab in patients with DSRCT or other B7H3 positive neoplasm with peritoneal involvement. The results with 48 treated patients with DSRCT showed this approach is tolerable and the maximum tolerated dose was not reached[134]. A phase II trial was advocated based on these results.

 doi:10.20944/preprints202012.0288.v1

The neurotrophic tyrosine kinase receptor (NTKR) fusions act as driver mutation is a myriad of neoplasms. The use of NTRK inhibitors has demonstrated robust activity across many histologies[135]. Sarcoma was one of the most common tumor type included in the larotrectinib trials [136] and the search for NTRK fusion in sarcoma patients are recommended based on a priority criteria [135]. Recently, it was demonstrated that *EWS-WT1* promotes direct NTRK3 transcription and in vivo and in vitro inhibition of NTRK3 cells with entrectinib resulted in significant decrease in cell growth. This finding opens the possibility for future trials with NTRK inhibitor in DSRCT[137].

5. Conclusion

In summary, DSRCT is a rare and aggressive disease and fatal for the majority of the patients. A modest improvement in the survival has been observed in more recent studies. For second line treatment, there is no standard of treatment and the results with conventional chemotherapy and novel agents are disappointing. The better understanding of the disease biology has identified potential targets to be explored in future clinical trials. It is paramount the work of cooperative group to organize prospective databanks and conduct clinical trials.

Conflicts of Interest: CAM declares advisory board and speaker fee from MSD, Roche, Bayer, Lilly, Servier, Merck Serono, Amgen. Clinical Trial honoraria from BMS, Servier, Roche.

Author Contributions

Conceptualization, CAM, FAC, TGS; methodology, writing—original draft preparation, CAM, TGS, FAC, MLGS.; writing—review and editing, CAM, GTT, TGS, FAC, AGN, AL, SAJ, CS, MPC.; supervision, CAM. All authors have read and agreed to the published version of the manuscript.

Funding

This study was supported by grants from National Institute of Science and Technology in Oncogenomics and Therapeutic Innovation (INCITO) funded by São Paulo Research Foundation (FAPESP – grant number 2014/50943-1), National Council for Scientific and Technological Development (CNPq – grant number 465682/2014-6) and Coordination for the Improvement of Higher Education Personnel (CAPES - 88887.136405/2017-00). TGS is supported by FAPESP grant (2018/25541-8) and GTT is supported by FAPESP grant (2018/06269-5).

ACCCC is member of the SELNET consortium, sponsored by a Horizon2020/EU (EU proposal 825806)

References

1. Gerald W et al. Case 2 Desmoplastic small cell tumor with divergent differentiation. Pediatr Pathol. 1989;9(2):177–83.
2. Dufresne A et al. Desmoplastic small round cell tumor: Current management and recent findings. Sarcoma. 2012;2012:1–5.
3. Bulbul A, Fahy BN, Xiu J, Rashad S, Mustafa A, Husain H, et al. Desmoplastic Small Round Blue Cell Tumor: A Review of Treatment and Potential Therapeutic Genomic Alterations. Sarcoma. 2017;2017.
4. Hayes-Jordan A et al. Management of Desmoplastic Small Round Cell Tumor. Semin Pediatr Surg. 2016;25(5):299–304.
5. Honoré C, Delhorme JB, Nassif E, Faron M, Ferron G, Bompas E, et al. Can we cure patients with abdominal Desmoplastic Small Round Cell Tumor? Results of a retrospective multicentric study on 100 patients. Surg Oncol. 2019 Jun;29:107–12.
6. US Food and Drug Administration. Orphan Drug Act—Relevant Excerpts.
7. Greenlee RT, Goodman MT, Lynch CF, Platz CE, Havener LA, Howe HL. The

occurrence of rare cancers in U.S. adults, 1995-2004. Public Health Rep. 2010;125(1):28–43.

8. Mallone S, De Angelis R, van der Zwan JM, Trama A, Siesling S, Gatta G, et al. Methodological aspects of estimating rare cancer prevalence in Europe: the experience of the RARECARE project. Cancer Epidemiol. 2013 Dec;37(6):850–6.
9. Lettieri CK et al. Incidence and Outcomes of Desmoplastic Small Round Cell Tumor: Results from the Surveillance, Epidemiology, and End Results Database. J Cancer Epidemiol. 2014;2014:1–5.
10. Sawyer JR et al. A Novel Reciprocal Chromosome Translocation t(11;22)(p13;q12) in an Intraabdominal Desmoplastic Small Round-Cell Tumor. Am J Surg Pathol. 1992;16(4):411–6.
11. Rodriguez E et al. A Recurring Translocation, t(11;22)(p13;q11.2), Characterizes Intra-Abdominal Desmoplastic Small Round-Cell Tumors. Cancer Genet Cytogent. 1993;21:17–21.
12. Gedminas JM, Chasse MH, McBrairty M, Beddows I, Kitchen-Goosen SM, Grohar PJ. Desmoplastic small round cell tumor is dependent on the EWS-WT1 transcription factor. Oncogenesis [Internet]. 2020;9(4):1–8. Available from: http://dx.doi.org/10.1038/s41389-020-0224-1
13. Scharnhorst V, van der Eb AJ, Jochemsen AG. WT1 proteins: functions in growth and differentiation. Gene. 2001 Aug;273(2):141–61.
14. Lee SB, Kolquist KA, Nichols K, Englert C, Maheswaran S, Ladanyi M, et al. The EWS-WT1 translocation product induces PDGFA in desmoplastic small round-cell tumour. Nat Genet. 1997 Nov;17(3):309–13.
15. Liu J et al. Molecular heterogeneity and function of EWS-WT1 fusion transcripts in desmoplastic small round cell tumors. Clin Cancer Res. 2000;6(9):3522–9.
16. Vignaud JM, Marie B, Klein N, Plénat F, Pech M, Borrelly J, et al. The role of platelet-derived growth factor production by tumor-associated macrophages in tumor stroma formation in lung cancer. Cancer Res [Internet]. 1994 Oct 15;54(20):5455–63. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/7923179

17. Ferreira EN, Barros BDF, De Souza JE, Almeida RV, Torrezan GT, Garcia S, et al. A genomic case study of desmoplastic small round cell tumor: comprehensive analysis reveals insights into potential therapeutic targets and development of a monitoring tool for a rare and aggressive disease. Hum Genomics. 2016;10(1).
18. Santiago L, Daniels G, Wang D, Deng F-M, Lee P. Wnt signaling pathway protein LEF1 in cancer, as a biomarker for prognosis and a target for treatment. Am J Cancer Res. 2017;7(6):1389–406.
19. Devecchi A, De Cecco L, Dugo M, Penso D, Dagrada G, Brich S, et al. The genomics of desmoplastic small round cell tumor reveals the deregulation of genes related to DNA damage response, epithelial-mesenchymal transition, and immune response 06 Biological Sciences 0604 Genetics. Cancer Commun [Internet]. 2018;38(1):1–14. Available from: https://doi.org/10.1186/s40880-018-0339-3
20. Xiu J, Bulbul A, Rashad S. Potential therapeutic genomic alterations in desmoplastic small round blue cell tumor. J Clin Oncol [Internet]. 2017 May 20;35(15_suppl):11066. Available from: https://doi.org/10.1200/JCO.2017.35.15_suppl.11066
21. Lal DR et al. Results of multimodal treatment for desmoplastic small round cell tumors. J Pediatr Surg. 2005;40(1):251–5.
22. Stiles ZE, Dickson P V, Glazer ES, Murphy AJ, Davidoff AM, Behrman SW, et al. Desmoplastic small round cell tumor: A nationwide study of a rare sarcoma. J Surg Oncol. 2018 Jun;117(8):1759–67.
23. Al-Ibraheemi A et al. Desmoplastic Small Round Cell Tumors With Atypical Presentations: A Report of 34 Cases. Int J Surg Pathol. 2018;00(0):1–8.
24. Thondam SK et al. Intracranial desmoplastic small round cell tumor presenting as a suprasellar mass. J Neurosurg. 2015;122:773–7.
25. Faras F et al. Primary desmoplastic small round cell tumor of upper cervical lymph nodes. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;120(1):4–10.
26. Xu J et al. Desmoplastic small round cell tumor of the middle ear. Medicine

(Baltimore). 2018;97(17):1–5.

27. Nakayama J et al. Desmoplastic small round cell tumor of the ovary: A rare but devastating disease in young women. Gynecol Oncol Reports. 2014;7:16–8.
28. Lettieri CK, Garcia-Filion P, Hingorani P. Incidence and Outcomes of Desmoplastic Small Round Cell Tumor: Results from the Surveillance, Epidemiology, and End Results Database. J Cancer Epidemiol. 2014;2014.
29. Honoré C et al. Abdominal Desmoplastic Small Round Cell Tumor: Multimodal Treatment Combining Chemotherapy, Surgery, and Radiotherapy is the Best Option. Ann Surg Oncol. 2015;22(4):1073–9.
30. Gani F, Goel U, Canner JK, Meyer CF, Johnston FM. A national analysis of patterns of care and outcomes for adults diagnosed with desmoplastic small round cell tumors in the United States. J Surg Oncol. 2019;119(7):880–6.
31. Stiles ZE et al. Desmoplastic small round cell tumor: A nationwide study of a rare sarcoma. J Surg Oncol. 2018;117(8):1759–67.
32. Gani F, Goel U, Canner JK, Meyer CF, Johnston FM. A national analysis of patterns of care and outcomes for adults diagnosed with desmoplastic small round cell tumors in the United States. J Surg Oncol. 2019 Jun;119(7):880–6.
33. Hayes-Jordan A et al. The diagnosis and management of desmoplastic small round cell tumor: a review. Curr Opin Oncol. 2011;23(4):385–9.
34. Bellah R et al. Desmoplastic Small Round Cell Tumor in the Abdomen and Pelvis: Report of CT Findings in 11 Affected Children and Young Adults. Am J Roentgenol. 2013;184(6):1910–4.
35. Arora VC et al. Characteristic imaging features of desmoplastic small round cell tumour. Pediatr Radiol. 2013;43(1):93–102.
36. Pickhardt PJ et al. Desmoplastic small round cell tumor of the abdomen: radiologic-histopathologic correlation. Radiology. 1999;210(3):633–8.
37. Kis B et al. Imaging of desmoplastic small round cell tumour in adults. Br J Radiol. 2012;85(1010):187–92.
38. Zhang W et al. CT, MRI, and FDG-PET/CT imaging findings of abdominopelvic

 doi:10.20944/preprints202012.0288.v1

desmoplastic small round cell tumors: Correlation with histopathologic findings. Eur J Radiol. 2011;80(2):269–73.

39. Goadsby PJ, Kurth T, Pressman A. FDG PET/CT Imaging of Desmoplastic Small Round Cell Tumor: Findings at Staging, During Treatment and at Follow Up. 2016;35(14):1252–60.

40. Hayes-Jordan A et al. Novel treatment for desmoplastic small round cell tumor: hyperthermic intraperitoneal perfusion. J Pediatr Surg. 2010;45(5):1000–6.

41. Lae ME et al. Desmoplastic Small Round Cell Tumor: A Clinicopathologic, Immunohistochemical, and Molecular Study of 32 Tumors. Am J Surg Pathol. 2002;26(7).

42. Ordóñez NG. Desmoplastic Small Round Cell Tumor: II: An Ultrastructural and Immunohistochemical Study with Emphasis on New Immunohistochemical Markers. Am J Surg Pathol. 1998;22(11).

43. Gerald WL, Ladanyi M, De Alava E, Cuatrecasas M, Kushner BH, LaQuaglia MP, et al. Clinical, pathologic, and molecular spectrum of tumors associated with t(11;22)(p13;q12): Desmoplastic small round-cell tumor and its variants. J Clin Oncol. 1998;16(9):3028–36.

44. Thway K et al. Desmoplastic Small Round Cell Tumor: Pathology, Genetics, and Potential Therapeutic Strategies. Int J Surg Pathol. 2016;24(8):672–84.

45. Machado I, Navarro L, Pellin A, Navarro S, Agaimy A, Tardío JC, et al. Defining Ewing and Ewing-like small round cell tumors (SRCT): The need for molecular techniques in their categorization and differential diagnosis. A study of 200 cases. Ann Diagn Pathol. 2016 Jun;22:25–32.

46. Izquierdo FJ, Plana A, Schuitevoerder D, Hayes-Jordan A, Deneve JL, Al-Kasspooles M, et al. The Chicago Consensus on peritoneal surface malignancies: Management of desmoplastic small round cell tumor, breast, and gastrointestinal stromal tumors. Cancer. 2020;126(11):2566–70.

47. Hassan I, Shyyan R, Donohue JH, Edmonson JH, Gunderson LL, Moir CR, et al. Intraabdominal desmoplastic small round cell tumors: a diagnostic and therapeutic

challenge. Cancer. 2005 Sep;104(6):1264–70.

48. Wong HH et al. Desmoplastic small round cell tumour: characteristics and prognostic factors of 41 patients and review of the literature. Clin Sarcoma Res. 2013;3(1):1–9.

49. Subbiah V et al. Multimodality treatment of desmoplastic small round cell tumor: Chemotherapy and complete cytoreductive surgery improve patient survival. Clin Cancer Res. 2018;24(19):4865–73.

50. Farhat F et al. Desmoplastic small round cell tumors: Results of a four-drug chemotherapy regimen in five adult patients. Cancer. 1996;77(7):1363–6.

51. Kushner BH et al. Desmoplastic small round-cell tumor: Prolonged progression-free survival with aggressive multimodality therapy. J Clin Oncol. 1996;14(5):1526–31.

52. Bertuzzi A et al. High-dose chemotherapy in poor-prognosis adult small round-cell tumors: Clinical and molecular results from a prospective study. J Clin Oncol. 2002;20(8):2181–8.

53. Koscielniak E, Kosztyla D, Dantonello T et al. No Title. Pediatr Blood Cancer. 2013;60(S3):32.

54. Scheer M, Vokuhl C, Blank B, Hallmen E, von Kalle T, Münter M, et al. Desmoplastic small round cell tumors: Multimodality treatment and new risk factors. Cancer Med. 2019;8(2):527–42.

55. Liu KX, Collins NB, Greenzang KA, Furutani E, Campbell K, Groves A, et al. The use of interval-compressed chemotherapy with the addition of vincristine, irinotecan, and temozolomide for pediatric patients with newly diagnosed desmoplastic small round cell tumor. Pediatr Blood Cancer [Internet]. 2020 Oct 19;67(10). Available from: https://onlinelibrary.wiley.com/doi/10.1002/pbc.28559

56. Atallah V, Honore C, Orbach D, Helfre S, Ducassou A, Thomas L, et al. Role of adjuvant radiation therapy after surgery for abdominal desmoplastic small round cell tumors. Int J Radiat Oncol Biol Phys. 2016;95(4):1244–53.

57. Goodman KA, Wolden SL, La Quaglia MP, Kushner BH. Whole abdominopelvic

radiotherapy for desmoplastic small round-cell tumor. Int J Radiat Oncol Biol Phys. 2002 Sep;54(1):170–6.

58. Desai NB et al. Reduced toxicity with intensity modulated radiation therapy (IMRT) for desmoplastic small round cell tumor (DSRCT): An update on the whole abdominopelvic radiation therapy (WAP-RT) experience. Int J Radiat Oncol Biol Phys. 2013;85(1):e67-72.

59. Hayes-Jordan AA et al. Desmoplastic Small Round Cell Tumor Treated with Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy: Results of a Phase 2 Trial. Ann Surg Oncol. 2018;25(4):872–7.

60. Hayes-Jordan A et al. Complete cytoreduction and HIPEC improves survival in desmoplastic small round cell tumor. Ann Surg Oncol. 2014;21(1):220–4.

61. Osborne EM, Briere TM, Hayes-Jordan A, Levy LB, Huh WW, Mahajan A, et al. Survival and toxicity following sequential multimodality treatment including whole abdominopelvic radiotherapy for patients with desmoplastic small round cell tumor. Radiother Oncol. 2016;119(1):40–4.

62. Campos F, Coutinho DL, Silva MLG, Lopes A, Nascimento A, Aguiar Júnior S, et al. Clinical Characteristics, Management, and Outcomes of 19 Nonpediatric Patients with Desmoplastic Small Round Cell Tumor: A Cohort of Brazilian Patients. Honoki K, editor. Sarcoma [Internet]. 2020;2020:8713165. Available from: https://doi.org/10.1155/2020/8713165

63. Lal DR, Su WT, Wolden SL, Loh KC, Modak S, La Quaglia MP. Results of multimodal treatment for desmoplastic small round cell tumors. J Pediatr Surg [Internet]. 2005 Jan;40(1):251–5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022346804006499

64. Forlenza CJ, Kushner BH, Kernan N, Boulad F, Magnan H, Wexler L, et al. Myeloablative Chemotherapy with Autologous Stem Cell Transplant for Desmoplastic Small Round Cell Tumor. Sarcoma [Internet]. 2015;2015:1–9. Available from: http://www.hindawi.com/journals/sarcoma/2015/269197/

65. Honoré C, Atallah V, Mir O, Orbach D, Ferron G, LePéchoux C, et al. Abdominal

desmoplastic small round cell tumor without extraperitoneal metastases: Is there a benefit for HIPEC after macroscopically complete cytoreductive surgery? PLoS One. 2017;12(2):1–12.

66. Subbiah V, Lamhamedi-Cherradi SE, Cuglievan B, Menegaz BA, Camacho P, Huh W, et al. Multimodality treatment of desmoplastic small round cell tumor: Chemotherapy and complete cytoreductive surgery improve patient survival. Clin Cancer Res. 2018;24(19):4865–73.

67. van der Graaf WTA, Blay J-Y, Chawla SP, Kim D-W, Bui-Nguyen B, Casali PG, et al. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet (London, England). 2012 May;379(9829):1879–86.

68. Loktev A, Shipley JM. Desmoplastic small round cell tumor (DSRCT): emerging therapeutic targets and future directions for potential therapies. Expert Opin Ther Targets [Internet]. 2020;24(4):281–5. Available from: https://doi.org/10.1080/14728222.2020.1738392

69. Nishio J, Iwasaki H, Ishiguro M, Ohjimi Y, Fujita C, Yanai F, et al. Establishment and characterization of a novel human desmoplastic small round cell tumor cell line, JN-DSRCT-1. Lab Invest. 2002 Sep;82(9):1175–82.

70. van Erp AEM, van Houdt L, Hillebrandt-Roeffen MHS, van Bree NFHN, Flucke UE, Mentzel T, et al. Olaparib and temozolomide in desmoplastic small round cell tumors: a promising combination in vitro and in vivo. J Cancer Res Clin Oncol [Internet]. 2020; Available from: https://doi.org/10.1007/s00432-020-03211-z

71. Gerald WL, Rosai J, Ladanyi M. Characterization of the genomic breakpoint and chimeric transcripts in the EWS-WT1 gene fusion of desmoplastic small round cell tumor. Proc Natl Acad Sci. 2006;92(4):1028–32.

72. Ito E, Honma R, Imai J, Azuma S, Kanno T, Mori S, et al. A Tetraspanin-Family Protein, T-Cell Acute Lymphoblastic Leukemia-Associated Antigen 1, Is Induced by the Ewing's Sarcoma-Wilms' Tumor 1 Fusion Protein of Desmoplastic Small Round-Cell Tumor. Am J Pathol. 2003 Dec;163(6):2165–72.

73. Li H, Smolen GA, Beers LF, Xia L, Gerald W, Wang J, et al. Adenosine Transporter ENT4 Is a Direct Target of EWS/WT1 Translocation Product and Is Highly Expressed in Desmoplastic Small Round Cell Tumor. Jin D-Y, editor. PLoS One. 2008 Jun;3(6):e2353.

74. Kang H-J, Park JH, Chen W, Kang SI, Moroz K, Ladanyi M, et al. EWS-WT1 Oncoprotein Activates Neuronal Reprogramming Factor ASCL1 and Promotes Neural Differentiation. Cancer Res. 2014 Aug;74(16):4526–35.

75. Hingorani P, Dinu V, Zhang X, Lei H, Shern JF, Park J, et al. Transcriptome analysis of desmoplastic small round cell tumors identifies actionable therapeutic targets: a report from the Children's Oncology Group. Sci Rep [Internet]. 2020;10(1):1–12. Available from: https://doi.org/10.1038/s41598-020-69015-w

76. Tirado OM, Mateo-Lozano S, Notario V. Rapamycin induces apoptosis of JN-DSRCT-1 cells by increasing the Bax : Bcl-xL ratio through concurrent mechanisms dependent and independent of its mTOR inhibitory activity. Oncogene. 2005 May;24(20):3348–57.

77. Hayes-Jordan AA, Ma X, Menegaz BA, Lamhamedi-Cherradi SE, Kingsley C V., Benson JA, et al. Efficacy of ONC201 in Desmoplastic Small Round Cell Tumor. Neoplasia (United States) [Internet]. 2018;20(5):524–32. Available from: https://doi.org/10.1016/j.neo.2018.02.006

78. Magnan HD, Chou T, LaQuaglia MP, Gerald W, Ladanyi M, Merchant MS. Elevated expression of VEGFR-2 and VEGFA in desmoplastic small round cell tumor (DSRCT) and activity of bevacizumab and irinotecan in a xenograft model of DSRCT. J Clin Oncol [Internet]. 2009 May 20;27(15_suppl):10016. Available from: https://ascopubs.org/doi/abs/10.1200/jco.2009.27.15_suppl.10016

79. Shi C, Feng Y, Zhang LC, Ding DY, Yan MY, Pan L. Effective treatment of apatinib in desmoplastic small round cell tumor: A case report and literature review. BMC Cancer. 2018;18(1):1–6.

80. Uboldi S, Craparotta I, Colella G, Ronchetti E, Beltrame L, Vicario S, et al. Mechanism of action of trabectedin in desmoplastic small round cell tumor cells. BMC Cancer.

2017 Dec;17(1):107.

81. Forni C, Minuzzo M, Virdis E, Tamborini E, Simone M, Tavecchio M, et al. Trabectedin (ET-743) promotes differentiation in myxoid liposarcoma tumors. Mol Cancer Ther. 2009 Jan;8(2):449–57.

82. Markides CSA, Coil DR, Luong LH, Mendoza J, Kozielski T, Vardeman D, et al. Desmoplastic small round cell tumor (DSRCT) xenografts and tissue culture lines: Establishment and initial characterization. Oncol Lett. 2013;5(5):1453–6.

83. Negri T, Brich S, Bozzi F, Volpi C V., Gualeni A V., Stacchiotti S, et al. New transcriptional-based insights into the pathogenesis of desmoplastic small round cell tumors (DSRCTs). Oncotarget. 2017;8(20):32492–504.

84. Frezza AM, Benson C, Judson IR, Litiere S, Marreaud S, Sleijfer S, et al. Pazopanib in advanced desmoplastic small round cell tumours : a multi-institutional experience. 2014;1–6.

85. Bétrian S, Bergeron C, Blay J-Y, Bompas E, Cassier PA, Chevallier L, et al. Antiangiogenic effects in patients with progressive desmoplastic small round cell tumor: data from the French national registry dedicated to the use of off-labeled targeted therapy in sarcoma (OUTC's). Clin Sarcoma Res. 2017;7(1):1–7.

86. Chow LQM, Eckhardt SG. Sunitinib: from rational design to clinical efficacy. J Clin Oncol Off J Am Soc Clin Oncol. 2007 Mar;25(7):884–96.

87. Menegaz BA, Cuglievan B, Benson J, Camacho P, Lamhamedi-Cherradi S-E, Leung CH, et al. Clinical Activity of Pazopanib in Patients with Advanced Desmoplastic Small Round Cell Tumor. Oncologist [Internet]. 2017/12/06. 2018 Mar;23(3):360–6. Available from: https://pubmed.ncbi.nlm.nih.gov/29212731

88. Stacchiotti S, Negri T, Zaffaroni N, Palassini E, Morosi C, Brich S, et al. Sunitinib in advanced alveolar soft part sarcoma: evidence of a direct antitumor effect. Ann Oncol [Internet]. 2011 Jul;22(7):1682–90. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0923753419384418

89. Italiano A, Kind M, Cioffi A, Maki RG, Bui B. Clinical activity of sunitinib in patients with advanced desmoplastic round cell tumor: a case series. Target Oncol. 2013

Sep;8(3):211–3.

90. Scott AJ, Messersmith WA, Jimeno A. Apatinib: A promising oral antiangiogenic agent in the treatment of multiple solid tumors. Drugs of Today [Internet]. 2015;51(4):223. Available from: http://journals.prous.com/journals/servlet/xmlxsl/pk_journals.xml_summary_pr?p_JournalId=4&p_RefId=2320599&p_IsPs=N

91. Tian Y, Cheng X, Li Y. Chemotherapy combined with apatinib for the treatment of desmoplastic small round cell tumors: A case report. J Cancer Res Ther [Internet]. 2020;16(5):1177. Available from: http://www.cancerjournal.net/text.asp?2020/16/5/1177/296439

92. Magnan HD, Price A, Chou AJ, Riedel E, Wexler LH, Ambati SR, et al. A pilot trial of irinotecan, temozolomide and bevacizumab (ITB) for treatment of newly diagnosed patients with desmoplastic small round cell tumor (DSRCT). J Clin Oncol [Internet]. 2017 May 20;35(15_suppl):11050. Available from: https://doi.org/10.1200/JCO.2017.35.15_suppl.11050

93. Fine RL, Shah SS, Moulton TA, Yu I-R, Fogelman DR, Richardson M, et al. Androgen and c-Kit receptors in desmoplastic small round cell tumors resistant to chemotherapy: novel targets for therapy. Cancer Chemother Pharmacol. 2007 Mar;59(4):429–37.

94. Bulbul A, Shen JP, Xiu J, Tamayo P, Husain H. Genomic and Proteomic Alterations in Desmoplastic Small Round Blue-Cell Tumors. JCO Precis Oncol. 2018;2.

95. Thijs AMJ, van der Graaf WTA van HC. Temsirolimus for Metastatic Desmoplastic Small Round Cell Tumor. Pediatr Blood Cancer. 2010;55:1431–2.

96. Wan X, Helman LJ. The biology behind mTOR inhibition in sarcoma. Oncologist. 2007 Aug;12(8):1007–18.

97. Helman LJ, Meltzer P. Mechanisms of sarcoma development. Nat Rev Cancer. 2003 Sep;3(9):685–94.

98. Felkai L, Krencz I, Kiss DJ et al. Characterization of mTOR Activity and Metabolic Profile in Pediatric Rhabdomyosarcoma. Cancers (Basel). 2020;12:1947.

99. Subbiah V, Brown RE, Jiang Y, Buryanek J, Hayes-Jordan A, Kurzrock R, et al. Morphoproteomic Profiling of the Mammalian Target of Rapamycin (mTOR) Signaling Pathway in Desmoplastic Small Round Cell Tumor (EWS/WT1), Ewing's Sarcoma (EWS/FLI1) and Wilms' Tumor(WT1). PLoS One. 2013;8(7):1–7.

100. Jiang Y, Subbiah V, Janku F, Ludwig JA, Naing A, Benjamin RS, et al. Novel secondary somatic mutations in Ewing's sarcoma and desmoplastic small round cell tumors. PLoS One. 2014;9(8):1–7.

101. Tarek N, Hayes-Jordan A, Salvador L et al. Recurrent desmoplastic small round cell tumor responding to an mTOR inhibitor containing regimen. Pediatr Blood Cancer. 2018;65(1):1–3.

102. Naing A, LoRusso P, Fu S et al. Insulin growth factor-receptor (IGF-1R) antibody cixutumumab combined with the mtor inhibitor temsirolimus in patients with refractory ewing's sarcoma family tumors. Clin Cancer Res. 2012;18(9):2625–31.

103. Katz D, Azraq Y, Eleyan F et al. Pazolimus: Pazopanib plus sirolimus following progression on pazopanib, a retrospective case series analysis. BMC Cancer. 2016;16:616.

104. Chow WA, Yee JK, Tsark W, Wu X, Qin H, Guan M, et al. Recurrent secondary genomic alterations in desmoplastic small round cell tumors. BMC Med Genet. 2020;21(1):1–8.

105. Jackson SP and Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461(22):1071–8.

106. SA MA and IS. PARP Inhibitors as Therapeutics: Beyond Modulation of PARylation. Cancers (Basel). 2020;12:394.

107. Murai J, Huang SN, Das BB et al. Differential trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res. 2012;72(7):1608–13.

108. Mellado-Lagarde M, Federico SM, Tinkle C, Shelat A, Stewart E. PARP inhibitor combination therapy in desmoplastic small round cell tumors. J Clin Oncol. 2017 May;35(15_suppl):e23212–e23212.

109. Liu J, Nau MM, Yeh JC, Allegra CJ, Chu E, Wright JJ. Molecular heterogeneity and

 doi:10.20944/preprints202012.0288.v1

function of EWS-WT1 fusion transcripts in desmoplastic small round cell tumors. Clin Cancer Res. 2000;6(9):3522–9.

110. Aune GJ, Takagi K, Sordet O, JoséeGuirouilh-Barbat, Antony S, Bohr V, et al. Von hippel-lindau-coupled and transcription-coupled nucleotide excision repair-dependent degradation of RNA polymerase II in response to trabectedin. Clin Cancer Res. 2008;14(20):6449–55.

111. Germano G, Frapolli R, Belgiovine C, Anselmo A, Pesce S, Liguori M, et al. Role of macrophage targeting in the antitumor activity of trabectedin. Cancer Cell. 2013 Feb;23(2):249–62.

112. Pignochino Y, Capozzi F, D'Ambrosio L, Dell'Aglio C, Basiricò M, Canta M, et al. PARP1 expression drives the synergistic antitumor activity of trabectedin and PARP1 inhibitors in sarcoma preclinical models. Mol Cancer. 2017;16(1):1–15.

113. Grignani G, D'Ambrosio L, Pignochino Y, Palmerini E, Zucchetti M, Boccone P, et al. Trabectedin and olaparib in patients with advanced and non-resectable bone and soft-tissue sarcomas (TOMAS): an open-label, phase 1b study from the Italian Sarcoma Group. Lancet Oncol. 2018 Oct;19(10):1360–71.

114. Ferracini R, Di Renzo MF, Scotlandi K et al. The Met/HGF receptor is over-expressed in human osteosarcomas and is activated by either a paracrine or an autocrine circuit. Oncogene. 1995;10(4):739–49.

115. Davis IJ, McFadden AW, Zhang Y et al. Identification of the Receptor Tyrosine Kinase c-Met and Its Ligand, Hepatocyte Growth Factor, as Therapeutic Targets in Clear Cell Sarcoma. Cancer Res. 2010;70(2):639–46.

116. Jo EB, Lee YS, Lee H et al. Combination therapy with c-met inhibitor and TRAIL enhances apoptosis in dedifferentiated liposarcoma patient-derived cells. BMC Cancer. 2019;19:496.

117. Ferracini R, Olivero M, Di Renzo MF et al. Retrogenic expression of the MET proto-oncogene correlates with the invasive phenotype of human rhabdomyosarcomas. Oncogene. 1996;12(8):1697–705.

118. Chen HM and Feng G. Use of anlotinib in intra-abdominal desmoplastic small round

cell tumors: a case report and literature review. Onco Targets Ther. 2019;12:57–61.

119. Finkeltov I, Kuhn S, Glaser T, Idelman G, Wright JJ, Roberts CT, et al. Transcriptional regulation of IGF-I receptor gene expression by novel isoforms of the EWS-WT1 fusion protein. Oncogene. 2002;21(12):1890–8.

120. Tap WD, Demetri G, Barnette P, Desai J, Kavan P, Tozer R, et al. Phase II study of ganitumab, a fully human anti-type-1 insulin-like growth factor receptor antibody, in patients with metastatic Ewing family tumors or desmoplastic small round cell tumors. J Clin Oncol Off J Am Soc Clin Oncol. 2012 May;30(15):1849–56.

121. Hollingsworth RE and Jansen K. Turning the corner on therapeutic cancer vaccines. NPJ Vaccines. 2019;8(4):7.

122. Pender A, Jones RL PS. Optimising Cancer Vaccine Design in Sarcoma. Cancers (Basel). 2019;11(1):1.

123. Geiger JD, Hutchinson RJ, Hohenkirk LF et al. Vaccination Of Pediatric Solid Tumor Patients with Tumor Lysate-pulsed Dendritic Cells Can Expand Specific T Cells and Mediate Tumor Regression. Cancer Res. 2001;61(23):8513–9.

124. Merchant MS, Bernstein D, Amoako M et al. Adjuvant Immunotherapy to Improve Outcome in High-Risk Pediatric Sarcomas. Clin Cancer Res. 2016;22(13):3182–91.

125. Sharma P, Allison JP. Dissecting the mechanisms of immune checkpoint therapy. Nat Rev Immunol [Internet]. 2020 Feb 10;20(2):75–6. Available from: http://www.nature.com/articles/s41577-020-0275-8

126. Lawrence MS, Stojanov P, Polak P, Kryukov G V., Cibulskis K, Sivachenko A, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature [Internet]. 2013 Jul 16;499(7457):214–8. Available from: http://www.nature.com/articles/nature12213

127. Tawbi HA, Burgess M, Bolejack V, Van Tine BA, Schuetze SM, Hu J, et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol. 2017 Nov;18(11):1493–501.

128. D'Angelo SP, Mahoney MR, Van Tine BA, Atkins J, Milhem MM, Jahagirdar BN, et al.

Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): two open-label, non-comparative, randomised, phase 2 trials. Lancet Oncol [Internet]. 2018 Mar;19(3):416–26. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1470204518300068

129. Petitprez F, de Reyniès A, Keung EZ, Chen TWW, Sun CM, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. Nature. 2020;577(7791):556–60.

130. Wilky BA, Trucco MM, Subhawong TK, Florou V, Park W, Kwon D, et al. Axitinib plus pembrolizumab in patients with advanced sarcomas including alveolar soft-part sarcoma: a single-centre, single-arm, phase 2 trial. Lancet Oncol [Internet]. 2019;20(6):837–48. Available from: http://dx.doi.org/10.1016/S1470-2045(19)30153-6

131. Negri T, Brich S, Bozzi F, Volpi C V, Gualeni A V, Stacchiotti S, et al. New transcriptional-based insights into the pathogenesis of desmoplastic small round cell tumors (DSRCTs). Oncotarget. 2017 May;8(20):32492–504.

132. van Erp AEM, Versleijen-Jonkers YMH, Hillebrandt-Roeffen MHS, van Houdt L, Gorris MAJ, van Dam LS, et al. Expression and clinical association of programmed cell death-1, programmed death-ligand-1 and CD8+ lymphocytes in primary sarcomas is subtype dependent. Oncotarget. 2017;8(41):71371–84.

133. Chen JL, Mahoney MR, George S, Antonescu CR, Liebner DA, Van Tine BA, et al. A multicenter phase II study of nivolumab +/- ipilimumab for patients with metastatic sarcoma (Alliance A091401): Results of expansion cohorts. J Clin Oncol [Internet]. 2020 May 20;38(15_suppl):11511–11511. Available from: https://ascopubs.org/doi/10.1200/JCO.2020.38.15_suppl.11511

134. Modak S, Zanzonico P, Grkovski M, Slotkin EK, Carrasquillo JA, Lyashchenko SK, et al. B7H3-Directed Intraperitoneal Radioimmunotherapy With Radioiodinated Omburtamab for Desmoplastic Small Round Cell Tumor and Other Peritoneal Tumors: Results of a Phase I Study. J Clin Oncol [Internet]. 2020 Oct 29;JCO.20.01974. Available from: https://ascopubs.org/doi/10.1200/JCO.20.01974

135. Demetri GD, Antonescu CR, Bjerkehagen B, Bovée JVMG, Boye K, Chacón M, et al. Diagnosis and management of tropomyosin receptor kinase (TRK) fusion sarcomas: expert recommendations from the World Sarcoma Network. Ann Oncol [Internet]. 2020 Nov;31(11):1506–17. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0923753420422975

136. Hong DS, DuBois SG, Kummar S, Farago AF, Albert CM, Rohrberg KS, et al. Larotrectinib in patients with TRK fusion-positive solid tumours: a pooled analysis of three phase 1/2 clinical trials. Lancet Oncol [Internet]. 2020 Apr;21(4):531–40. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1470204519308563

137. Ogura K, Somwar R, Hmeljak J, Magnan H, Benayed R, Momeni-Boroujeni A, et al. Therapeutic potential of NTRK3 inhibition in desmoplastic small round cell tumor. Clin Cancer Res [Internet]. 2020 Nov 23;clincanres.2585.2020. Available from: http://clincancerres.aacrjournals.org/lookup/doi/10.1158/1078-0432.CCR-20-2585