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Pharmacogenomics of soft tissue sarcomas: New horizons to understand efficacy and toxicity

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ABSTRACT

Clinical responses to anticancer therapies in advanced soft tissue sarcoma (STS) are unfortunately limited to a small subset of patients. Much of the inter-individual variability in treatment efficacy and risk of toxicities is as result of polymorphisms in genes encoding proteins involved in drug pharmacokinetics and pharmacodynamics. Therefore, the detection of pharmacogenomics (PGx) biomarkers that might predict drug response and toxicity can be useful to explain the genetic basis for the differences in treatment efficacy and toxicity among STS patients. PGx markers are frequently located in transporters, drug-metabolizing enzyme genes, drug targets, or HLA alleles. Along this line, genetic variability harbouring in the germline genome of the patients can influence systemic pharmacokinetics and pharmacodynamics of the treatments, acting as predictive biomarkers for drug-induced toxicity and treatment efficacy. By linking drug activity to the functional complexity of cancer genomes, also systematic pharmacogenomic profiling in cancer cell lines and primary STS samples represents area of active investigation that could eventually lead to enhanced efficacy and offer a powerful biomarker discovery platform to optimize current treatments and improve the knowledge about the individual's drug response in STS patients into the clinical practice.

Introduction

Soft tissue sarcomas (STS) are heterogeneous rare malignancies accounting for less than 1% of all cancers [1]. They include more than 70 histological and molecular subtypes with varying biology, molecular aberrations, and variable response to treatment [2]. Generally, surgery with or without radiation, only in selected cases of localized disease, represents the preferred treatment of STS [3]. Anthracycline-based therapy is the standard first-line treatment [I, A]. There are no formal data that multi-agent chemotherapy (ChT) is related to an improved overall survival (OS) than single-agent ChT with doxorubicin. Nevertheless, a higher response rate and longer progression free survival (PFS) can be estimated in a number of selected for expected better sensitivity histological types in light of evidences from several randomised, clinical trials [4]. Doxorubicin plus dacarbazine is an optional multi-agent first line regimen for leyomiosarcoma, in which the activity of ifosfamide is debacted [5]. Gemcitabine represents a therapeutic option, alone or in combination with docetaxel [6,7]. Notably, a phase III study compared single-agent doxorubicin with the combination of gemcitabine-docetaxel as first-line regimen in advanced STS patients of all types. The combination did not show improvement in PFS and objective response rate (ORR) and is not recommended as upfront therapy for advanced STS patients [7] including uterine LMS. Taxanes can be a valid treatment options for angiosarcoma, which has shown high sensitivity to this ChT [8]. Neurotrophic tyrosine receptor kinase (NTRK) inhibitor larotrectinib represent standard treatment of those rare patients with locally-advanced or metastatic NTRK rearranged sarcomas [9,10,11]. The evidence for systemic therapy beyond the

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Table 1
Most relevant SNPs emerged as biomarkers related to toxicity in STS.

GENE	SNP	Cancer type	Therapy	Toxicity	Reference
SLC22A16	rs714368 rs6907567 rs723685	ASTS	doxorubicin	reduced frequency of grade 3–4 AE (rs723685)	*Seddon 2017*
ABCB1	rs1128503	ASTS	trabectedin	decreased risk of severe hepatic cytolysis	*Maillard 2020*
	rs2032582	ASTS	trabectedin	decreased risk of overall hepatotoxicity	*Maillard 2020*
ABCC2	rs717620 rs8187707 rs8187710	LPS	trabectedin	Irreversible hepatotoxicity	*Laurenty 2013*
	rs2273697	ASTS	trabectedin	increased risk of hepatic cytolysis	*Maillard 2020*
	rs17222723	ASTS	trabectedin	decreased risk of hepatic cytolysis	*Maillard 2020*
ABCC3	rs2072365	ASTS	trabectedin	increased the risk of overall hepatotoxicity and especially severe cytolysis	*Maillard 2020*
ABCC4	rs9516519	ASTS	trabectedin	decreased risk of overall hepatotoxicity	*Maillard 2020*
ABCG2	rs7699188	ASTS	trabectedin	increased risk of hepatic cytolysis	*Maillard 2020*
CYP3A5	rs776746	ASTS	trabectedin	increased risk to present a grade 3/4 hepatic event	Maillard 2020
ITGA	rs1126643	STS	apatinib	surgical wound complications and spontaneously pneumothorax	Bao Q 2019

Abbreviations: SNP= single nucleotide polymorphism; STS= soft tissue sarcoma; AE=adverse event; ASTS= advanced STS; LPS= liposarcoma.

first-line is less robust. Patients who have already received ChT may be treated with ifosfamide if they did not progress on it previously [12, 13]. Trabectedin is an option in advanced STS in second and further lines of treatment, with some degree of efficacy in LMS and liposarcoma [14]. A randomised trial showed a benefit in PFS averaging 3 months for tirosyne kinase inhibitor (TKI) pazopanib, which exerts its clinical antitumor effects through inhibiting vascular endothelial growth factor receptor (VEGFR)-mediated angiogenesis, fibroblast growth factor receptors (FGFRs), and c-kit in previously treated, advanced STS patients (excluding liposarcomas) [15].Thus, it represents an option in non-adipogenic STS in second and further lines setting of treatment. In addiction, a randomised phase III trial showed that eribulin was superior to dacarbazine in patients with liposarcomas and LMS. The median difference in OS was 2 months and reached 7 months in liposarcomas [16]. The combination of dacarbazine-gemcitabine or gemcitabine-docetaxel is an option in doxorubicin pretreated patients. One trial showed that gemcitabine-docetaxel is more effective than gemcitabine alone as second- or furtherline ChT, especially in LMS patients; in both trials, toxicity was superior with the combination of docetaxel-gemcitabine [17]. Gemcitabine showed antitumour activity also in LMS, angiosarcoma and epithelioid sarcomas given as a single agent [6; 18]. A randomized trial reported improved OS and PFS with combinatorial treatment of gemcitabine-dacarbazine over dacarbazine alone [19].

The most frequent adeverse events associated with drug used for STS treatment were hematologic (leucopenia; neutropenia; anemia:; thrombocypenia), asthenia, neurotoxicity, alopecia, diarrhea, nausea, fever, hepatotoxicity. Rarely, trabctededin treatment is associated with elevated creatine kinase and rhabdomyolysis [14]. Tretament with TKI pazopanib might be associated with hypertension [15].

However, clinical responses to anticancer therapies continue to be limited to a small subset of patients. Much of the inter-individual variability in drug efficacy and risk of toxicities is due to polymorphisms in genes encoding proteins involved in drug pharmacokinetics and pharmacodynamics [19, 20]. In light of this evidence, the detection of pharmacogenomics (PGx) biomarkers that can predict drug response and toxicity can be useful to explain the genetic basis for the differences in treatment efficacy and toxicity among patients [20]. PGx markers predicting efficacy or risk to develop toxicity are frequently located in transporters, drug-metabolizing enzyme genes, drug targets, or HLA alleles [20]. In this scenario, genetic variability harbouring in the germline genome of the patients can influence systemic pharmacokinetics and pharmacodynamics of the treatments, acting as predictive biomarkers for drug-induced toxicity and treatment efficacy [20]. The most common germline variations, that can be used as suitable biomarkers for toxicity and drug response, are represented by very penetrant predisposed mutations and frequent genetic variants mostly hereditary single-nucleotide polymorphisms (SNPs) [20, 21, 22,23]. By linking drug activity to the functional complexity of cancer genomes, also systematic pharmacogenomic profiling in cancer cell lines may offer a powerful biomarker discovery platform to optimize current treatments and improve the knowledge about the individual's drug response in STS

Table 2
Most relevant SNPs emerged as prognostic/predictive biomarkers in STS.

GENE	SNP	Cancer type	Therapy	PFS	OS	Reference
SLC22A16	rs714368 rs6907567 rs723685	ASTS	doxorubicin	↓		*Seddon 2017*
SLC29A1	rs9394992	ASTS	gemcitabine and docetaxel	↓	↓	*Seddon 2017*
ABCB1	rs1045642	ASTS	gemcitabine and docetaxel	↓		*Seddon 2017*
CDA	rs2072671	ASTS	gemcitabine and docetaxel		↓	*Seddon 2017*
PRDX4	rs518329	ASTS	doxorubicin		↑	*Seddon 2017*
CMPK1	rs4492666	ASTS	gemcitabine and docetaxel		↑	*Seddon 2017*
BRCA1	rs16941 rs16942 rs1799966 rs799917 (AAAG)	ASTS	trabectedin	↑	↑	*Italiano 2011*
GSTA1	rs3957357C>T (TT)	ASTS	doxorubicin	↑		Gelderblom 2013
CYP2B6	rs2279343	RMS	cyclophosphamide	↑		Labib 2016
PER2	rs7602358	LPS	–		↑	Benna 2018
VEGFR2	rs2071559	STS	apatinib	↑		Bao Q 2019

Abbreviations: SNP= single nucleotide polymorphism; STS= soft tissue sarcoma; PFS= progression free survival; OS=overall survival; ASTS= advanced STS; RMS= rhabdomyosarcoma; LPS= liposarcoma.

patients.

Seversl studies showed a correlation between genetic variations, clinical outcome and toxicity in several cancer types. TSER*2, TSER*3 variations in the thymidylate synthetase gene has been reported to influence efficacy and toxicity in patients with colorectal, bladder and gastric cancer treated with 5-fluorouracil and capecitabine. Analogously 667 C > T, 1298A > C variations in the methylene tetrahydrofolate reductase gene influence efficacy and toxicity in patients with colorectal, ovarian and gastric cancer treated with 5-fluorouracil and methotrexate and 496 C > T, 8092 C > A, 19,007 T > C variations in the excision repair cross complementing group 1 (ERCC1) gene influence efficacy and toxicity in patients with non-small cell lung carcinoma and bladder cancer treated with platinum containing anti-cancer drugs. CYP2D6 variant alleles in the cytochrome P450 2D6 gene influence efficacy and toxicity in patients with breast cancer treated with tamoxifen [24]. The implementation of pharmacogenomics data in oncology is essential to select more suitable therapies to predict toxicity and accordingly modify drug doses without affecting efficacy. In light of the correlation between pharmacogenomics and several types of cancer, combined with the knowledge that clinical responses to anticancer therapies in advanced STS are unfortunately limited to a small subset of patients, we collected data derived from pharmacogenomics studies in STS patients.

In this review, we report recent studies on PGx biomarkers that have been described to be active on modulation of clinical outcomes and/or toxicity (Table 1 and 2) of anti-cancer drugs in several STS histotypes.

The human solute carrier (SLC)

The solute carrier family (SLC) are critical membrane transport proteins carrying different solutes such as inorganic ions, amino acids, lipids, neurotransmitters and drugs [14,15]. Among the SLC family the organic cations transporters (OCTs) and of nucleoside transporters (NTs) have been studied in STS patients.

Organic cation transporters (OCTs) are influx transporters belonging to the SLC family 22 (SLC22), which contains four subtypes, OCT1 (SLC22A1), OCT2 (SLC22A2), OCT3 (SLC22A3), and OCT6 (SLC22A16). OCTs were shown to exert a critical role in the cellular uptake of antineoplastic drugs such as cisplatin, oxaliplatin and gemcitabine [25]. Several studies suggest that alteration in OCT6 expression and activity may have profound implications on the pharmacokinetic and pharmacodynamic of doxorubicin in cancer patients [26]. In light of this evidence, we herein discuss about OCT6 (SLC22A16).

OCT6 (SLC22A16). OCT 6 (SLC22A16) is constitutively expressed in leukemic as well as a variety of solid cancer cell lines. In vitro studies in *Xenopus oocytes* expressing SLC22A16 demonstrated a dose-dependent and saturable kinetics with regards to influx of doxorubicin. Moreover, Jurkat T-cell lymphoma cells overexpressing SLC22A16 are increasingly susceptible to the cytotoxic effects of doxorubicin, and this was postulated to result from the increased influx of the drug. These studies suggest that alterations in the expression and activity of SL22A16 may have profound implications on the pharmacokinetics and pharmacodynamics of doxorubicin in cancer patients [26, 27].

GeDDiS phase 3 trial (who has randomized patients with untreated unresectable or metastatic STS to receive front-line therapy with gemcitabine and docetaxel versus doxorubicin) demonstrates that SNPs in the gene SLC22A16 (rs714368, rs6907567, rs723685) are associated with reduced efficacy following doxorubicin treatment. Three of the four SNPs analyzed in the SLC22A16 gene were associated with a worse progression free survival (PFS) (rs714368 and rs6907567 AG $n = 45$ with HR = 1.75, GG $n = 4$ with HR = 1.33 and rs723685 CT $n = 21$ with HR = 1.72). Furthermore, the SLC22A16 rs723685 minor allele was associated with a reduced frequency of grade 3–4 adverse events (AEs) compared with wild type (WT) (10 of 21 patients[48%] vs 69 of 97 [71%] patients) in the doxorubicin treatment group ($p = 0.04$) [28].

Nucleoside Transporters (NTs) belong to the SLC family and are transmembrane proteins that act as the cell's gatekeepers for purine and pyrimidine nucleobases and nucleosides [29]. The NTs are classified into two major classes, sodium-dependent concentrative transporters (CNTs; SLC28) and equilibrative (ENTs; SLC29) NTs. Human ENT1 (hENT1; SLC29A1) is the best-studied SLC29 member [30, 31].

Human ENT1 (hENT1; SLC29A1). hENT1 (SLC29A1) was detected in erythrocytes and hence was expressed in heart, brain, mammary gland and placenta, and also in fetal liver and spleen, mediating both influx and efflux of nucleosides across the membrane (equilibrative transporter). Thus, it is also critical for cellular uptake of gemcitabine, who is a cytotoxic pyrimidine antimetabolite used as a first-line chemotherapeutic agent for the treatment of a wide range of solid tumors and hematologic malignancies [30]. SLC29A1 is a transmembrane protein that is thought to be responsible for the intracellular uptake of the prodrug gemcitabine into tumor cells [30]. The association between hENT1 expression and gemcitabine efficacy was demonstrated in different tumor types including cholangiocarcinoma [31, 32], bladder cancer [33] and non-small cell lung cancer (NSCLC) [34]. Recently, a retrospective study has analyzed the relationship between hENT1 expression and clinical outcome in leiomyosarcoma and angiosarcoma patients treated with gemcitabine [35]. Patients with high levels of hENT1 showed a better outcome in terms of PFS and overall survival (OS) both in leiomyosarcoma (PFS: 6.8 vs 3.2 months; OS: 14.9 vs 8.5 months) and angiosarcoma (PFS was 9.3 vs 4.5 months; OS 20.6 vs 10.8 months) compared to those with low hENT1 levels [35]. Since gemcitabine, as a single agent or in combination with docetaxel, has been widely adopted also in leiomyosarcoma and angiosarcoma [6,7], the identification of molecular markers such as hENT1 could be useful to select patients with a high likelihood of benefiting from this CT regimen. Furthermore, the GeDDiS phase III study has shown that SNPs in the gene SLC29A1 (rs9394992, rs760370) are related to decreased efficacy of gemcitabine and docetaxel treatment. More in detail, one of the two SNPs analyzed was associated with a worse PFS (rs9394992 CT $n = 51$ with HR = 1.04, TT $n = 9$ with HR = 1.92); both rs9394992 and rs760370 were associated with a worse OS (rs9394992 CT $n = 51$ with HR = 1.11, TT $n = 9$ with HR = 1.86; rs760370 AG $n = 56$ HR 1.02, GG $n = 15$ HR 1.19). No differences in frequency of grade 3–4 AEs related to gemcitabine and docetaxel were observed [7].

ATP-binding cassette (ABC)

The ATP-binding cassette (ABC) superfamily consists of membrane proteins that transport a wide variety of substrates across membranes [30]. Members of the ATP-binding cassette family are involved in the multidrug resistance (MDR) process, which is responsible for the failure of cancer CT since the tumor cells can efflux antineoplastic agents and therefore reduce the intracellular drug levels [31,32]. The ABC transporter family can be divided into seven subfamilies (A-G) according to their genome sequences and transmembrane domain (TMD) structures [33]. We will here discuss ABCB11, ABCC and ABCG2 family.

ABCB family. The ABCB1 gene encodes p-glycoprotein (Pg-p) was the first transporter belonging to the ABC superfamily whose expression was associated with MDR in light of its crucial role in the extrusion of CT drugs out of the cells, thereby lowering their intracellular concentration [34]. Increased expression levels of Pg-p have been found in many tumor cells including breast, colon, gastric, kidney, leukemic, liver and pancreatic cells [36–37]. The CT substrates of Pg-p include anthracyclines, taxanes, vinka alkaloids and tyrosine kinase inhibitors (TKIs), such as imatinib and sorafenib, which are involved in the treatment of STS [38, 39]. A prospective study has analyzed Pg-p mRNA expression in both normal and tumor tissues from 28 newly diagnosed pediatric STS showing that the expression of the Pg-p was significantly higher in malignant tissue than in the normal tissues of patients with STS. In addition, high Pg-p expression was significantly associated with local recurrences, as well as poor response to treatment. These results might imply that a significant mRNA level of Pg-p gene was intrinsically

present in STS before exposure to chemotherapeutic drugs, suggesting that may be an important contributor to innate chemoresistance of this tumor type [40]. Polymorphic variants of ABC transporter genes that impair substrate efflux could be associated with a higher cancer incidence, due to decreased xenobiotic efflux and impaired normal tissue protection because of decreased transporter efficacy. On the other hand, they could also be associated with better outcome following cancer systemic treatments, because of reduced CT drug efflux. However, not all cancer chemotherapeutics are substrates for transporters. Moreover, the information on the transport activity of polymorphic variants are currently incomplete and clinical outcome may be influenced by coexisting polymorphic variants in multiple genes. As a result, the literature for outcomes associated with polymorphic variants of ABCB1 is contradictory [38,39]. Maillard et al. in an ancillary pharmacogenetic study of the TSAR trial evaluated the possible correlation between trabectedin treatment and the occurrence of hepatotoxicity in 63 patients with advanced STS. The patients were genotyped by next-generation sequencing (NGS) for 11 genes, and genotype–toxicity association analyses were evaluated. Two SNPs in the ABCB1 gene emerged as relevant in this analysis. The ABCB1 rs1128503 (c.1236C > T) was present in heterozygosity in 26 patients and in homozygosity in 14 patients and was associated with a decreased risk of severe hepatic cytolysis (p = 0.015, OR = 0.25) and the ABCB1 rs2032582 (c.2677 G > T,A) was present in heterozygosity in 34 patients and in homozygosity in 11 patients and was associated with a decreased risk of overall hepatotoxicity (p = 0.027 and OR = 0.22) [41].

ABCC family. The ABCC family comprises of six pumps, among them the MRP1 is the most abundant one. The MRP1 is a co-conveyer which extrudes non-ionic lipophilic drugs and amphipathic anions conjugated with sulfate, glucuronic acid or glutathione. Initially, it was believed that doxorubicin resistance is associated with MRP1 but it was explored that the MRP1 also confer cellular resistance to anthracyclines such as daunorubicin, methotrexate, epipodophyllotoxins and vinca alkaloids [42]. Another MDR modulator is MRP2, who is known to reduce the oral absorption and boosts hepatobiliary clearance of therapeutics [41]. Several evidences suggested a correlation between the presence of particular SNPs in ABCC family members and AEs related to trabectedin treatment. A case report describes a 60-year old male patient treated for metastatic STS with second-line trabectedin [42]. After the second cycle, the patient's transaminases increased six times over the upper normal limit, with no normalization after stopping the treatment. A liver biopsy was performed, showing findings compatible with drug-induced damage (cholangitis and cholangiolitis). Patients genotyping revealed a deficient variant genotype of ABCC2 (c.−24TT rs717620; c.4488CT rs8187707; c.4544GA rs8187710), suggesting a possible correlation between the presence of these SNPs and the liver toxicity developed after treatment with trabectedin [42]. Analysis of the gemcitabine and docetaxel treatment group in the phase III study performed by Seddon et al. indicated a possible association of the ABCB1 rs1045642 minor allele with worse PFS [7]. Specifically, 68 patients were heterozygous (CT) for this SNP and their relative OS HR was 1.62, whereas for homozygous (CC) patients (N = 23) the HR was 1.87 [7]. In the pharmacogenetic study conducted by Maillard et al., the analysis of SNPs present in the ABCC2 gene found that carriers of the SNP rs2273697 (c.1249 G > A; GA = 21, AA=1) were associated with an increased risk of hepatic cytolysis, while a decreased risk is related with the presence of the SNP rs17222723 (c.3563T > A; TA = 9, AA = 1), respectively, with an OR = 3.63 and an OR = 0.11 [41]. The pharmacogenetic study has also characterized ABCC3 and ABCC4 isoforms. The ABCC3 rs2072365 (c.2714 + 29C > T) was present in heterozygosity in 29 out of 63 and in homozygosity in 6 patients and was associated with an increased risk of overall hepatotoxicity (p = 0.003 and OR=5.92). The ABCC4 rs9516519 (c.*3261A > G) was present in heterozygosity in 16 patients and in homozygosity in one patient and was associated with a decreased risk of overall hepatotoxicity (p = 0.048 and OR=0.31) [42].

ABCG family. Members of the ABCG group are involved in the regulated transport of hydrophobic compounds across cellular membranes. Five ABCG family members have been identified. ABCG2 is one of at least three so-called MDR ABC transporters expressed in humans, and its activity is associated with decreased efficacy of anti-cancer agents in several tumor types [43]. The pharmacogenetic study conducted by Maillard et al. also characterized the gene ABCG2. Of 63 total patients, 19 were heterozygous carriers of the variant rs7699188 (c.−15994C > T) and 2 were homozygous and the presence of this SNP was associated with an increased risk of hepatic cytolysis (p = 0.034; OR = 3.41) [41].

Pyrimidine metabolism

Pyrimidine is a basic and indispensable substrate for nucleic acids, phospholipid, glucose metabolism, and protein glycosylation. Pyrimidine antagonists, for example, 5-fluorouracil, cytarabine and gemcitabine, are widely used in chemotherapy regimes for several cancers and additionally it has been reported that KRAS mutant, PTEN deficiency as well as p53 deficiency cells exhibits increased pyrimidine de novo synthesis flux.

Cytidine deaminase (CDA) .*CDA* gene encodes the CDA protein, a ubiquitous enzyme whose major role is to participate in the recycling of free pyrimidines. CDA is considered one of the major gemcitabine inactivation enzymes and a high systemic CDA level was associated with the poor efficacy of this drug. Furthermore, low CDA levels were associated with increased risk of toxicity [44]. Analysis of the gemcitabine and docetaxel treatment group within the GeDDiS trial indicated that the CDA rs2072671 was associated with worse OS. More in detail, 42 patients were heterozygous (AC) for this SNP and their relative OS HR was 1.44, whereas for homozygous (CC) patients (n = 15) the HR was 2.34 [7].

Pyrimidine nucleoside monophosphate kinase

Pyrimidine nucleoside monophosphate kinase [UMP/CMP kinase (UMP/CMPK)] plays a crucial role in the formation of UDP, CDP, and dCDP, which are required for cellular nucleic acid synthesis. Family studies provided evidence for 3 alleles–UMPK1, UMPK2, and UMPK3–at an autosomal locus. The CMPK1 allele was associated with about 3 times the catalytic activity of the UMPK2 allele, so that UMPK2 homozygotes are relatively deficient of the enzyme. Notably, CMPK1 performs a crucial role in the activation of deoxycytidine and cytidine analogues, such as 1-β-D-arabinofuranosylcytosine, 5-azacytidine, and 2′,2′-difluorodeoxycytidine (gemcitabine), were shown to be useful for the treatment of patients with leukemia, lymphoma, or solid tumors. Some genetic polymorphisms of CMPK1 have also been reported as a prognostic marker for NSCLC and pancreatic cancer patients treated with a gemcitabine-based ChT [45]. Analysis of the gemcitabine and docetaxel treatment group in the GEDDIS trial indicated that the CMPK1 rs4492666 SNP was associated with improved OS in both heterozygotes (n = 60) and homozygotes (n = 28), respectively, with an HR=0.53 and an HR=0.56 [7].

CYP450 family

The cytochrome P450 (CYP) family is a group of oxidative/dealkylating enzymes localized in the microsomes of many tissues including the bowel and liver, responsible for the metabolism of several anticancer drugs. Over-expression of CYP is considered one of the major mechanisms of chemoresistance in solid tumors [46]. We will focus on CYP2B6, which is the primary CYP450 responsible for alkylating chemotherapeutic agents' activation [47, 48] and CYP3A5.

CYP2B6

The CYP2B6 gene is highly variable with over 38 named alleles and

can have multiple sequence variations. It was identified as the major cyclophosphamide 4-hydroxylase catalyzing the metabolism of cyclophosphamide. Thus, SNPs in CYP2B6 might affect the functionality of this enzyme in activating cyclophosphamide, which could be reflected on the response to treatment. Labib et al. have carried out a pharmacogenetic study on rhabdomyosarcoma pediatric population aiming to investigate the influence of CYP2B6 variants on cyclophosphamide treatment outcome. This retrospective analysis, which has enrolled 73 pediatric rhabdomyosarcoma, has examined three particular SNPs of CYP2B6 (rs2279343, rs3745274, and rs3211371) involved in cyclophosphamide activation pathway and that are thought to influence response to treatment. The main finding in this study was that patients who carried at least one mutant allele CYP2B6rs2279343 (carrying G mutant allele; $n = 46/73$), had a better objective clinical response (ORR) compared to the homozygous wild allele carriers ($p = 0.01$) and a significantly longer event-free survival (EFS) ($p = 0.034$) but not OS (p-= 0.48) compared to those carrying the homozygous wild allele (A allele) [49].

CYP3A4 and CYP3A5. CYP3A4 is quantitatively the most important CYP enzyme in adults. It is expressed to a major extent in the human liver (95%) but also in the small intestine thus contributing to presystemic and systemic metabolism of approximately 50% of all drugs [50]. Four members of the CYP3A subfamily have been identified in humans: CYP3A4, CYP3A5, CYP3A7, and CYP3A43. Several genetic variants have been described for the CYP3A5 gene, of which the CYP3A5×3 allele (gA6986G), the most common form and leading to the loss of CYP3A5 activity, has been extensively investigated in the aspect of pharmacokinetics and disease risk [51]. Maillard et al. revealed that the WT allele A of CYP3A5 rs776746 (c.6986A > G*)* coding for the CYP3A5×1 genotype was associated with overall hepatotoxicity related to treatment with trabectedin for STS. The incidence of severe hepatic toxicity was higher in heterozygous *1/*3 patients (A/G vs. A/A vs. G/G, $p = 0.012$, OR = 5.75 (1.16–28.55)). Indeed, 85.7% of the heterozygous patients (12/14) had at least one severe elevation of hepatic enzymes. On the contrary, two homozygous *1/*1 (A/A) patients did not experience any hepatotoxic event [41]. Moreover, it was conducted an observational retrospective study (NCT00514345), who had investigated the genes expressed in samples of blood from young patients with cancer treated with ifosfamide. This trial aimed to identify risk factors for kidney damage, looking at the possibility of a relationship between CYP3A5 genotype and ifosfamide nephrotoxicity; results are awaited.

Others

Peroxiredoxins (PRDXs)

PRDXs belong to redox family proteins; members of this family are characterized by a cysteine residue that is involved in the reduction of peroxides, which are ubiquitously expressed in most organisms [52]. Currently, there are six known mammalian PRDX members, specifically PRDX1, PRDX2, PRDX3, PRDX4, PRDX5, PRDX6 [52]. Some studies report that PRDXs play critical roles in the process of carcinogenesis and in the development of drug resistance. In particular, increased or decreased levels of PRDXs mRNA expression levels were found among 33 tumor types, such as PRDX1, PRDX2, PRDX4 and PRDX5 increased in several cancer types, while PRDX3 and PRDX6 decreased in kidney cancer (KIRP, KIRC and KICH). Regarding drug sensitivity, clinical outcome related to pazopanib was found to be associated with PRDX1, PRDX3, PRDX4, PRDX6, which suggested that the PRDXs may be strongly associated with the EGF and VEGF signaling pathways. Moreover, gemcitabine and doxorubicin were significantly associated with PRDXs [52]. In the GeDDiS trial, the SNP rs518329 in PRDX4 gene was analysed, reporting an improved OS with minor allele in the doxorubicin group in both heterozygotes ($n = 43$, HR = 0.89) and homozygotes ($n =$ 32, HR = 0.43) [7]. These findings suggest that reasonable use of drugs such as pazopanib, doxorubicine, gemcitabine can effectively treat some cancers with aberrant expression of PRDXs.

BRCA1

The breast cancer 1 (BRCA1) gene encodes for a tumor suppressor protein that plays a key role in the DNA damage response and repair pathways and functions as a negative regulator of tumor growth [49]. BRCA1 mutations in the germline represent a hallmark for hereditary breast and ovarian cancers, but it has also been typified in other cancers, including sarcoma [53]. Prospective studies have shown an improved prognosis and a superior disease control rate (DCR) in sarcoma patients where the tumor expression of BRCA1 appears inversely related to the response to trabectedin [53]. Italiano et al. have studied the relationship of precise haplotypes associated with trabectedin sensitivity to specific SNPs within the BRCA1 gene. In this study, the four BRCA1 SNPs (rs16941, rs16942, rs1799966, and rs799917) were genotyped in 59 advanced STS patients. Two haplotypes, AAAG and GGGA, represented approximately 90% of the population. Patients were distinguished into two categories based on the presence of the most frequent haplotype (AAAG): those who had at least 1 AAAG allele (46 patients) and those who had no AAAG allele (13 patients). They demonstrated that advanced STS harboring at least one AAAG allele on BRCA1's haplotype displayed a statistically significantly longer PFS and OS, compared with STS without AAAG allele [53, 54].

GSTA1

The glutathione-S-transferases (GST) are a group of enzymes catalysing the addition of glutathione to target electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress [55]. GSTA1 is a relatively small gene (around 11Kb) harbouring 7 SNPs with an allele frequency of more than 5% in the general European population [55]. It is most abundantly expressed in the hepatocytes, kidney proximal tubules, adrenal glands, pancreas, and testis. Regarding pharmacogenomics, GSTA1 is involved in the metabolic pathway of many important chemotherapeutic agents such as doxorubicin and cyclophosphamide [55,56]. Several SNPs have been detected analysing the proximal promoter of the gene encoding GSTA1, which is believed to affect its expression. These genetic polymorphisms consist of two leading haplotypes, GSTA1*A and GSTA1*B, containing 3 linked base substitutions in the proximal promoter, at positions -52, -69, and -567 [56, 56]. A phase II study has investigated brostallicin versus doxorubicin as first-line CT in patients with advanced or metastatic STS. In the doxorubicin arm, the PFS at 26 weeks success rate was significantly higher for the GSTA1 rs3957357C>T (TT) [57] genotype (success rate 62.5%; 95% confidence interval (CI) [24.49–91.48]) (as compared with C/C 12.5 [0.32–52.65]; and C/T12.5 [1.55–38.35]) ($p = 0.023$), showing a relationship between GSTA1 TT genotype and efficacy of doxorubicin. The low expression variant TT genotype was in fact report to be associated with a reduced risk of death after treatment with various alkylating agents in various tumor types. This finding can be explained considering the role of GSTA1, which mediates conjugation of glutathione to alkylating agents. Thus, GSTA1 TT germline genotype might predict the efficacy of doxorubicin, even is this conclusion needs further confirmation [57].

PER2

The Period2 (Per2) gene is a member of the Period family of genes consisting of Per1, Per2, and Per3, and is mainly expressed in the peripheral and central nervous system including the suprachiasmatic nucleus [58]. Benna et al. conducted a retrospective study to test the hypothesis that genetic variation (in terms of candidate SNPs) of the circadian pathway might be associated with the susceptibility and the prognosis of patients affected with sarcoma ($n = 162$). In particular,

rs7602358, located upstream PER2 of the circadian pathway was significantly associated with liposarcoma survival (HR: 1.98; 95% CI 1.02–3.85; $P = 0.04$) [59].

ITGA2

Integrin alpha 2 (ITGA2) is the alpha subunit of a transmembrane receptor for collagens and related proteins. ITGA2 frequently forms a heterodimer α2β1 with a β subunit, which mediates the adhesion of platelets and other cells to the extracellular matrix (ECM) [60]. ITGA2 is overexpressed in several types of tumor and evidence that ITGA2 might play an essential role in modulating tumor cell migration, invasion, and metastasis have been reported [60]. Bao et al. carried out a retrospective study to determine the impact of germline mutations of the angiogenesis pathways on the therapeutic response to anti-angiogenic therapy with apatinib in STS patients ($n = 79$). In 67 (84%) of these patients, twenty previously reported SNPs in the angiogenesis pathway were genotyped to screen for potential toxicity and predictive biomarkers. Interestingly, they observed a strong correlation of ITGA2 rs1126643 polymorphism and surgical wound complications (C/C 4% vs C/T 24% vs T/T 33%, $p = 0.008$) as well as spontaneously pneumothorax (C/C 13.8% vs C/T 36.4% vs T/T 66%), suggesting that integrin mechanisms might underlie both toxicities [61].

VEGFR2

Vascular endothelial growth factor receptor 2 (VEGFR2) is a type V receptor tyrosine kinase mainly known to be expressed in vascular endothelial cells and encoded by the KDR gene. This receptor responds to the signal of vascular endothelial growth factor (VEGF) binding, which initiates a phosphorylation cascade that ultimately involves nuclear regulatory targets resulting in enhancement of endothelial proliferation and migration [62]. The retrospective analysis carried out by Bao et al. among STS patients ($n = 79$) treated with apatinib, reported that VEGFR2 rs2071559 polymorphism might be a sensitivity biomarker for PFS (mutation vs WT, 12 months vs 5 months), regardless of the sarcoma subtype. Thus, VEGFR2 (rs2071559), as well as ITGA (rs1126643), might serve as pan-sarcoma biomarkers for VEGFR2-targeted therapy and warrant further validation for its biological and clinical implication [63].

New treatments

In addition to the evaluation of the activity of conventional chemotherapeutic agents for patients with STS, new treatments need to be explored. Currently, there are a number of ongoing trials exploring the possible value of TKI in patients with STS (Table 4). Anlotinib is a new, orally administered TKI that targets VEGFR, FGFR, platelet-derived growth factor receptors (PDGFR), and c-kit. In a phase IIb study (ALTER-0203) for patients with refractory STS, anlotinib showed remarkable antitumor activity: the 12-week progression-free rate (PFR 12 weeks) and the overall response rate (ORR) were 68% and 13%, respectively, and the PFS and overall survival (OS) were 5.6 months and 12 months, respectively [64]. Anlotinib is being currently evaluated in a single arm phase II trial (ALTER-S006; NCT03890068) as maintenance therapy among patients with STS whose disease had not progressed with first-line anthracyclines treatment. Furthermore, an ongoing phase II study (NCT04172805) is evaluating anlotinib in combination with toripalimab, an anti-programmed death receptor-1 (PD-1) monoclonal antibody (mab): the results are awaited. Surufatinib, a multi-kinase inhibitor of VEGFR 1–3, FGFR 1 and Colony stimulating factor 1 receptor (CSF-1R), is also being tested in STS [65]. An ongoing phase I-II study (NCT05093322) is evaluating the combination of surufatinib and gemcitabine in pediatric patients with recurrent or refractory sarcoma.

While multitargeted TKIs have shown activity in a broad range of histologic subtypes, a robust understanding of the molecular drivers of disease pathogenesis will hopefully result in design of novel clinical trials to improve patient outcomes. Epithelioid sarcoma, as an example, comprises <1% of STS and is often associated with loss of integrase interactor 1 (INI1) resulting in dependency on enhancer of zeste homolog 2 (EZH2), an epigenetic modifier [66]. An understanding of this biology led to an international phase II study of the tazemetostat, an EZH2 inhibitor, in patients with epithelioid sarcoma resulting in an observed response rate of 15% and a disease control rate of 26% [67]. US Food and Drig Administration (FDA) has approved tazemetostat for epithelioid sarcomas on January 2020. Disease-agnostic molecular observations have also advanced treatment in STS. For example, malignancies harboring NTRK fusions (including STS) have shown unparalleled, durable responses to the tropomyosin receptor kinase (TRK) inhibitor, larotrectinib [10]. Even if the value of universal molecular profiling to guide STS management is debated, retrospective studies seem to suggest that targeted next-generation sequencing can help to identify alternative treatment options [77]. The ongoing randomized, phase III MULTISARC clinical trial (NCT03784014) compares the standard of care therapy with the utilization of next-generation sequencing (NGS) to enroll patients into sub-arms of targeted therapies. The results of this trial may lead us toward subtype-agnostic approaches that target individualized molecular findings or identify additional histology-specific molecular characteristics that facilitate drug discovery.

Poly (ADP-ribose) polymerase-1 inhibitors (PARP1-i) represent an emerging therapeutic option in tumors with genomic instability. Although STS do not have a characterized defect in BRCA1/2, their genomics is complex in more than half of the cases, suggesting genomic instability and eventual possible deficiency in DNA damage repair and a high level of inherent DNA damage [78.]. Thus, STS could be efficiently targeted with PARP inhibitors. In sarcomas, promising preclinical data have been reported, notably in Ewing sarcoma and in STS [68]. A number of trials are currently evaluating PARP1-i combined with chemotherapy, especially with trabectedin. Trabectedin is an alkylating drug with a unique mechanism of action causing single-strand and double-strand DNA breaks that activate DNA damage-response pathways. Based on preclinical data, it was hypothesised that PARP1-i might be an ideal partner of trabectedin. The TOMAS phase Ib study has proved feasibility and shown preliminary signs of activity of trabectedin and olaparib PARP inhibitor as combinatorial second- or further-line treatment in patients with bone and STS [69]. The ongoing TOMAS2 trial (NCT03838744) is a phase II study of trabectedin plus olaparib versus trabectedin alone in advanced, metastatic or unresectable STS after failure of standard treatments: results are awaited. Table 5

PARP1-i stimulates immunomodulatory pathways in cancer cells and may reshape the tumor microenvironment. These findings have raised interest in the potential of combining PARP1-i with immune checkpoint inhibitors (ICI); several trials are ongoing to explore this hypothesis [70]. ICI against anti- PD1/PD-ligand (L)1 and Cytotoxic T-Lymphocyte Antigen 4 (CTLA4) have shown some degree of anti-tumor activity in a subset of patients with STS. The pioneer study was SARC028 phase II trial with pembrolizumab flat dose at 200 mg every 3 weeks, with clinical activity in patients with UPS and dedifferentiated liposarcoma [71].

Maximizing the number of patients who can benefit from immunotherapuetic approach undoubtedly requires additional efforts. Along this line, immunotherapy combinations and the association of immunotherapeutic agents with targeted therapy, oncolytic viral-therapy, tumor microenvironment modulators, and epigenetic drugs, as summarized in table 6, represent areas of active investigation that could eventually lead to enhanced efficacy. For example, cabozantinib is being explored in a randomized study with or without dual PD1/CTLA4 checkpoint blockade, with a broader spectrum TKI potentially more impactful to the tumor microenvironment than narrow VEGF inhibitors (NCT04551430). Furthermore, the use of adoptive T-cell transfer with enhanced affinity for tumor-specific antigens (such as New York

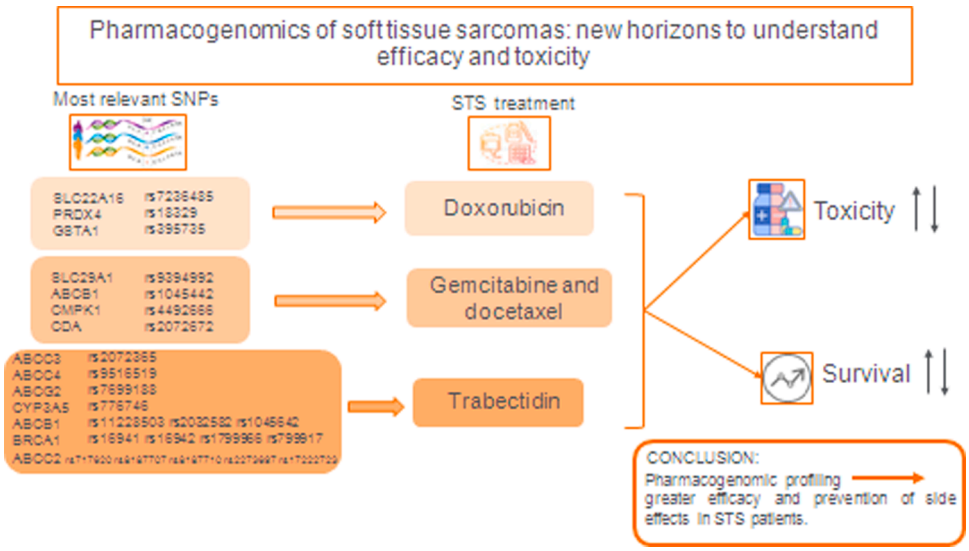

Fig. 1. Visual Abstract. Pharmacogenomics of soft tissue sarcomas: new horizons to understand efficacy and toxicity.

Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) and melanoma antigen gene type A4 (MAGE-A4)) are also showing early promise in STS, particularly in synovial sarcoma. Lentiviral (LV)-based vaccine approaches, such as CMB305, which combines LV305, a dendritic cell-targeting LV encoding NY-ESO-1, with a toll-like receptor 4 agonist, G305, are also under investigation in clinical trials. A phase II study has shown that the co-administration of CMB305 with atezolizumab led to increases in an anti-NY-ESO-1 immune response and appeared to fare better by imaging than those without such an immune response. On the other hand, the combination did not result in a statistically significant improvement in PFS and OS [72].Therefore, prime-boost vaccines such as CMB305 alone or in combination with anti-programmed death ligand-1 therapies merits further evaluation.

Overall, it is critical to support preclinical and translational laboratory research with these and other ongoing studies to better understand mechanisms of response and resistance in treated patients with STS, and to develop biomarkers for specific immune subsets of STS to better tailor combination therapies.

Clinical implications of Pgx findings

Clinical applications of genomic biomarkers have been rapidly expanded and developed.

A recent retrospective study performing NGS analysis of several genes related to cancers in pretreatment tumor biopsy from patients with advanced STS treated with anti-VEGFR agents (pazopanib and sunitinib) [73] has shown the importance of TP53 and RB1 genes in regulating the outcome of TKI treatments. TP53 mutations were shown to have significant association with a longer PFS respect to TP53 wild-type. Predictors factors of pazopanib effectiveness and toxicity in STS patients are related to cytokines and circulating angiogenic factors in serum [73]. Indeed, PFS observed after 12 weeks of treatment was positively correlated to high levels of interleukin (IL)-12 and mitochondrial pyruvate carrier 3 (MPC3) levels at baseline, and negatively associate with low soluble VEGFR2 and high placental growth factor (PGF) levels [74].

Another recent retrospective analysis reported that improvements in PFS and OS of leiomyosarcoma and angiosarcoma patients treated with gemcitabine was related to high hENT1 tumor expression levels [30], which have been proved to be responsible for intracellular uptake of prodrug gemcitabine into tumoral cells. Thus, evaluation of hENT1 expression level could allow to select patients that appeared to benefit more from gemcitabine-based regimens.

Also somatic alterations in the homologous repair system could determine a deeper and longer activity of trabectedin in STS patients with drug response negatively correlated with the BRCA1 mRNA levels [75]. Moreover, a significant correlation between improved clinical efficacy of trabectedin (DCR 56 vs. 36%, $p = 0.04$; median PFS7.1 vs. 2.5 months, $p = 0.002$) was also observed in patients with high expression level of ERCC5/XPG complex [21], supporting the hypothesis of a direct link between DNA damage repair system functionality and efficacy of trabectedin, differently from other DNA interacting agents.

About adverse events, as summarized in Table 1, SNPs in genes like SLC22A16 or ABCB1 could be associated to decreased risk of toxicity related to doxorubicin and trabctedin, respecitively.

Furthermore, a genome-wide SNP analysis of the epithelial and spindle cell components of 12 formalin-fixed paraffin-embedded biphasic synovial sarcoma (BSS) samples, found some significant mutations in genes involved in cell adhesion, ECM–ECM receptor interactions, the TGF-β signaling pathway and cell junctions and signaling [76]. Such findings could help to shed light in the mechanisms of tumorogenesis and provide new therapeutic targets. Fig. 1

Conclusions

Pharmacogenomics studies of anti-cancer drugs in STS play an important role in identifying patients avoiding AEs optimizing drug dose and maximizing the efficacy outcomes aiming to hopefully guide to regarding STS treatment. Development in NGS technologies has opened a new opportunity for characterizing the genomic landscape of STS, together with the future possibility to apply the genetic diagnostic tests to choose the best personalized treatment regimens. Due to the finding that STS with different morphologies or biological behaviours may share the same genotype [70], the evaluation of both histopathological findings and molecular features together seems to be very relevant to pursue the goal of personalized medicine. The study of SNP) performed by the Real Time PCR genotyping method allows obtaining immediate results; it is a fast and simple analysis for the interpretation of the data. This method can be defined as a target analysis, limited at only a pair of bases belong to the known regions of DNA, instead of the next generation sequence (NGS) that through the sequencing of the DNA, allows investigating a broad range of many variants, mutations at the same time. The high- throughput of NGS leads to simultaneous analysis of wide regions, also the unknown regions of DNA. On the contrary, the possible limitations of this procedure include the specificity that leads to study only small, specific regions of interest, leading to consider together, on the

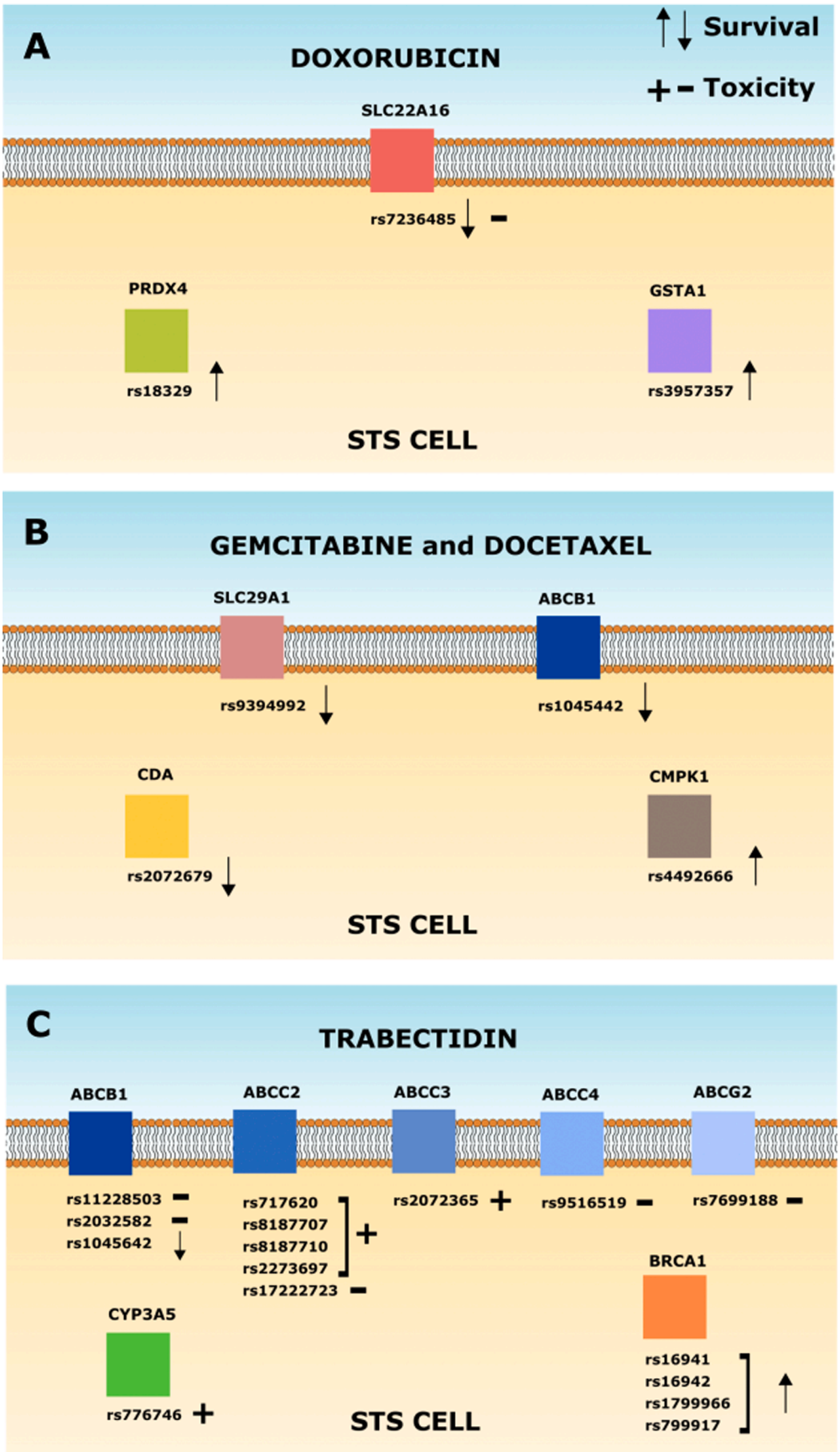

Fig. 2. Impact of SNPs on toxicity and efficacy in STS treated with doxorubicin (A), gemcitabine and docetaxel (B), and trabectedin (C).

Table 3
Selected trials analyzing SNPs as biomarkers[a] in STS.

NCT trial	Study	Objectives	Type of study	Status
NCT03058289	A Phase 1/2 Safety Study of Intratumorally Dosed INT230–6 (IT-01)	Exploratory: Blood, Genetic and Tissue Biomarker Identification from cell flow phenotyping, tissue analysis, genetic SNP analysis.	Interventional	Recruiting
NCT02110069	A Randomized Phase 2 Study of Vincristine Versus Sirolimus to Treat High Risk Kaposiform Hemangioendothelioma (KHE)	Secondary: to identify Genetic Variants in Drug Metabolism Enzymes. SNPs array analysis to obtain genetic information on variants in drug metabolism enzymes that affect sirolimus and vincristine metabolism.	Interventional	Completed
NCT01050296	Molecular Analysis Of Solid Tumors (MAST)	Secondary: to perform analysis of focal alterations in the genome including amplification, deletion and LOH. DNA isolated from the tumor and blood samples will be hybridized to SNP chips.	Observational Prospective	Recruiting
NCT02398058	A Phase Ib Study on the Combination of Trabectedin and Olaparib in Unresectable Advanced/Metastatic Sarcomas After Failure of Standard Therapies	Secondary: Biomarkers (composite outcome) Several gene assessments (expression, amplification/ deletion, SNPs) on DNA-damage response-related markers (including but not limited to BRCA 1–2, ERCC 1–2–5, XRCC 1–2–3, RAD51 and 53BP1, PARP 1–2, P-histone H2AX and others) will be conducted. Statistical analysis will be performed to investigate the association between trial outcomes and SNPs of these genes.	Interventional	Completed
NCT00954473	Retrospective Study of Genetic Risk Factors for Osteosarcoma	Primary: Hardy-Weinberg equilibrium on all SNPs. SNPs associated with OS.	Observational Retrospective	Completed
NCT00601406	Radiogenomics: Assessment of Polymorphisms for Predicting the Effects of Radiotherapy (RAPPER)	Primary:To test the hypothesis that an association between common genetic variations, reported by SNPs in relevant candidate genes, is associated with individual patient variability in normal tissue radiation response and toxicity. Secondary:To compare a detailed 3D dose-volume analysis in a subset of patients with late effects and SNP results.	Interventional	Unknown
NCT01430637	Studying Genes in Samples From Younger Patients With Desmoplastic Small Round Cell Tumor Registered on COG-D9902 or COG-ABTR01B1	Primary: Identification of novel mutations, single nucleotide polymorphisms, and copy number changes associated with DSRCT Identification of genomic regions involved in pathogenesis of DSRCT	Observational Retrospective	Completed
NCT01567046	Studying Genes in Tissue Samples From Younger and Adolescent Patients With Soft Tissue Sarcomas	Correlative studies: Archived DNA tissue samples are analyzed for frequency of genetic mutations, including SNPs, SNVs, and small deletions and/or insertions.	Observational Retrospective	Completed
NCT01504360	Predictive Study of Radiation Induced Sarcoma (SARI)	Primary: To determine the predictive clinical and biological risk factors to develop a sarcoma on irradiated territory. Clinical data of initial radiation therapy, radiation-induced apoptosis of CT8+ lymphocytes and polymorphism of the genes involved in reparation of DNA by using chip screening of SNPs will be studied for each patient included.	Interventional	Completed
NCT01802125	Biomarker Differences in Samples From Patients With Undifferentiated Sarcomas	Primary:To determine whether undifferentiated sarcoma can be subdivided into separate and distinct pathologic entities that are distinguishable by light microscopy, SNP array profiling, or clinical features. To determine whether undifferentiated sarcomas with specific "actionable mutations" can be identified based on their histologic appearance, immunohistochemical staining characteristics, or SNP array profiling features.	Observational Retrospective	Completed

[a]as of 7, Jan 2022. Source: clinicaltrials.gov.
Abbreviations: STS= soft tissue sarcoma; SNPs: single nucleotide polymorphisms; LOH: loss of heterozygosity; DSRCT: desmoplastic small round cell tumor.

Table 4
Selected multitargeted TKI trials in sarcoma[a].

Multitargeted TKI	Other agents	NCT trial number	Phase	Status
Tazemetostat (EZH2[b]-i)	Doxorubicin	NCT04204941	III	Recruiting
Sirolimus (mTOR[c]-i)	Pexidartinib (TKI of CSF-1R, Kit (c-Kit), and FLT3	NCT03190174	I	Recruiting
Larotrectinib (TRK[d]-i)	–	NCT02576431	II	Recruiting
Abemaciclib (CDK [e] 4-i)	-	NCT04040205	II	Recruiting
	Temozolomide and Irinotecan	NCT04238819	I	Recruiting

[a] as of Jan 7,2022. Source: clinicaltrials.gov.
[b] enhancer of zeste homolog 2.
[c] mammalian target of rapamycin.
[d] neurotrophic tyrosine receptor kinase.
[e] cyclin-dependent kinase.

Table 5
Selected PARP- inhibitors trials in sarcoma[a].

PARP- inhibitors	Other agents	NCT trial number	Phase	Status
Olaparib	Radiation therapy	NCT02787642	I	Recruiting
Olaparib	Temozolomide	NCT01858168	I	Recruiting
Olaparib	Durvalumab	NCT03784014	III	Recruiting
Olaparib	Trabectedin	NCT04076579	II	Recruiting
Olaparib	Pembrolizumab	NCT04123366	II	Recruiting
Olaparib	–	NCT03233204	II	Recruiting
Rucaparib	–	NCT04171700	II	Recruiting

[a] as of Jan 7,2022. Source: clinicaltrials.gov.

NGS analyses, samples from different STS histotypes where panels with a limited number of covered genes are used. Thus, in light of this particular evidence, the implementation of a panel containing an increased number of genes seems to be compulsory for a better daily diagnostic routine in STS and the development of novel molecular target therapies for these rare and complex cancers (Fig. 2) [11]. Finally, the results of ongoing and future trials (Table 3) in this field should be considered to identify and validate drug-sensitivity test systems for routine use that

Table 6
Selected immunotherapy ongoing trials in sarcoma[a].

Immunotherapy	Other agents	NCT trial number	Phase	Status
Immune checkpoint inhibitors (ICI) in combination with other immunotherapy agents				
Pembrolizumab	IDO-1[¶] Inhibitor (epacadostat)	NCT03414229	II	Active, not recruiting
Nivolumab	Ipilimumab (anti-CTLA-4[ϛ])	NCT04741438	III	Recruiting
	Relatlimab (anti-LAG-3[ϛϛ])	NCT04095208	II	Recruiting
	Bempegaldesleukin (PEGylated IL-2[#], NKTR-214)	NCT03282344	II	Active, not recruiting
Immune checkpoint inhibitors in combination with chemotherapy or radiation therapy				
Atezolizumab	Radiation therapy	NCT03474094	II	Recruiting
Nivolumab	Paclitaxel	NCT04339738	II	Recruiting
	Trabectedin	NCT03590210	II	Active, not recruiting
	T-VEC[¶¶] (Oncolytic herpes virus) and Trabectedin	NCT03886311	II	Recruiting
Pembrolizumab	Radiation therapy	NCT03338959	I-II	Recruiting
	Eribuline	NCT03899805	II	Active, not recruiting
	T-VEC[¶¶]	NCT03069378	II	Recruiting
	Lenvatinib(VEGFR[**]-i)	NCT04784247	II	Recruiting
	Axitinib (VEGFR[**]- i)	NCT02636725	II	Active, not recruiting
Immune checkpoint inhibitors in combination with other agents				
Atezolizumab	Bevacizumab (VEGF-i)	NCT03141684	II	Recruiting
	Rucaparib (PARP1[§]- i)	NCT04624178NCT04216953	I-II	Recruiting
	Cobimetinib (MEK[§§]-i)		II	Recruiting
Dual immune checkpoint inhibitors therapy in combination with other agents				
Nivolumab plus ipilimumab	Cabozantinib(VEGFR/-i)	NCT04551430	II	Recruiting
	Trabectedin	NCT03138161	I-II	Recruiting
Adapted T-cell therapies				
NY-ESO-1 (TCR Affinity Enhancing Specific T cell Therapy)	–	NCT03462316	I	Recruiting
Afamitresgene autoleucel (previously ADP-A2M4)	–	NCT04044768	II	Recruiting

[a] as of Jan 7, 2022. Source: clinicaltrials.gov.
[¶] Indoleamine-2,3 dioxygenase 1.
[ϛ] Cytotoxic T-Lymphocyte Antigen 4.
[ϛϛ] Lymphocyte Activating 3.
[#] interleukin-.
[¶¶] Talimogene Laherparepvec.
[**] vascular endothelial growth factor receptor.
[§] Poly (ADP-ribose) polymerase-1.
[§§] mitogen-activated protein kinase.

include known specific PGx markers in the common clinical management of STS.

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CRediT authorship contribution statement

Elisabetta Gambale: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Anna Boddi:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Adriano Pasqui:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Domenico Andrea Campanacci:** Data curation, Formal analysis, Writing – original draft. **Guido Scoccianti:** Data curation, Formal analysis, Writing – original draft. **Ilaria Palchetti:** Data curation, Formal analysis, Writing – original draft. **Andrea Bernini:** Data curation, Formal analysis, Writing – original draft. **Lorenzo Antonuzzo:** Conceptualization, Data curation, Formal analysis, Writing – original draft. **Serena Pillozzi:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

Elisabetta Gambale has not conflict of interest to declare; Anna Boddi has not conflict of interest to declare; Adriano Pasqui has no conflict of interest to declare; Domenico Andrea Campanacci has not conflict of interest to declare; Guido Scoccianti has not conflict of interest to declare; Ilaria Palchetti has not conflict of interest to declare; Andrea Bernini has not conflict of interest to declare; Lorenzo Antonuzzo has not conflict of interest to declare; Serena Pillozzi has not conflict of interest to declare;

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