Expert Opinion on Therapeutic Targets

ISSN: 1472-8222 (Print) 1744-7631 (Online) Journal homepage: https://www.tandfonline.com/loi/iett20

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To cite this article: Anastasia Loktev & Janet M. Shipley (2020): Desmoplastic small round cell tumour (DSRCT): emerging therapeutic targets and future directions for potential therapies, Expert Opinion on Therapeutic Targets, DOI: 10.1080/14728222.2020.1738392

To link to this article: https://doi.org/10.1080/14728222.2020.1738392

Accepted author version posted online: 03 Mar 2020.

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Opinion on Therapeutic Targets*

DOI: 10.1080/14728222.2020.1738392

Desmoplastic small round cell tumour (DSRCT): emerging therapeutic targets and future directions for potential therapies

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Keywords: DSRCT, sarcoma, targeted therapy, EWS-WT1 fusion, DNA damage repair, immunotherapy

1. Introduction

1.1. Molecular characteristics of DSRCT

Desmoplastic Small Round Cell Tumours (DSRCTs) are a rare soft tissue sarcoma (850 patients reported in medical literature by 2018[1]) which predominantly occurs in male children and adolescents. It typically presents with multiple intra-abdominal tumours and a distinctive desmoplastic stroma. The exact tissue of origin is unknown and very few druggable molecular targets have been identified so far[2,3]. There is no standard treatment regimen and current treatment strategies rely on surgery, radiation and high-dose chemotherapy. The molecular hallmark of DSRCT is a chromosomal translocation which results in the fusion of the *EWS* gene with *WT1*. As a result of alternative splicing different isoforms of the fusion protein exist, with and without the insertion of three additional amino acids lysine, threonine, and serine (KTS) between zinc fingers 3 and 4 (Figure 1)[4].

1.2. Similarity to Ewing's sarcoma

Ewing's sarcoma (ES) also involve a gene fusion of *EWS* but with members of the ETS domain family of transcription factors, and appears to activate similar oncogenic pathways as *EWS-WT1*[5,6]. Due to this similarity, therapeutic strategies and clinical treatment regimens for DSRCT have been derived from ES treatments. Yet, survival rates for DSRCT patients are significantly lower than for ES patients[7]. Due to the rarity of the tumour, DSRCT patients are included in clinical studies designed for patients with other sarcomas and not based on the specific molecular features of DSRCT. It is therefore crucial to identify characteristic biomarkers and tumour-associated druggable targets for DSRCT to enable the

delivery of targeted therapeutics that may improve the poor outcome of this very aggressive disease.

2. Potential therapeutic targets for DSRCT

Wildtype *WT1* encodes a zinc finger-containing protein which acts as a repressor of transcription. In the EWS-WT1 fusion, loss of the zinc finger region of WT1 leads to transcriptional activation of at least 35 target genes (reviewed by Bulbul *et al.* [6]). These include those encoding growth factors and their receptors such as PDGFα, IGF-1-R and EGFR, regulators of transcription, e.g. c-MYC, N-MYC, PAX2-2 and ENT4 and genes encoding extracellular proteins such as Syndecan-1, E-cadherin and TALLA-1. These proteins potentially contribute to tumour growth and therapy resistance and thus represent interesting target structures for the development of novel treatments. However, it is unclear in what way these molecular events contribute to DSRCTs' pathophysiology and therefore research addressing their potential as therapeutic targets is required.

Cell surface proteins offer potential for the targeted delivery of immunotherapy, chemotherapy or radiotherapy. CD276, also known as neoantigen B7H3, is expressed in about 96% of DSRCT cells. It exerts an inhibitory effect on T cells, thus contributing to tumour cell immune evasion and plays an important role in tumour growth and metastasis. There are monoclonal antibodies targeting CD276, which are being tested in a recent clinical trial in combination with therapeutic radionuclides[1].

GD2 is a glycosphingolipid expressed in different solid tumours, including DSRCT. Its function is not completely understood, but it is thought to play an important role in the attachment of tumour cells to extracellular matrix proteins. GD2 is used as a target structure for immunotherapy and radioimmunotherapy in melanoma, neuroblastoma and osteosarcoma[8]. However, only two of 20 DSRCT samples were shown to be GD2-positive. GD3, which is a ganglioside further

upstream in the biosynthesis of GD2, has been shown to be expressed in 14 of the 20 samples analysed. GD3 is associated with proliferation, adhesion and invasive activity of melanoma cells and might also be of interest for targeted therapy of DSRCT[9].

VEGF-dependent angiogenesis seems to play an important role for DSRCT tumour biology. Overexpression of VEGFR-2 and VEGFA was demonstrated in DSRCT xenografts, which showed strong response to bevacizumab treatment[3].

Other cell surface proteins are mainly considered as immunotherapy targets but are also addressed with drug and radionuclide antibody conjugates. These include LRRC15, whose role for tumour progression is yet unclear. Poliovirus receptor (PVR, CD155) as well as PVR-related 2 (PVRL2, CD112) are both described as NK ligands[6]. Increased expression levels were also shown for the androgen receptor (AR, expressed in 59% of 35 DSRCT samples) and connective tissue growth factor CCN2, which is associated with the production of abundant extracellular matrix and may have autocrine or paracrine roles in disease progression[6,10]. However, expression levels vary widely, requiring thorough predictive patient stratification. Immune checkpoint inhibition by targeting CTLA-4 and PD-1 (CD279) was described as a potential novel therapeutic strategy for the treatment of DSRCT. However, despite PD-1 expression, expression of PD-L1 in DSRCT appears very limited. According to Bulbul et al., none of the patients in a recently described DSRCT cohort had identifiable tumoral PD-L1 expression[6,11].

Recent analyses of mutational profiles revealed the deregulation of genes related to immune response, epithelial–mesenchymal transition (EMT) and the DNA damage response (DDR) in DSRCT[12]. Whole-exome sequencing of six DSRCT samples identified a total of 137 unique somatic mutations, of which 133 mutated genes were case-specific, and two genes were mutated in two cases but in different positions. These results highlight the inter-tumoural heterogeneity of the DSRCT

genome in addition to the pathognomonic fusion gene. However, there were two main biological categories affected in all samples. Among the 135 mutated genes, 27% were related to EMT/mesenchymal-epithelial reverse transition (MErT) and the DDR network. These include proteins with a crucial function in the cell cycle such as Ataxia telangiectasia and Rad3-related protein (ATR) and Ribonucleoside-diphosphate reductase subunit M2 (RRM2)[12]. Consistent with the latter, Mellado et al. demonstrated sensitivity of DSRCT to PARP inhibition combination therapy in patient derived xenograft models. This strategy has also been shown to work in ES, where PARP inhibition in combination with standard of care treatment (irinotecan and temozolomide) resulted in more than 80% complete response compared to 100% mortality with standard of care alone. This is not surprising since the interference of the *EWS* fusion gene with wild-type *EWS* in both ES and DSRCT might result in similar replication stress and defects in the DDR system. This may create similar vulnerabilities to DDR targeting compounds.

3. Expert opinion

To improve the dismal outcome of DSRCT therapy, the use of targeted approaches based on biological rationale is urgently required. A number of targeted compounds have been included in recent clinical trials but most treatment protocols have been adapted from other tumour types and the limited number of patients poses a big challenge for the implementation of clinical trials.

Little is known about the underlying molecular mechanisms driving DSRCT and undoubtedly, more fundamental research needs to be done in order to establish solid rationale for therapeutic developments. To support this, the development of more models is needed that better represent the heterogeneity of tumours and the complexity of the interplay between tumour and stromal cells. So far, only one established cell line exists and patient xenografts are rare and difficult to culture *in vitro*. It might be of significance to target the abundant desmoplastic stroma, especially as corresponding therapies have been successfully used in other tumour

types. In addition, it could be of clinical benefit to use liquid biopsies and particularly circulating tumour DNA (ctDNA), including the tumour-specific EWS-WT1 fusion, for the detection of minimal residual disease and recurrence as well as in the assessment of therapy efficacy[13].

Based on molecular findings in DSRCT so far, promising targets are related to immune response, the DDR network or epithelial–mesenchymal and mesenchymal-epithelial transition (Figure 2, Table 1). Due to the heterogeneity of the tumours, the advanced disease at diagnosis and frequent therapy resistance that emerges, combination treatments could significantly improve therapeutic outcomes. Within the limits of currently available DSRCT models, preliminary preclinical studies combining established chemotherapeutics with DDR or immune checkpoint inhibitors look promising.

Funding

The work of the authors is funded by the Kelly Turner Foundation.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

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**** First study to identify unique somatic mutations, of which some might be relevant as potential therapeutic targets**

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*** First evidence for successful treatment targeting DNA damage repair in DSRCT in vivo**

Table 1: Potential therapeutic targets for DSRCT

Target	Description	Lit.
Downstream effectors of the fusion protein		
Platelet Derived Growth Factor Subunit A (PDGFα)	Growth factor, involved in regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis	6,14
Insulin-like growth factor 1 receptor (IGF-1-R)	Receptor tyrosine kinase, crucial for tumour transformation and survival of malignant cell	6,14
Epidermal growth factor receptor (EGFR)	Receptor tyrosine kinase binding ligands of the EGF family	6,14
Myc proto-oncogene protein (c-MYC)	Transcription factor regulating growth-related genes, binds to the VEGFA promoter	6,14
N-myc proto-oncogene protein (N-MYC)	Transcription factor	6,14
Paired box protein Pax-2 (PAX2-2)	Transcription factor with critical role in the development of the urogenital tract, the eyes, and the central nervous system	6,14

Equilibrative nucleoside transporter 4 (ENT4)	Polyspecific organic cation transporter	6,14
Syndecan-1	Cell surface proteoglycan, links the cytoskeleton to the interstitial matrix	6,14
E-cadherin	Cell adhesion protein	6,14
T-cell acute lymphoblastic leukemia-associated antigen 1 (TALLA-1)	Cell surface glycoprotein, regulating cell adhesion, migration and metastasis	6
Leucine-rich repeat-containing protein 15 (LRRC15)	Type I membrane protein, function unclear	10,15
Immune response		
CD276 (B7H3)	Immune checkpoint, modulates T-cell-mediated immune responses	1
Poliovirus receptor-related protein 2 (PVRL2, CD112)	Modulator of T-cell signalling, NK ligand	6
Programmed cell death protein 1 (PD-1, CD279)	Immune checkpoint, inhibitory receptor on antigen activated T-cells	10
Transmembrane proteins		
Cellular communication network factor 2 (CCN2, CTGF)	Matricellular protein, involved in cell adhesion, migration, proliferation and angiogenesis	16
Ganglioside G2 (GD2)	Glycosphingolipid involved in the attachment of tumour cells to extracellular matrix proteins	8
Poliovirus receptor (PVR, CD155)	Type I transmembrane glycoprotein, establishment of intercellular adherens junctions between epithelial cells, NK ligand	6

Vascular endothelial growth factor receptor 2 (VEGFR-2)	Tyrosine-protein kinase that acts as a cell-surface receptor, regulation of angiogenesis, vascular development, vascular permeability and embryonic haematopoiesis	3
DNA damage repair pathways		
Ataxia telangiectasia and Rad3-related protein (ATR)	Serine/threonine protein kinase which activates checkpoint signalling upon genotoxic stresses	12
Poly(ADP-ribose)-polymerase (PARP)	DNA repair enzyme, involved in base excision and double-strand break repair	17
Probable ubiquitin carboxyl-terminal hydrolase FAF-X (USP9X)	Promotes the repair of alkylated DNA lesions	12
Ribonucleoside-diphosphate reductase subunit M2 (RRM2)	Catalyses the biosynthesis of deoxyribonucleotides from the corresponding ribonucleotides, inhibits Wnt signalling	12
Other		
Androgen receptor (AR)	DNA-binding transcription factor	6

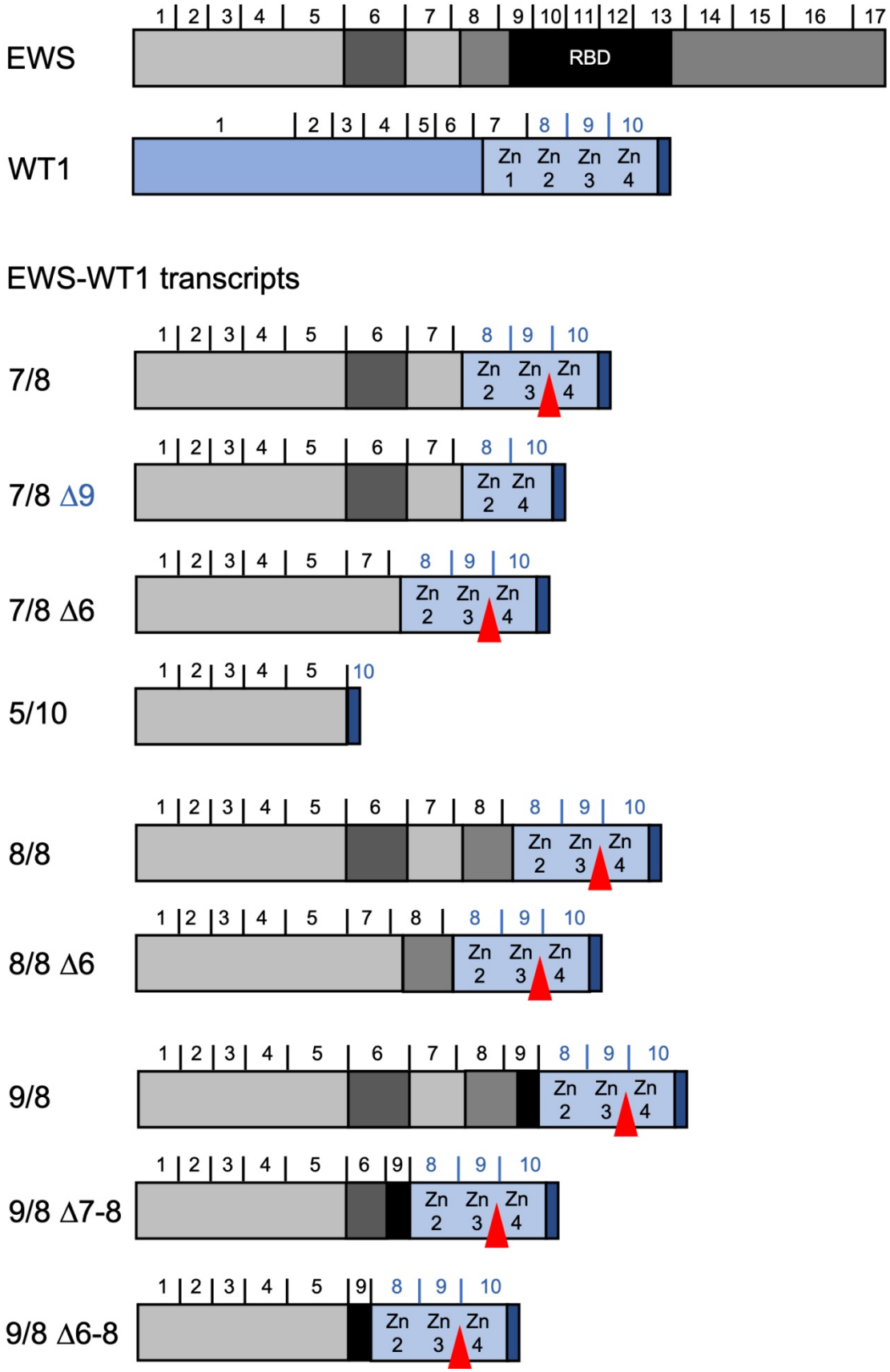

Figure 1. Schematic structures of EWS-WT1 fusion proteins. Due to alternative

splicing, the fusion of EWS and WT1 results in the expression of different isoforms. In most Desmoplastic Small Round Cell Tumours (DSRCTs), prototypical EWS-WT1 fusion proteins include exons 1–7 or 1–8 of EWS fused to exons 8–10 of the WT1 gene4. Alternative splicing of exon 9 leads to WT1 domains containing or lacking the amino acids lysine, threonine and serine (KTS) between zinc-fingers (Zn) 3 and 4 (represented as red triangle). Depending on the presence or absence of KTS in the DNA-binding domain, different target genes are activated, contributing to the heterogeneity of DSRCTs. RBD: RNA-binding domain.

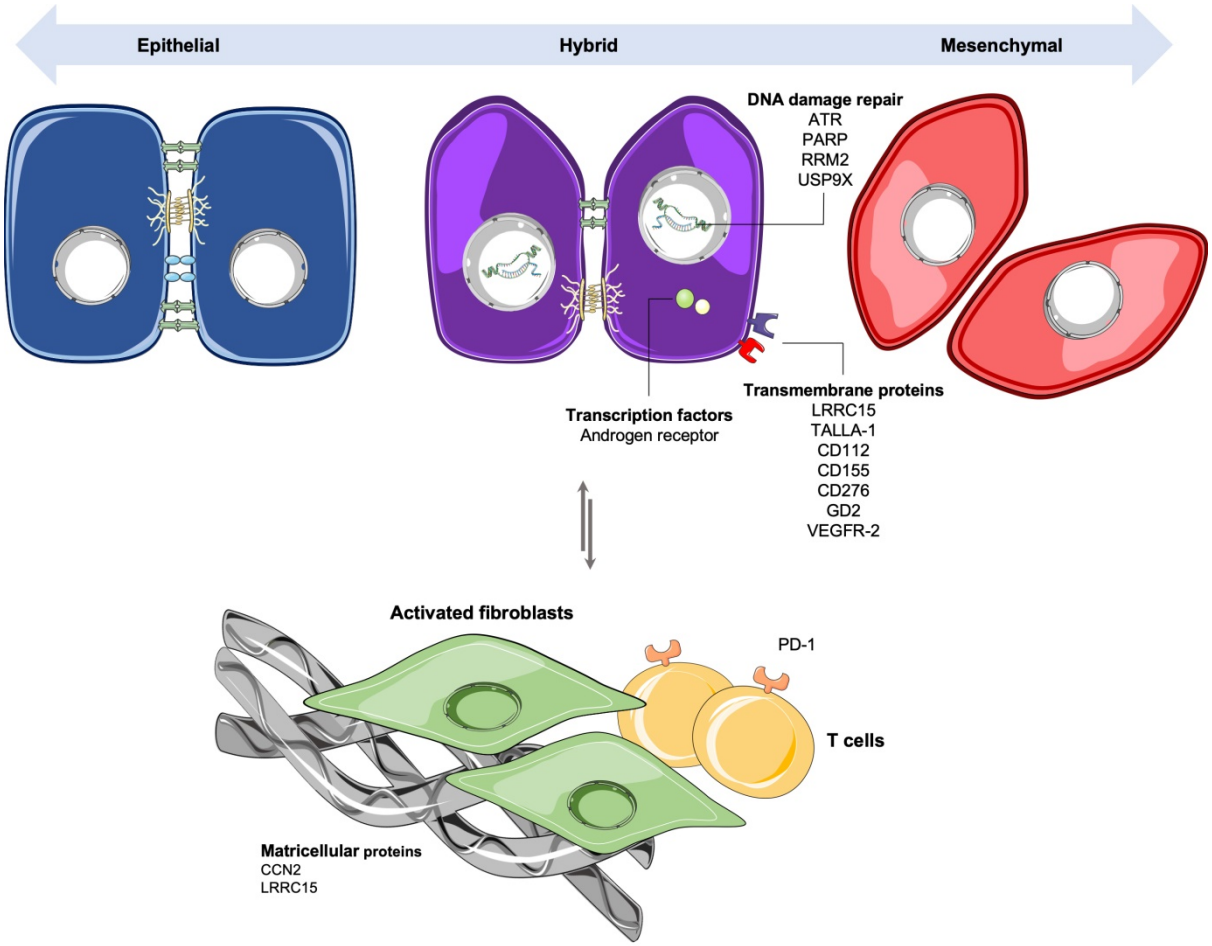

Figure 2. Potential molecular targets for the treatment of DSRCT. These include cell surface molecules, which can be used for the targeted delivery of immunotherapy, chemotherapeutics or radionuclides. Some of these cell surface proteins are transcriptional targets of the fusion protein, e.g. Leucine-rich repeat-containing protein 15 (LRRC15). Although roles for epithelial–mesenchymal transition and mesenchymal-epithelial reverse transition are yet unclear, it may prove possible and effective to target these transitions. The desmoplastic character of these tumours is expected to play a key supporting role, suggesting therapeutic targeting of the tumour stroma. Immunotherapy via unsuppressing T cell function and perturbing the extracellular matrix may ultimately prove useful in therapeutic strategies.