Review Article

Current Approaches for Personalized Therapy of Soft Tissue Sarcomas

Kirill I. Kirsanov, Ekaterina A. Lesovaya, Timur I. Fetisov, Beniamin Yu Bokhyan, Gennady A. Belitsky, and Marianna G. Yakubovskaya

1 N. Blokhin Cancer Research Center, Moscow, Russia
2 RUDN University, Moscow, Russia
3 I. P. Pavlov Ryazan State Medical University, Ryazan, Russia

Correspondence should be addressed to Ekaterina A. Lesovaya; lesovenok@yandex.ru

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Soft tissue sarcomas (STS) are a highly heterogeneous group of cancers of mesenchymal origin with diverse morphologies and clinical behaviors. While surgical resection is the standard treatment for primary STS, advanced and metastatic STS patients are not eligible for surgery. Systemic treatments, including standard chemotherapy and newer chemical agents, still play the most relevant role in the management of the disease. Discovery of specific genetic alterations in distinct STS subtypes allowed better understanding of mechanisms driving their pathogenesis and treatment optimization. This review focuses on the available targeted drugs or drug combinations based on genetic aberration involved in STS development including chromosomal translocations, oncogenic mutations, gene amplifications, and their perspectives in STS treatment. Furthermore, in this review, we discuss the possible use of chemotherapy sensitivity and resistance assays (CSRA) for the adjustment of treatment for individual patients. In summary, current trends in personalized management of advanced and metastatic STS are based on combination of both genetic testing and CSRA.

1. Introduction

Soft tissue sarcomas (STS) present a highly heterogeneous cancer group with more than 50 subtypes in terms of anatomical location, histology, molecular characteristics, and prognosis [1]. STS originate from mesenchymal cells of a number of tissue lineages, such as adipose, muscle, fibrous, cartilage, and vasculature [2]. Surgery is the standard of care for primary soft tissue sarcomas, while for locally advanced or metastatic STS, chemotherapy is generally the principal treatment modality [3]. Factors to consider when selecting first-line chemotherapy for advanced STS include, besides histological state, disease bulk, and patient-related factors such as age and presence of comorbidities, genetic and molecular-biological peculiarities of specific tumors. Recent discoveries of the underlying molecular and genomic mechanisms of different STS histology subtypes have enabled to reclassify these tumors and to optimize cytotoxic chemotherapeutic regimens as well as to encourage development of novel targeted chemotherapeutic agents in treating STS. Thus, the development of low molecular weight inhibitors of chimeric kinase ETV6-NTRK3, specific for congenital fibrosarcoma/mesoblastic nephroma, is now at the Phase I clinical trial ([4] and Table 1). Efficacy of tyrosine kinase inhibitors imatinib and sunitinib was approved for COL1A1-PDGFB-positive dermatofibrosarcoma treatment or involved in clinical trials of Phase I-III ([5–7] and Table 1). The first part of this review will summarize the approaches of targeted STS therapy based on genetic alteration associated with distinct tumor types. However, the prognosis of personalized chemosensitivity and resistance of STS presents certain difficulties, as accumulated data are insufficient to provide the efficacy of prescribed therapy of at least 40% or more. Moreover, less than 40% of STS subtypes could
| Tumor type                        | Translocation       | Fusion product          | Targeted therapy approach based on genetic testing                                                                 | Stage of investigations        | Reference |
|----------------------------------|---------------------|-------------------------|------------------------------------------------------------------------------------------------------------------|------------------------------|-----------|
| Alveolar rhabdomyosarcoma        | t(2; 13) (q35; q14) | PAX3-FOXO1A (aberrant transcription) | Inhibition of regulatory networks (phosphorylation, transcription, coactivation, acetylation) | *In vitro*/*in vivo* studies | [32, 79]  |
|                                  | t(1; 13) (p36; q14) | PAX7-FOXO1A (aberrant transcription) | Not described                                                                                                      | Not described                 | [80]      |
| Alveolar soft part sarcoma       | t(X; 17) (p11.2; q25) | TFE3-ASPL (aberrant transcription) | Not described                                                                                                      | Not described                 | [84]      |
| Angiomatoid fibrous histiosarcoma| t(12; 16) (q13; p11) | FUS-ATF1 (aberrant transcription) | Not described                                                                                                      | Not described                 | [85, 86]  |
| Chondroid lipoma                 | t(11; 16) (q13; p12-13) | C11orf95-MKL2 | Not described                                                                                                      | Not described                 | [87]      |
| Clear cell sarcoma               | t(12; 22) (q13; q12) | EWS-ATF1 (aberrant transcription) | Inhibition of EWS-ATF1 downstream target c-Met, an oncogenic receptor tyrosine kinase, with small-molecule inhibitor SU11274 or a neutralizing antibody to its ligand HGF AMG 102 Inhibition of EWS-ATF1 downstream target proto-oncogene FOS, with FOS-targeted siRNA | *In vitro*/*in vivo* studies | [88]      |
| Congenital fibrosarcoma/mesoblastic nephroma | t(12; 15) (p13; q25) | ETV6-NTRK3 (ligand-independent kinase activation) | Inhibition of ETV6-NTRK3 with LOXO-101, an experimental, highly selective inhibitor of TRK | Phase I clinical trial | [4]       |
| Dermatofibrosarcoma              | t(17; 22) (q22; q13) | COL1A1-PDGFB (increased expression of kinase) | Inhibition of PDGFRB with imatinib Approval for systemic treatment of dermatofibrosarcoma Trials for use in case of imatinib-resistant dermatofibrosarcoma | [5]                          |
| Desmoplastic small round cell tumor | t(11; 22) (p13; q12) | EWS-WT1 (aberrant transcription) | Inhibition of EWS-WT1 expression with trabectedin Inhibition of EWS-WT1 downstream target IGFI1 with monoclonal antibody ganitumab Inhibition of EWS-WT1 downstream targets (mTOR, Notch, PDGFRB) with known approved inhibitors | *In vitro*/*in vivo* studies | [38]      |
| Endometrial stromal sarcoma, low grade | t(7; 17) (p15; q21) | JAZF1-JJAZ1 (aberrant transcription) | Not described                                                                                                      | Not described                 | [91]      |
| Epithelioid hemangioendothelioma  | t(1; 3) (p36; q25)  | WWTR1-CAMTA1 (aberrant transcription) | Not described                                                                                                      | Not described                 | [81]      |
|                                  | t(X; 11) (p11.2; q13) | YAP1-TFE3 (aberrant transcription) | Not described                                                                                                      | Not described                 | [92]      |
### Table 1: Continued.

| Tumor type | Translocation | Fusion product | Targeted therapy approach based on genetic testing | Stage of investigations | Reference |
|------------|---------------|----------------|--------------------------------------------------|-------------------------|-----------|
| Ewing sarcoma and peripheral primitive neuroectodermal tumor | t(21; 22) (q22; q12) | EWS-FLI1 (aberrant transcription) | Inhibition of EWS-FLI1 activity with low molecular weight compound YK-4-279 | In vitro/in vivo studies | [19, 93, 94] |
| | | | Inhibition of EWS-FLI1 activity with analogues of myt4ramycin | In vitro/in vivo studies | [95] |
| | | | Inhibition of EWS-FLI1 phosphorylation with EnglerinA | In vitro studies | [29] |
| | | | Inhibition of EWS-FLI1 expression with antisense oligodeoxynucleotides, siRNAs | In vitro/in vivo studies | [27, 96, 97] |
| | | | Inhibition of EWS-FLI1 downstream target IGF1R with monoclonal antibodies R1507, MK-0646, cixitumumab, Ganitumab, figitumumab | Phase I-II clinical trials | [27] |
| | | | Inhibition of EWS-FLI1 downstream targets Aurora kinase (AURK) family members (A, B, and C) with AURKA inhibitors alisertib and tozasertib | In vitro/in vivo studies | [102] |
| | | | Inhibition of EWS-FLI1 activity and its downstream targets with trabectedin and its analogues | In vitro/in vivo studies | [40, 98-101] |
| | | | Inhibition of EWS-FLI1 downstream target CDK7/12/13 with low molecular weight CDK7/12/13 inhibitor THZ71/THZ531 | In vitro/in vivo studies | [102] |
| | | | Inhibition of EWS-FLI1 using PARP inhibitor olaparib | In vitro/in vivo studies | [30, 31] |
| | | | Inhibition of EWS-FLI1 using HDAC and DMT inhibitors | In vitro/in vivo studies | [27] |
| | | | Inhibition of EWS-FLI1 using combination therapy with PARP inhibitors and trabectedin | In vitro/in vivo studies | [103, 104] |
| | | | Same approaches as in case of EWS-FLI1-positive disease could be used as differences in the C-terminal partner in gene fusions are not associated with significant phenotypic differences | In vitro/in vivo studies | [105, 106] |
| Ewing sarcoma and peripheral primitive neuroectodermal tumor | t(11; 22) (q24; q12) | EWS-ERG (aberrant transcription) | Not described | Not described | [107] |
| | t(17; 22) (q12; q12) | EWS-ELAF (aberrant transcription) | EWS-FEV (aberrant transcription) | EWS-ETV1 (aberrant transcription) | EWS-ETV4 (aberrant transcription) | Not described | Not described | Not described | Not described | [81] |
| | inv(22) (q12; q12) | EWS-PATZ1 (aberrant transcription) | EWS-SP3 (aberrant transcription) | EWS-NFATC2 (aberrant transcription) | EWS-SMARCA5 (aberrant transcription) | Not described | Not described | Not described | Not described | [110] |
| | t(2; 22) (q31; q12) | FUS-ERG (aberrant transcription) | FUS-FEV (aberrant transcription) | Inhibition of FUS-ERG downstream targets CDK4/6 and IGFR1 with linsitinib and palbociclib | In vitro/in vivo studies | [112] |
| | t(2; 16) (q16; p11) | Not described | Not described | | Not described | Not described | [81] |
| | t(4; 22) (q31; q12) | Not described | Not described | | Not described | Not described | [111] |
| | t(16; 21) (p11; q22) | Not described | Not described | | Not described | Not described | [81] |
| Tumor type                                    | Translocation       | Fusion product                          | Targeted therapy approach based on genetic testing                                                                 | Stage of investigations | Reference |
|----------------------------------------------|---------------------|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------|-------------------------|-----------|
| Extraskeletal myxoid chondrosarcoma           | t(9; 17) (q22; q11) | RBPP56-NR4A3 (aberrant transcription)   | Not described                                                                                                           | Not described           | [113]     |
|                                              | t(9; 15) (q22; q21) | TCF12-NR4A3 (aberrant transcription)     | Not described                                                                                                           | Not described           | [114]     |
|                                              | t(2; 22) (q34; q12) | EWS-FEV (aberrant transcription)         | Not described                                                                                                           | Not described           | 30776935  |
|                                              | t(9; 22) (q22; q12) | EWS-NR4A3 (aberrant transcription)       | Correlation in survival after sunitinib-based therapy and the presence of EWS-NR4A3 translocation                   | Clinical report         | [115, 116]|
| Fibromyxoid sarcoma, low grade               | t(7; 16) (q33; p11) | FUS-CREB3L2 (aberrant transcription)     | Inhibition of FUS-CREB3L2 and FUS-CREB3L1 potential downstream target CD24                                               | In silico studies       | [117]     |
|                                              | t(11; 16) (p11; p11) | FUS-CREB3L1 (rare) (aberrant transcription) | Not described                                                                                                           | Not described           | [118]     |
|                                              | t(11; 22) (q11; q12) | EWS-CREB3L1 (aberrant transcription)     | Not described                                                                                                           | Not described           | [118]     |
| Glomus tumor                                 | t(1; 5) (p13; q32)  | MIR143-NOTCH2 (aberrant transcription)   | Not described                                                                                                           | Not described           | [81]      |
|                                              | t(5; 9) (q32; q34.3) | MIR143-NOTCH1 (aberrant transcription)   | Not described                                                                                                           | Not described           | [81]      |
| Inflammatory myofibroblastic tumor           | t(2; 19) (p23; p13.1) | TPM4-ALK (aberrant transcription)       | Inhibition of the expression of ALK fusion genes by low molecular weight compounds of natural and synthetic origin       | Phase II-III clinical trials | [23]     |
|                                              | t(2; 2) (p23; q23)  | TPM3-ALK (aberrant transcription)       | Inhibition of the expression of ALK fusion genes by ALK inhibitor crizotinib                                             | In silico/in vitro studies | [22]     |
|                                              | t(2; 11) (p23; p15) | CLTC-ALK (aberrant transcription)        | Inhibition of the expression of ALK fusion genes by low molecular weight compounds of natural and synthetic origin       | Phase II-III clinical trials | [23]     |
|                                              | inv(2) (p23; q35)   | RANBP2-ALK (aberrant transcription)      | Inhibition of the expression of ALK fusion genes by ALK inhibitor crizotinib                                             | In silico/in vitro studies | [22]     |
|                                              | t(2; 4) (p23; q21)  | CAR5-ALK (aberrant transcription)        | Inhibition of the expression of ALK fusion genes by ALK inhibitor crizotinib                                             | In silico/in vitro studies | [22]     |
|                                              | t(2; 12) (p23; p12) | ATIC-ALK (aberrant transcription)        | Inhibition of the expression of ALK fusion genes by ALK inhibitor crizotinib                                             | In silico/in vitro studies | [22]     |
|                                              | t(8; 8) (q13; q21)  | HEY1-NCOA2 (aberrant transcription)      | Not described                                                                                                           | Not described           | [119]     |
| Mesenchymal chondrosarcoma                   | t(6; 22) (p21; q12) | EWS-POUSF1 (aberrant transcription)      | Not described                                                                                                           | Not described           | [120]     |
| Myoepithelial tumors                         | t(19; 22) (q13; q12) | EWS-ZNF444 (aberrant transcription)      | Not described                                                                                                           | Not described           | [81]      |
|                                              | t(1; 22) (q23; q12) | EWS-PBX1 (aberrant transcription)        | Not described                                                                                                           | Not described           | [81]      |
| Myxoinflammatory fibroblastic sarcoma/       | t(1; 10) (p33; q34) | TGFB8R3-MGFA5 (amplification of VGLL3)   | Not described                                                                                                           | Not described           | [81]      |
| hemosiderotic fibrolipomatous tumor          | and amplification of 3p11-12 |                                    | Not described                                                                                                           | Not described           | [81]      |
| Tumor type | Translocation | Fusion product | Targeted therapy approach based on genetic testing | Stage of investigations | Reference |
|------------|---------------|----------------|---------------------------------------------------|-------------------------|-----------|
| Myxoid liposarcoma | t(12; 16) (q13; p13) | FUS-DDIT3 (aberrant transcription) | Inhibition of FUS-DDIT3 expression with siRNAs | In vitro studies | [121] |
| | t(12; 22) (q13; q11-q12) | EWS-DDIT3 (aberrant transcription) | Inhibition of FUS-DDIT3 activity by direct binding of Trabectedin | In vitro studies | [39] |
| | | | Inhibition of FUS-DDIT3 downstream targets IGF-1R/PI3K/Akt with their known inhibitors | In vitro studies | [121] |
| | t(17; 22) (p13; q13) | MYH9-USP6 (aberrant transcription) | Not described | Not described | [122] |
| Ossifying fibromyxoid tumor | t(6; 12) (p21.2; q24.33) | EP400-PHF1 (aberrant transcription) | Not described | Not described | [123] |
| | t(1; 6) (p34.3; p21.2) | MEAIF-PHF1 (aberrant transcription) | Not described | Not described | [124] |
| | t(X; 22) (p11; q13) | ZC3H7B-BCOR (aberrant transcription) | Not described | Not described | [123] |
| | t(6; 10) (p21.2; p11) | EPC1-PHF1 (aberrant transcription) | Not described | Not described | [123] |
| Pericytoma | t(7; 12) (p22; q13) | ACTB-GLI1 (aberrant transcription) | Not described | Not described | [125] |
| Pseudomyogenic hemangiendothelioma | t(7; 19) (q22; q13) | SERPINE1-FOSB (aberrant transcription) | Inhibition of SERPINE1 with VEGFR1-4/PDGFR inhibitor telatinib | In vitro studies | [126] |
| Sclerosing epithelioid fibrosarcoma | t(7; 16) (q34; p11) | FUS-CREBL2 (aberrant transcription) | Inhibition of FUS-CREBL2 potential downstream target CD24 | In silico studies | [117] |
| Soft tissue angiofibroma | t(5; 8) (p15; q13) | AHRR-NCOA2 (aberrant transcription) | Not described | Not described | [127] |
| Solitary fibrous tumor | 12q13(inversion) | NAB2-STAT6 (aberrant transcription) | Not described | Not described | [128] |
| Congenital/infantile spindle cell rhabdomyosarcoma | t(2; 8) (q35; q13) | PAX3-NCOA2 (aberrant transcription) | Not described | Not described | [83] |
| | t(6; 8) (p12; q13) | SRF-NCOA2 (aberrant transcription) | Not described | Not described | [129] |
| | t(8; 11) (q13; p15) | TEAD1-NCOA2 (aberrant transcription) | Not described | Not described | [130] |
be characterized by specific chromosomal translocation, oncogenic mutation, or gene amplification. The adjustment of effective therapy strategy for STS forms without any molecular-genetic peculiarity remains a lottery game with unpredictable outcome. Phenotypic or functional screening can be an alternative to overcome this gap. It refers to the identification of antisarcoma activity of individual drugs or drug combinations using cell- or tissue-based models: chemosensitivity and resistance assays (CSRA). The development of CSRA was started in 1970s for identification of anticancer drugs for individual patients, and the first assays were based on colony-forming efficiency of tumor-derived cells in the presence of various drugs [8, 9]. Furthermore, CSRA were incorporated in a variety of detection systems but shared similar principles and general procedures: (a) tumor cells were isolated and established in an in vitro medium; (b) cells were incubated with the chemotherapeutic agent(s) of interest; (c) cell survival (or death) was assessed; and (d) a report detailing sensitivity and/or resistance to tested drugs was generated. Now, CSRA and patient-derived xenografts (PDX) are considered as an efficient approach to identify treatments or new therapeutic indications for approved drugs [10, 11]. In the second part of the review, we will discuss possible use of CSRA for the optimization of sarcoma treatment and current progress in the field.

### 2. Molecular Targeting Therapies for STS

#### 2.1. Inhibition of Target Oncogenic Protein Expression or Activity

Design and development of small molecule inhibitors have resulted in remarkable progress for treatment of certain cancers, particularly with drugs targeting protein kinases. Direct inhibitors are expected to work by specific binding and promotion of degradation and/or by specific binding and blocking sites required for target protein activation or interaction with other critical effector proteins. Certain progress has been made in directly targeting many transcription factors, in particular, chimeric kinases and mutant proteins involved in cancer development ([12] and Tables 1 and 2). However, the design of direct inhibitors for wild-type and fusion transcription factors can be attributed in part to the large protein-protein interaction interfaces and absence of deep protein pockets that are common targetable sites for drug design [13, 14]. Only a few molecules were

| Tumor type | Translocation | Fusion product | Targeted therapy approach based on genetic testing | Stage of investigations | Reference |
|------------|---------------|----------------|---------------------------------------------|------------------------|-----------|
| Synovial sarcoma | t(X; 18) (p11; q11) | SS18-SSX2 (aberrant transcription) | Inhibition of SS18-SSX2 downstream signaling pathways VEGFA, IGFR1, Wnt/b-catenin, and chromatin remodeling proteins with their known inhibitors (Wnt inhibitor monoclonal antibody FZD10, IGFR1 inhibitor cixutumumab, VEGFA inhibitor bevacizumab, HDAC inhibitors, trabectedin and sorafenib for multiple pathways) | Phase I-II clinical trials | [132] |
| SS18-SSX4 (rare) (aberrant transcription) | Inhibition of SS18-SSX4 downstream signaling pathways VEGFA, IGFR1, Wnt/b-catenin, and chromatin remodeling proteins with their known inhibitors (Wnt inhibitor monoclonal antibody FZD10, IGFR1 inhibitor cixutumumab, VEGFA inhibitor bevacizumab, HDAC inhibitors, trabectedin and sorafenib for multiple pathways) | Phase I-II clinical trials | [132] |
| Tenosinovial giant cell tumor | t(1; 2) (p13; q35-37) | COL6A3-CSF1 (aberrant transcription) | Not described | Not described | [133] |
| Undifferentiated round cell sarcoma | t(4; 19) (q35; q13) | CIC-DUX4 (aberrant transcription) | Not described | Not described | [134] |
| Xp11 (inversion) | BCOR-CCNB3 (aberrant transcription) | Not described | Not described | Not described | [135] |
### Table 2: Oncogenic mutations and gene amplifications in STS.

| Tumor type                      | Gene          | Therapy approach                                      | Stage of investigations       | Reference |
|---------------------------------|---------------|-------------------------------------------------------|-------------------------------|-----------|
| **Activating mutations**        |               |                                                       |                               |           |
| Gastrointestinal stromal tumor  | c-KIT         | Inhibition of c-KIT with imatinib, nilotinib, and      | Phase I-III clinical trials   | [52, 136, 137] |
|                                 | PDGFRA        | Inhibition of PDGFRA with olaratumab, imatinib,       | Phase I-III clinical trials   |           |
|                                 |               | pazopanib, pazopanib, regorafenib, sorafenib,         |                               | [138–141] |
|                                 |               | and sunitinib                                          |                               |           |
|                                 | BRAF          | Inhibition of BRAF with dabrafib and vemurafenib      | In vitro/in vivo studies;     |           |
| Myxoid round cell liposarcoma   | PI3CA         | Inhibition of PI3K/Akt signaling with multiple         | In vitro/in vivo studies      |           |
| **Inactivating mutations**      |               | known inhibitors                                      |                               |           |
| Malignant peripheral nerve      | NF-1          | Inhibition of NF-1 downstream Ras-dependent targets   | In vitro/in vivo studies      | [146]     |
| sheath tumor                    |               | Src kinase (CGP77675) and MEK-1 (U0126)               |                               |           |
| Rhabdoid tumors                 | INI1          | Inhibition of INI1 downstream targets, epigenetic     | In vitro/in vivo studies      | [50]      |
| Perivascular epithelioid cell   | TSC1/2        | Inhibition of mTOR signaling with known mTOR          | Phase I-II clinical trials    | [24]      |
| tumors                          |               | inhibitors temsirolimus, ridaforolimus, everolimus,   |                               |           |
|                                 |               | and sirolimus                                         |                               |           |
| **Gene amplifications**         |               |                                                       |                               |           |
| Dedifferentiated and well-      | MDM2          | Inhibition of MDM2 with antagonist RG7388             | In vitro/in vivo studies      | [26]      |
| differentiated liposarcoma      | CDK4          | Inhibition of CDK4 with palbociclib                   | Phase I-II clinical trials    | [25]      |
|                                 | c-JUN         | Not described                                         | Not described                 | [148]     |
| Intimal sarcomas                | MDM2          | Inhibition of MDM2 with antagonist RG7388             | In vitro/in vivo studies      | [26]      |
|                                 | CDK4          | Inhibition of CDK4 with palbociclib                   | Phase I-II clinical trials    | [25]      |

Described, designed as inhibitors of STS-specific aberrant proteins, which reached clinical trial. For example, LOXO10, an experimental, highly selective inhibitor of tropomysin-related kinases (TRK), is now involved in clinical trials of Phase I for treatment of infantile fibrosarcoma [4], which is nearly always characterized by a t(12; 15) (p13; q25) translocation [15]. This translocation fuses the ETS variant gene 6 (ETV6) in chromosome 12 with the neurotrophin 3 receptor gene (NTRK3) kinase domain, resulting in activation of multiple signaling cascades including the RAS and PI3K-AKT pathways [16]. Another small molecule, YK-4-279, is able to disrupt binding between fusion protein EWS-FLI1, specific for Ewing’s sarcoma [17], and RNA Helicase A (RHA), which is necessary to enhance the oncogenic activity of EWS-FLI1 [18]. Therapeutic efficacy of YK-4-279 was proved in in vitro and in vivo models [19]. The YK-4-279 analog TK216 is currently being used in a Phase I clinical trial in patients with relapsed or refractory Ewing’s sarcoma [20]. Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase involved in the genesis of several human cancers, in particular, inflammatory myofibroblastic tumor (IMT), which is characterized by ALK-based chromosomal translocations t(2; 19) (p23; p13.1), t(1; 2) (q22-23; p23), t(2; 17) (p23; q23), t(2; 2) (p23; q23), etc. [21]. To date, FDA-approved ALK inhibitor Crizotinib is undergoing clinical trials of Phase II-III for IMT treatment ([22] and clinical trial NCT03874273), and several small molecules with the potency to inhibit ALK are under investigation [23]. Liposarcomas and intimal sarcomas form distinct STS subset, where MDM2 and CDK4 gene amplifications serve as oncogenic drivers as well as therapeutic targets [24]. Low molecular weight inhibitors were described for both genes, in particular, CDK4 inhibitor palbociclib is currently involved in clinical trials [25], and experimental MDM2 inhibitor demonstrated anticancer activity in vitro and in vivo [26].

Genetic inhibition of fusion gene by antisense oligonucleotides or siRNA could be another option. Thus, it was reported that antisense oligonucleotides and siRNA inhibited expression of EWS-FLI1, chimeric gene specific for Ewing’s sarcoma, in cell cultures and in the xenograft model in vivo [27], as well as decrease of SS18-SSX1 gene expression in synovial sarcoma [28].

As modeling and designing of direct inhibitors for known fusion genes/proteins are challenging, other strategies have been explored. There has been significant progress in several approaches, such as targeting transcriptional coactivators, phosphorylation modulators, and upstream regulators of chimeric kinases. Phosphorylation of EWS-FLI1 could be disrupted by Engerlin A, an active constituent of the plant Phyllanthus engleri, as it was demonstrated in vitro [29]. Furthermore, epigenetic regulators histone deacetylases (HDACs) and demethylases (DMT) and DNA repair enzyme poly-(ADP-ribose) polymerase 1 (PARP1) are transcriptional partners of EWS-FLI1, which formed a solid ground for use of PARP inhibitor olaparib, HDAC inhibitor entinostat, and DMT inhibitor HCl-2509 in preclinical and clinical studies for Ewing’s sarcoma therapy [27, 30, 31].
Efforts have been made to find the kinases, which are responsible for PAX3-FOXO1 phosphorylation, and to attenuate their activity using siRNA or known inhibitors [32]. Moreover, epigenetic regulators influence PAX3-FOXO1 interaction with transcriptional partners as well. Therefore, use of HDAC inhibitors in the treatment of PAX3-FOXO1-positive rhabdomyosarcoma represents attractive therapeutic strategy [32].

Known FDA-approved low molecular weight inhibitors, such as already mentioned PARP and HDAC inhibitors, form a separate group of targeted drugs for STS treatment. Drug repurposing refers to the application of a drug for another indication than it was originally approved for. It has received increasing interest as an alternative strategy to de novo drug synthesis [33] as usage of known therapeutics gone through preclinical and clinical studies could drastically decrease the time and the cost of investigations. Moreover, it meets the demand of social changes from overconsumption to rational reuse and recycle. Despite the main problem in the drug repurposing approach associated with inability to patent the known drugs for novel application and, therefore, with certain difficulties in fundraising and absence of massive studies, there are a number of repurposed medical, in particular, in cancer treatment. There are different approaches to drug repurposing including target-based, integrating disease-associated proteins, biomarkers, and pathway knowledge to identify a specific new target or mechanism and therapeutic use; drug-based, identifying similarities between molecular structures of existing drugs; and disease-based, finding new strategies to drug intervention in a disease. Significant example of the identification of a new target is imatinib mesylate, initially designed as a BCR-ABL inhibitor and used in patients with chronic myeloid leukemia (CML). Imatinib was found to specifically target PDGFRB tyrosine kinase, and so its use was approved for treating COL1A1-PDGFRB-positive dermatofibrosarcoma [5]. Based on the same strategy, tyrosine kinase inhibitor suntinib was included in clinical trials for imatinib-resistant dermatofibrosarcoma therapy [6, 7]. Furthermore, the drug repurposing approach is even more applicable for STS with oncogenic mutations: gastrointestinal stromal tumors (GIST) with activating mutations in c-KIT, PDGFA and BRAF, myxoid round cell liposarcoma with activating mutation in PI3K/Akt signaling component PI3CA ([24] and Table 2). Multitargeted low molecular weight inhibitors as imatinib, nilotinib, pazopanib, sorafenib, sunitinib, dabrafenib, vemurafenib, mTOR, and PI3K/Akt inhibitors have demonstrated their anticaner activity and therapeutic potential for treatment of STS in preclinical and clinical studies [34].

Special attention should be given for marine-derived natural product trabectedin, initially isolated from the marine ascidian Ecteinascidia turbinata [35]. Currently, trabectedin is a validated option for the treatment of patients with advanced STS as target molecule [36]. The compound was described to interfere directly with activated transcription, to poison the nucleotide excision repair system, and to generate double-strand DNA breaks (DSBs) [36]. The drug has shown a high selectivity for myxoid liposarcoma, characterized by the translocation t(12; 16) (q13; p11) leading to the expression of FUS-DDIT3 fusion gene. Trabectedin appears to bind directly with the chimeric protein and to impair transactivating activity of FUS-CHOP [37]. Similar results were obtained for trabectedin and EWS-WT1-positive desmoplastic small round cell tumor [38], myxoid liposarcoma with EWS-DDIT3 [39], and Ewing’s sarcoma with EWS-FLI1 translocation [40].

2.2. Inhibition of Downstream Effectors of Oncogenic Protein.
All oncogenic genetic alterations in STS start aberrant activation of transcription through multiple downstream targets whose expression is proposed to promote tumorigenesis. High-throughput technologies such as DNA microarray, RNA sequencing, and chromatin immunoprecipitation sequencing (ChIP-Seq) have enabled generation of comprehensive signatures of downstream targets expressed in specific STS subtypes driven by oncogenic mutation or chromosomal translocation. Moreover, this approach is also applicable for sarcomas with complex karyotypes, which often lack tumour-specific genetic abnormalities.

The insulin-like growth factor (IGF) signaling contributes to tumorigenesis through IGF1R phosphorylation and activation of several cancer-related pathways to regulate cell growth and tumorigenesis in a variety of malignancies [41]. It is known that EWS-FLI1 protein, which is characteristic for Ewing’s sarcoma, binds the promoter of insulin-like growth factor binding protein 3 (IGFBP3) to suppress the expression of IGFBP3, which sequesters circulating IGF1. These results suggest a crosstalk between the oncogenic function of EWS-FLI1 and the IGF1R signaling [27]. Similar observation was made for EWS-WT1-positive desmoplastic small round cell tumor [42]. Based on these findings, the studies of anticancer activity of IGF1R inhibitors, monoclonal antibodies R1507, MK-0646, cixutumumab, ganitumab, and figitumumab were started for several STS subtypes; some of them reached clinical trials [27, 42].

Endoglin, coreceptor of the ‘’TGFβ family, is an interesting and perspective target for Ewing’s sarcoma as its high expression is associated with poor prognosis of the disease. Thus, monoclonal endoglin-targeting antibodies, TRC105, OMTX503 and OMTX703, have demonstrated the decrease in tumor growth in Ewing sarcoma cell line-derived xenografts and patient-derived xenografts as well as in angiosarcoma in clinical trials of Phase I/II [43, 44].

It was reported that EWS-FLI1 protein upregulated the expression of Aurora kinases A and B (AURKA, AURKB) by direct binding to their promoters [45]. As AURKA and AURKB are serine/threonine kinases, regulators of mitosis, and diverse signal transduction pathways, and their overexpression is associated with tumorigenesis, they have become a promising therapeutic option in cancer therapy [46]. Efficacy of antitumor action of AURK inhibitors MLN8823 and tozasertib was demonstrated in vitro on Ewing’s sarcoma cell lines and in vivo in xenograft models [27].

Ezrin, an ezrin-radixin-moesin protein, presents another interesting target for STS therapy. This protein links
the membrane and cytoskeletal actin to promote cell mobility, adherence, signal transduction, and activation of tyrosine kinases [47]. As increased metastatic potential and decreased survival have been observed in rhabdomyosarcoma, Ewing’s sarcoma, myxofibrosarcoma, chondrosarcoma, etc., it was proposed that targeting ezrin is expected to prevent metastatic progression [2]. Direct inhibitors of ezrin, small molecules NSC305787 and NSC668394, demonstrated statistically significant reduction in tumor growth in vitro using the model of osteosarcoma [48]. Moreover, ezrin is a downstream target of SMARCB1/INI1, regulator of chromatin remodeling, and potential tumor suppressor [49]. Inactivating mutation in SMARCB1/INI1 is an oncogene driver in rhabdoid tumors, and the loss of functional SMARCB1/INI1 leads to increase in ezrin expression [50]. In vitro and in vivo studies demonstrated the efficacy of ezrin inhibitor DZNep in treatment of this subset of STS [50].

Besides the abovementioned pathways and molecules, Notch, Wnt/β-catenin, PI3K/Akt/mTOR, VEGF, and other signaling pathways may also promote tumor cell proliferation, survival, migration, angiogenesis, and metastasis in sarcomas. Moreover, multiple studies have already demonstrated the perspectives of the suppression of key components of these cascades for the therapy of STS (Tables 1 and 2). However, reaching therapeutic efficacy of newly developed as well as approved drugs on patient tumors is still challenging and demands additional approaches to find out the effective treatment. Use of chemotherapy sensitivity and resistance assays together with genetic testing could be a step to significant improvement of STS therapy.

2.3. Immunotherapy of Soft Tissue Sarcomas. Nowadays, inhibitors of immune system checkpoints are considered as the most promising drug category for many malignancies, and they have been already applied in STS therapy also [51]. The expression of ligand of programmed death-1 (PD-L1) and PD-L2 was considered as one of the most important biomarkers for PD-1 inhibitor assignment; a high expression of PD-L1 could be a predictive factor of response to anti-PD-1 therapy, and in some malignancies, such as non-small-cell lung cancers, the evaluation of PD-L1 expression was in separably linked to the indication of immunotherapy [52]. The expression of PD-L1 in soft tissue sarcomas has been evaluated, and anti-PD-1 therapies showed clinical evidence of benefit [53]. However, in prospective clinical trials, anti-PD-1 therapy for STS resulted in minimal patient responses [54, 55]. Activation of indoleamine 2,3-dioxygenase 1 (IDO1) by anti-PD-1 therapy could be a new target of combined immunotherapy [56]. Immunotherapy with chimeric antigen receptor-modified T cells (CART) or dendritic cells has been also investigated [52]. Targeted immunotherapy with the cancer-testis antigen NY-ESO-1 for synovial sarcoma has shown especially promising results for patients with a specific human leukocyte antigen (HLA) haplotype, HLA-A*0201 [57].

A critical challenge of evaluation of immunotherapy application in STS management is the rarity of the disease and heterogeneity of its subtypes. Multicenter preclinical and clinical study collaborations are needed to efficiently enroll enough patients to assess efficacy of specific therapy. It appears that STS subtypes exhibit varying sensitivity to a particular immunotherapeutic strategy. Therefore, clinical trials should ideally be conducted for a specific STS subtype rather than collectively for all STS. Similarly, preclinical research should focus on an understanding of the native immune response and inhibitory mechanisms present in the tumor microenvironment that are unique and specific to each STS subtype. With the differences in STS biology, clinical behavior, and response to therapy, it is very likely that the immune response is also distinct between subtypes of disease. These immunologic differences need to be recognized and appropriately incorporated into the design of immunotherapeutic strategies for each STS subtype.

3. Drug Sensitivity Testing on Patient-Derived STS Cells

The majority of the CSRA has been developed during the past 20–30 years. Some of them have been revised, improved, and currently in use in clinical trials. However, none of these assays is in the routine clinical use due to their complex design and still lacking a strong correlation between results of testing in vitro and therapeutic outcome in vivo. Personalized treatment approaches take into account individual tumor characteristics: oncogenic mutation, chromosomal translocation, specific gene amplifications, and protein expression levels. Personalized CSRA testing could be a further step in identifying the appropriate chemotherapeutics and molecular targeting agents.

Studies describing CSRA in soft tissue sarcoma patients are largely missing. There are multiple studies describing cytotoxicity assays in sarcoma cell lines and anticancer activity in vivo, mainly in xenograft models [58–61]. More than 600 established sarcoma cell lines are available for screening, and, as STS is a highly heterogeneous group of cancer, there needs to be even a larger number of cell lines, with various histological subtypes, to better benefit sarcoma research [62].

There are a number of studies based on patient-derived cells (PDC) and patient-derived xenograft (PDX) model in vivo. However, most of the described investigations are focused on establishment of new cell line derived from patient tumor (for example, see [63–67], on preclinical studies of novel or repurposed drug/combination of drugs in vitro [68–71] and in vivo [72–74]). Only a few studies address the optimization of STS treatment. Thus, the efficacy of temozolomide treatment was demonstrated in the PDX model of doxorubicin-resistant undifferentiated spindle-cell sarcoma [75]. Moreover, Igarashi et al. concluded that the PDX model used in the study could identify promising therapies with significantly greater efficacy than first-line therapy for this recalcitrant disease. In another study of predictive models for response to therapy, 29 samples of patient tumors were engrafted in immunodeficient mice (“TumorGraft” method) and 22 (76%) of them were
subsequently engrafted in mice for drug sensitivity testing. The most relevant finding was that TumorGraft could predict response to treatment in 13 of 16 cases of sarcoma patients undergoing treatment. The main disadvantage of this model system was the fact that period duration from tumor engraftment to drug sensitivity assay fulfillment was several months [76], which presents a dramatic limitation for patients with progressive disease. However, these results demonstrate that patient-derived sarcoma cells or xenografts are relevant models that can be used to identify effective treatments for sarcoma patients. In line with this study, Brodin et al. performed the genomic profile and drug sensitivity testing of samples from sarcoma patients and showed that drug sensitivity of the patient sarcoma cells ex vivo correlated with the response to the actual treatment. ATP-TCA assay was used for evaluation of the viable cell number [77].

A growing evidence suggests that more complex three-dimensional (3D) models are necessary to properly mimic many of the critical hallmarks of soft tissue sarcoma. A number of innovative methods are being studied to fabricate biomimetic sarcoma tumors, encompassing both the surrounding cellular milieu and extracellular matrix. For example, certain advantages were described for 3D models of Ewing’s sarcoma [78]. These pilot studies show that patient-derived sarcoma cells can be isolated from biopsies and expanded in vitro for drug sensitivity testing. This rapid approach does not require budget- and time-consuming immunodeficient animals and can predict the patient response to standard or experimental treatments. However, trials with larger cohorts need to be performed to confirm its clinical value.

4. Conclusion

Given the genetic and histological diversity of this large family of cancers, the treatment of STS calls for a multi-disciplinary approach to achieve optimal outcomes. Future studies in the field should be focused on identification of known specific molecular markers in patient tumor tissue, identification and validation of new molecular targets, and validation and prospective use of drug-sensitivity test systems in vitro.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Kirill I. Kirsanov and Ekaterina A. Lesovaya have equally contributed to this paper.

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