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Molekulargenetische Charakterisierung von Sarkomen zur Identifizierung prognostischer Risikogruppen und potentieller therapeutischer Angriffspunkte

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ABC	ATP-binding cassette
ADM	Adriamycin
AIDS	Acquired Immunodeficiency Syndrome
AJ	Adherens junctions
AJCC	American Joint Committee on Cancer
ALT	Alternative lengthening of telomeres
AML	Acute myeloid leukemia
APC	Adenomatous Polyposis Coli
ARID	AT-Rich Interactive Domain-containing
AS	Angiosarcoma
ASS	Acetylsalicylic acid
ATRX	Alpha thalassemia/mental retardation X-linked
BARD1	BRCA1-associated RING Domain 1
BFB	Fusion-bridge breakage
BIRC	Baculoviral inhibitor of apoptosis protein repeat containing
BMI	Body mass index
Bp	Base pair
BRCA	Breast cancer gene
CCC	Comprehensive cancer center
CD	Cluster of differentiation
CDKN	Cyclin-dependent kinase inhibitor
CHEK	Checkpoint kinase
CKS1B	Cyclin-dependent kinase regulatory subunit 1B
CNAs	Copy number alterations
CNV	Copy number variants
COPII	Coat protein complex II
CPI	Checkpoint inhibitor
CT	Computed Tomography
CTNNB1	Catenin Beta 1
DDLPS	Dedifferentiated liposarcoma
DNA	Deoxyribonucleic Acid
DOT1L	Disruptor of telomeric silencing-1-like
DSRCT	Desmoplastic small round cell tumor

DTIC	Dacarbazine
EBNA	Epstein-Barr nuclear antigen
EBV	Epstein-Barr virus
EGFR	Epidermal growth factor receptor
ER	Endoplasmic reticulum
ERBB	Erythroblastic oncogene B
ERCC	Excision Repair Cross-Complementing
ES	Ewing sarcoma
ESMO	European Society for Medical Oncology
FANCA	Fanconi anemia, complementation group A
FANCG	Fanconi anemia, complementation group G
FANCI	Fanconi anemia, complementation group I
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FGFR-1	Fibroblast growth factor receptor 1
FH	Fumarate Hydratase
FNCLCC	Federation Nationale des Centers de Lutte Contre le Cancer
G	Histopathological grading
GIST	Gastrointestinal stromal tumor
Gy	Gray
HHV 8	Human herpes virus 8
HIF-1-alpha	Hypoxia-inducible factor 1-alpha
HIFU	High-intensity focused ultrasound
HIPEC	Hyperthermic intraperitoneal
HMGA2	High mobility group A2
HPF	High-power field
HRD	Homologous recombination deficiency
i.e.	id est
IDCS	Interdigitating dendritic cell sarcoma
IDH1	Isocitrate dehydrogenase 1
IDH2	Isocitrate dehydrogenase 2
IFS	Ifosfamide
IGF-1	Insulin-like growth factor 1
IGF-1R	Insulin-like growth factor 1 receptor
IHC	Immunohistochemistry

IL	Interleukin
ILP	Isolated limb perfusion
INPPL1	Inositol polyphosphate-5 phosphatase-like 1
iTME	immuno-tumor microenvironment
KDR	Kinase insert domain receptor
Kg	Kilogramm
L	Liter
LDH	Lactate dehydrogenase
LFS	Li-Fraumeni syndrome
LMS	Leiomyosarcoma
LOH	Loss of heterozygosity
LST	Large-scale transitions
LZTR1	Leucine-zipper-like transcriptional regulator 1
m^2	square meter
Mb	Megabase
MDM	Mouse double minute
MET	Mesenchymal epithelial transition factor receptor
MFH	Malignant fibrous histiocytoma
MGUS	Monoclonal gammopathy of undetermined significance
MGUS	Monoclonal gammopathy of undetermined significance
MHC	Major histocompatibility complex
MMR	Mismatch repair
MMR-D	Mismatch repair-deficient
MPNST	Malignant peripheral nerve sheath tumors
MPSS	Massively parallel signature sequencing
MRI	Magnetic resonance imaging
MSH6	MutS homolog 6
MSI	Microsatellite instability
MSS	Microsatellite stability
MTAP	5-Methylthioadenosine phosphorylase
MTAP	S-methyl-5'-thioadenosine phosphorylase
NCHS	National Centers for Health Statistics
NER	Nucleotide excision repair
NF-1	Neurofibromatosis type 1
NGS	Next-generation sequencing

NHEJ	Joining of non-homologous ends
NOS	Not otherwise specified sarcoma
NPM	Nucleophosmin
NSCLC	Non-Small Cell Lung Cancer
NTRK	Neurotrophic Tyrosine Receptor Kinase
ORR	Objective response rate
OSS	Overall survival
PARP	Poly-adenosine diphosphate-ribose-polymerase
PCR	Polymerase chain reaction
PDGFRA	Platelet-derived growth factor (PDGF) receptor alpha
PET	Positron emission tomography
PFO	Patent foramen ovale
PIK3	Phosphoinositide-3-kinase
PTEN	Phosphatase and tensin homolog
PVC	Polyvinyl chloride
R	Resection
RB	Retinoblastoma
RNA	Ribonucleic Acid
RSF1	Remodeling and spacing factor 1
RTK	Receptor tyrosine kinase
RTS	Rothmund-Thompson syndrome
SBRT	Stereotactic body radiotherapy
SCNA	somatic CAN
SEER	Surveillance, Epidemiology and End Results
SF3B1	Splicing factor 3B subunit 1
SH2-like	SWI/SNF related, Matrix associated, Actin dependent Regulator of Chromatin, subfamily B, member 1
SIADH	Syndrome of inappropriate antidiuretic hormone secretion
SMARCB1	SWI/SNF related, Matrix associated, Actin dependent Regulator of Chromatin, subfamily B, member 1
SPINK	Serine protease inhibitor Kazal-type
SPTA	Spectrin alpha
SS	Synovial sarcoma
STDEV	Standard deviation
STS	Soft tissue sarcoma

TAI	Telomeric allelic imbalance
TCGA	The Cancer Genome Atlas Research
TERT	Telomerase reverse transcriptase
TET2	Ten-Eleven Translocation 2
TFIIH	Transcription factor II Human
TGF	Transforming growth factor
TLS	Tertiary lymphoid structures
TMB	Tumor mutational burden
TME	Tumor microenvironment
TMN	primary tumor (T), lymph nodes (N), and distant metastases (M)
TNF	Tumor necrosis factor
TP53BP1	Tumor suppressor p53-binding protein-1
U	Unit
UGT1A1	Uridine diphosphate glucuronosyltransferase 1A1 enzyme
UICC	Union for International Cancer Control
ULMS	Uterine leiomyosarcoma
UPS	Undifferentiated pleomorphic sarcoma
USP9X	Ubiquitin-specific protease 9X
VEGF	Vascular endothelial growth factor
VHL	Von Hippel-Lindau
VRE	Vancomycin-resistant enterococci
WBRT	Whole brain radiotherapy
WES	Whole exome sequencing
WGS	Whole genome sequencing
WHO	World Health Organization
WS	Werner syndrome
XPO1	Exportin-1
YAP1	Yes-associated protein 1
µl	Microliter

1. Introduction

1.1. Sarcoma, background

1.1.1. Definition and history overview

Sarcomas are a relatively rare and yet very heterogeneous group of malignancies, most probably derived through transformed cells of mesenchymal origin (1). There are currently over one hundred described sarcoma types. Sarcoma can arise primarily from bone, cartilage, fat, fibrous vascular or related tissues (1) and should be distinguished from tumors that can secondarily metastasize in these tissues (2).
The word sarcoma comes originally from the Greek language and is derived from the word sarx (σάρξ), which means “flesh”, a fleshy excrescence (3). The first to use the term osteosarcoma in a clinical setting was Alexis Boyer (1757-1833), which was then included in eminent textbooks of the time (4).

His colleague John Abernethy (1764-1831) attempted to put together a classification of tumors according to the macroscopic appearance of the tissue. He listed eight types of sarcomas: 1) common vascular or organized, 2) adipose, 3) pancreatic, 4) cystic, 5) mammary, 6) tuberculated, 7) pulpy or medullary, and 8) carcinomatous. Retrospectively, this classification offers no differentiation between cancers and malignancies that are nowadays called sarcomas.

The next big historical step came after the development of cellular pathology and differentiation between primary and metastatic tumors, especially of bone tumors, by J.C.A. Recamier (1774-1852). Through the work of Rudolf Virchow (1821-1902), a distinction of sarcomas from cancers was achieved. Moreover, he defined them as a variety of tumors that evolved from non-epithelial and non-hematogenous tissues. Virchow historically distinguished six major types of sarcomas: 1) fibrosarcoma, 2) myxosarcoma, 3) gliosarcoma, 4) melanosarcoma, 5) chondrosarcoma, and 6) osteosarcoma.

The first great clinical-pathological correlation of a large number of bone sarcoma cases was carried out by Samuel Weissel Gross (1837-1889). In his seminal ar-

ticle published in the American Journal of Medical Sciences in 1879, entitled “Sarcoma of the long bones: Based on a study of 165 cases”, he presented histology, general pathology, symptomatology, diagnosis, prognosis, and treatment. He noted the tendency of these tumor entities to spread hematogenously, predominantly to lungs and at the same time, the low incidence of local lymphatic involvement. He advised amputating well above the lesion because of the high incidence of local recurrence. The treatment of bone or soft tissue sarcomas had historically been mainly surgical, involving wide local excision or amputation. The operative procedures were usually performed too late and thus, alas, were rarely curative.

The way for further advances in diagnosis and treatment was paved by important scientific discoveries of the time. The discovery of X-rays by Wilhelm Conrad Roentgen (1845-1923) in 1895 was followed rapidly by their introduction into medical practice as a standard diagnostic aid, which allowed the creation of the first Bone Sarcoma Registry (4).

The discovery of radium by the Curies in 1898 enabled the therapeutic use of radium in oncology just five years later. This caught the eye of pathologist James Ewing. Accumulation of his substantial experience led to the publishing of the book Neoplastic Diseases in 1919. In 1920, he described the diffuse bone endothelioma, which was later christened Ewing’s tumor by Codman in 1981. Ewing became enthralled with the treatment of malignancies by radiation. Through his profound influence at the time, radiotherapy received staunch support as a treatment of choice for the control of malignant lesions rather than surgery, which consequently delayed the development of surgical treatment.

Despite increased understanding and awareness, the treatment of bone and soft tissue sarcomas remained relatively ineffective. Only after the introduction of cancer chemotherapy as an adjuvant to surgical treatment in the next decade could there come to a noteworthy improvement in the long-term survival rates.

Although somatic tissues of mesenchymal origin account for more than two-thirds of total body mass, sarcomas paradoxically represent less than 1% of adult solid malignancies (5). In comparison to the much more prevalent epithelial-derived

malignancies, the histological and biological spectrum of sarcomas is truly remarkable. Sarcomas should be seen as a large family of multiple unique histological subtypes that can potentially occur at any age and any location in the human body, i.e., a multitude of distinct malignancies with a common mesenchymal origin, rather than a single entity. Further, sarcomas as a family exhibit some unique clinical behaviors that differentiate them from epithelial malignancies, nonetheless, individual subtypes can differ widely in comparison to each other. (5) This very diversity, in combination with the relative rarity of the disease, makes studying sarcomas very challenging and often only possible in big, specialized centers.

1.1.2. Etiology

The occurrence of most sarcoma cases is to be seen as sporadic and etiologically indeterminate. There are, however, several observed host-environment interactions at the micro- and macromolecular levels that are suspected to play a relevant role in sarcomagenesis. The identified etiological factors can be roughly divided into host-related and environmental (5):

The *host-related etiologies* can be divided into nonspecific and genetic. The *nonspecific etiologies* include chronic inflammation and host immune suppression. Chronic inflammation has been well-established in literature as an etiological factor for a plethora of malignancies. Exempli gratia, Stewart-Treves syndrome is characterized by chronic upper extremity lymphedema as a consequence of mastectomy with radiotherapy. An increased risk for subsequent development of sarcomas, primarily angiosarcoma (6) (7) has been established. Chronic irritation (for example foreign-body-induced) is linked in some experimental data to sarcomagenesis (8). Another well-established risk factor for the development of malignancies, including sarcomas, is chronic immunosuppression. For example, a long well-known association of Kaposi sarcoma and AIDS (Kaposi sarcoma is typically associated with human herpes virus 8 infection (HHV 8) and tends to regress with improvement of host immunosuppression) (9). Solid organ transplant recipients are also shown to have an increased Kaposi sarcoma incidence. This

is particularly observed in geographic locations with endemic HHV-8 exposure (10). Moreover, there are several case reports and a large retrospective study demonstrating an increased incidence of non-Kaposi sarcomas in organ recipients compared to the general population (11). In the past decades, there has been an increasing number of case reports describing the phenomenon of Epstein-Barr virus (EBV)-induced leiomyosarcomas, particularly in immunocompromised patients (12). A direct role of EBV in the development of smooth muscle tumors was observed in multiple studies (12) (13). The Epstein-Barr nuclear antigen-2 (EBNA-2) was demonstrated to have a fairly consistent expression in smooth muscle cells of immunocompromised individuals (13). Decreased immune surveillance, increased expression of the EBV receptor, and high plasma EBV levels are all postulated to be at the very least relevant contributing factors (12).

Genetic factors associated with sarcoma development can be divided broadly into three major categories: germline genetic diseases/mutations, discrete somatic genetic alterations (simple karyotypes) and complex genetic alterations (complex karyotypes). It has been well established that individuals with certain genetic syndromes are predisposed to develop sarcomas (5). The most common *genetic syndromes* associated with sarcomagenesis are the following:

Familial gastrointestinal stromal tumor (GIST) syndrome. GIST, the most prevalent mesenchymal neoplasm of the gastrointestinal tract, that is presumably arising from the Cajal interstitial cells and commonly highly resistant to conventional chemotherapy (14), is characterized by a high incidence (75-80% of the cases) of c-kit mutations. These mutations result in constitutive kinase activation, which is postulated to lead to tumorigenesis. Advances in understanding the underlying molecular biology of this entity enabled the development of targeted therapy with Imatinib mesylate and its derivates (15).

Li-Fraumeni syndrome (LFS) and germline mutations in the p53 gene. LFS is defined as a familial cancer syndrome with a proband who had a sarcoma diagnosis at an age under 45 years and a first- or second-degree relative who had any malignancy under 45 years of age or a sarcoma at any age (16). This is one

of the first described familial cancer syndromes. At the basis of this syndrome lies a germline mutation in the p53 tumor suppressor gene. The product of this gene, the p53 protein, is a transcription factor, which in a physiological setting inhibits cell growth and stimulates apoptosis when induced by cellular stress (17). Loss of function mutations in p53 that consequently block this essential pathway to apoptosis are most commonly described (18). In addition to sarcomas, these patients tend to develop many other types of malignancies such as breast, brain and adrenocortical tumors as well as leukemias (16). Aberrations in multiple regulators and effectors of p53 have been experimentally shown to play a significant role in sarcomagenesis. Exempli gratia, one of the better-researched regulators is the mouse double minute 2 protein (MDM2) – a nuclear phosphoprotein with a key role in cellular growth and death, as well as in the transformation of normal cells into tumor cells. MDM2 protein is a product of the MDM2 gene and can inactivate p53 via binding to pRB and consequently decreasing the levels of p53 at the transcriptional level. Overexpression of MDM2 has been observed in a variety of sarcomas, especially frequently noted in well-differentiated liposarcomas and osteosarcomas (19) (20).

Morbus von Recklinghausen, i.e., Neurofibromatosis type 1 (NF-1) is an autosomal dominant process that disrupts the NF1 gene function and its product neurofibromin, that acts as a tumor suppressor via guanosine triphosphatase mediated stimulation of the proto-oncogene RAS activity. Patients with Morbus von Recklinghausen are notoriously prone to the development of malignant peripheral nerve sheath tumors (MPNST) with a cumulative lifetime risk of up to 10% (21). The loss of neurofibromin has been postulated to lead to functional RAS pathway up-regulation. It was observed that NF-1 lesions (such as neurofibromas and neurogenic sarcomas) compared to non-NF-1 lesions have significant elevations of activated RAS levels, which is associated with increased tumor vascularity, which is possibly related to an increased vascular endothelial growth factor (VEGF) secretion (22). As a promoting factor in tumor progression, mutations in p53 are often seen in MPNST, leading to increased cell survival and genomic instability, which is suspected to work synergistically with the described increased RAS activity (22).

Retinoblastoma (RB). This is a rare childhood cancer of the eye. The hereditary retinoblastoma is caused by an inactivating germline mutation in one allele of the RB1 tumor suppressor gene. Unlike those with non-hereditary RB, patients with a loss of heterozygosity at the wild-type allele in the RB1 gene have an significantly elevated risk of developing sarcomas (particularly osteosarcoma), brain cancer or melanoma (23) (24) (25). Secondary malignancies are particularly prevalent in prior radiation treatment fields, which is likely to be a strong predisposing factor (23). The product of the RB1 gene (13q14) is the RB protein (pRB), which negatively regulates progression from G0/G1 into the S phase and is dysregulated in most human malignancies (26). During the G1 phase of the cell cycle, pRB binds to E2F1, E2F2 and E2F3 transcription factors. Sequential hypophosphorylation of pRB by cyclin-dependent kinases results in a release of the E2F and subsequent transcription of genes required for progression to the S phase of the cell cycle (26).

Werner syndrome (WS). This is a rare autosomal recessive disease caused by a mutation in a single gene, WRN (8p12-p11.2) which encodes a protein containing a highly conserved 3´ to 5´ DNA helicase domain of the RecQ family (27). The members of this protein family have diverse roles, including involvement in DNA recombination, replication and repair (28). Patients with Werner syndrome don´t tend to show pathologies until after their second decade of life, when they start to develop diseases and conditions that mimic many aspects of human aging, such as alopecia, bilateral ocular cataracts, hypogonadism, ischemic heart disease, osteoporosis and type 2 diabetes mellitus. Furthermore, these patients have an increased risk of developing rare non-epithelial malignancies, especially mesenchymal-derived, such as sarcomas (29). They die usually in their fourth decade due to cardiovascular events or malignancy. It has been shown in vitro that fibroblasts isolated from patients with Werner syndrome exhibit characteristically premature senescence (30) and display increased chromosomal aberrations (31).

Two other diseases with pathologies in the RecQ helicase family of proteins are Bloom syndrome and Rothmund-Thompson syndrome, which are associated with genomic instability.

Bloom Syndrome is a rare autosomal recessive disease with characteristically marked genetic instability associated with a greatly increased predisposition to a diverse spectrum of malignancies, including sarcomas (32). It occurs most frequently in the population of the Ashkenazi Jews and has a mutation in the BLM gene (15q26.1) in its root, which is encoding RecQ DNA helicase.

Rothmund-Thompson syndrome (RTS) is another rare autosomal recessive disease with a mutation in the RecQ4 gene (8q24.3), encoding the RecQ4 DNA helicase in its root. Patients with this syndrome typically develop small stature, poikiloderma and skeletal dysplasias during childhood and are at an increased risk for developing malignancies, especially skin cancer and osteosarcoma (33) (34) (35). Interestingly, the RecQ4 gene is located adjacent to the MYC gene on chromosome 8, a frequent site of amplification in osteosarcomas. Table 1 presents the most prevalent inherited diseases, which commonly predispose to sarcoma development.

Inherited diseases that commonly predispose to development of sarcoma

Disease	**Gene**	**Location**	**Function**
BS	BLM (RecQL3)	15q26.1	DNA helicase
Familial GIST	KIT	4q12	Receptor tyrosinase kinase
FH leiomyosarcoma and renal cell carcinoma syndrome	FH	1q43	FH
LFS	P53	17p13.1	DNA damage response
Neurofibromatosis	NF1	17q11.2	GTPase-activator
Rb	RB1	13q14.2	Cell cycle checkpoint
RTS	RTS (RecQL4)	18q24.3	DNA helicase

WS	WRN (RecQL2)	8p12-p11.2	DNA helicase, Exonuclease activity

Table 1: Inherited diseases that commonly predispose to the development of sarcoma, cited from (5)

Discrete genetic alterations – fusion genes. The majority of currently recognized sarcoma-associated genetic alterations are non-random chromosomal translocations that result in two genes being fused with a consequent formation of a chimeric protein. These translocations usually (but not always) involve genes encoding transcription factors, most frequently in one or both breakpoints, with one of the genes often contributing a DNA-binding domain and the other one providing an activation domain. Consequently, the resulting fusion proteins are often aberrant transcription factors that dysregulate gene expression (36). As a result, diverse key cellular pathways are altered, such as cell cycle control, apoptosis and differentiation. Fusion proteins are to be considered oncogenic as they are able to induce cell transformation in culture (37) (38) (39). To further support this, it has been shown that specific fusion genes are required for the growth of corresponding cell lines in vitro. Furthermore, in animal studies, it has been shown that a subcutaneous injection of transfected cells into immunodeficient mice can result in tumorigenesis (40) (41). It would seem, however, that only specific cell types are susceptible to the transforming effects of fusion proteins. Only the phenotypes of a specific mesenchymal cell or a precursor can be modified by a gene fusion. It has been postulated that this phenomenon could represent a type of lineage-dependent oncogenesis (42). For example, Ewing sarcoma (ES) serves as a model of the association between fusion genes and sarcomagenesis and was the first solid malignancy proved to have an identifiable recurrent translocation. The earliest described fusion was the one of EWSR1 (22q12) and FLI1 (11q24) genes (43), where the FLI1 gene as a member of the ETS family encodes a transcription factor and the EWSR1 gene a nuclear protein. The created chimeric fusion protein is comprised of the N-terminal portion of the EWSR1 protein linked to the DNA-binding domain of FLI1, has a transforming activity in cell cultures assays (44) and is considered to be the principal event leading to the ES

development, mainly through transcriptional dysregulation. The presence of fusion transcript seems to invoke an extensive program of altered gene expression, which then leads to the development of malignancies.

The following Table 2 provides an overview of known fusion genes in sarcomas.

Cytogenetic and molecular alterations in sarcoma		
Complex molecular/cytogenetic profile	**Cytogenetic Alternations**	**Molecular alternations**
Angiosarcoma	Complex	
Chondrosarcoma	Complex	
Leiomyosarcoma	Complex (frequent deletion of 1p)	
MPNST	Complex	
Osteosarcoma	Complex	
Pleomorphic rhabdomyosarcoma	Complex	
Pleomorphic sarcoma, NOS (MFH)	Complex	
Simple molecular/cytogenetic profile		
Alveolar Rhabdomyosarcoma	t(2;13)(q35;q14)	*PAX3-FOXO1A fusion*
	t(1;13)(p36;q14), double minutes	*PAX7-FOXO1A fusion*
	t(2;2)(q35;p23)	*PAX3-NCOA1 fusion*
	t(X;2)(q35;q13)	*PAX3-AFX fusion*
Alveolar soft part sarcoma	t(X;17)(p11;q25)	*TFE3-ASPSCR1 fusion*
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	*EWSR1-CREB1 fusion*
	t(12;22)(q13;q12)	*FUS-ATF1 fusion*
	t(2;22)(q33;q12)	*EWSR1-ATF1 fusion*
Embryonal rhabdomyosarcoma	Trisomy 2q, 8 and 20	*Loss of heterozygosity at 11p15*
Clear cell sarcoma	t(12;22)(q13;q12)	*EWSR1-ATF1 fusion*
	t(2;22)(q33;q12)	*EWSR1-CREB1 fusion*
Desmoids fibromatosis	Trisomy 8 and 20 and loss of 5q21	*CTNNB1 or APC mutation*
Desmoplastic small round cell	t(11;22)(p13;q12)	*EWSR1-WT1 fusion*

tumor		
Dermatofibrosarcoma protuberans	Ring forms of chromosomes 17 and 22	*COLIA1-PDGFB fusion*
Endometrial stromal sarcoma	t(7;17)(p15;q21)	*JAZF1-JJAZ1 fusion*
	t(6;7)(p21;p15)	*JAZF1-PHF1 fusion*
	t(6;10)(p21;p11)	*EPC1-PHF1 fusion*
Epithelioid hemangioendothelioma	t(1;3)(p36;7p25)	*Unknown fusion*
ES/primitive neuroectodermal tumor	t(11;22)(q24;q12)	*EWSR1-FLI1 fusion*
	t(21;22)(q12;q12)	*EWSR1-ERG fusion*
	t(2;22)(q33;q12)	*EWSR1-FEV fusion*
	t(7;22)(p22;q12)	*EWSR1-ETV fusion*
	t(17;22)(q12;q12)	*EWSR1-E1AF fusion*
	inv(22)(q12;q12)	*EWSR1-ZSG fusion*
	t(2;22)(q31;q12)	*EWSR1-SP3 fusion*
	t(16;21)(p11;q22)	*FUS-ERG fusion*
	t(2;16)(q33;p11)	*FUS-FEV fusion*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	*EWSR1-NR4A3 fusion*
	t(9;17)(q22;q11)	*TAF2N-NR4A3 fusion*
	t(9;15)(q22;q21)	*TCF12-NR4A3 fusion*
	t(3;9)(q11;q22)	*TFG-NR4A3 fusion*
Fibrosarcoma, infantile	t(12;15)(p13;q26)	*ETV6-NTRK3 fusion*
	Trisomy 8,11,17 and 20	
GIST	Monosomies 14 and 22	*KIT mutation*
	Deletion of 1p	
Inflammatory myofibroblastic tumor	t(1;2)(q22;p23)	*TPM3-ALK fusion*
	t(2;19)(p23;p13)	*TPM4-ALK fusion*
	t(2;17)(p23;q23)	*CLTC-ALK fusion*
	t(2;2)(p23;q13)	*RANB2-ALK fusion*
Low-grade fibromyxoid sarcoma	t(7;16)(q33;p11)	*FUS-CREB3L2 fusion*
	t(11;16)(p11;p11)	*FUS-CREB3L1 fusion*
Myxoid/round cell liposarcoma	t(12;16)(q13;p11)t	*FUS-DDIT3*

	(12;22)(q13;q12)	*EWSR1-DDIT3*
Myxofibrosarcoma (myxoid MFH)	Ring form of chromosome 12	
Synovial sarcoma		
Biphasic	t(x;18)(p11;q11)	*Predominately SS18-SSX1 fusion*
Monophasic	t(x;18)(p11;q11)	*SS18-SSX1,SS18-SSX2 or SSX4 fusion*
Well-differentiated liposarcoma	Ring form of chromosome 12	*Others*

Table 2: Cytogenetic and molecular alterations in sarcoma, cited from (5)

Complex genetic alterations. The presence of highly complex unbalanced karyotypes without specific genetic translocations characterizes a diverse group of sarcomas, including leiomyosarcoma, osteosarcoma, malignant fibrous histiocytoma (MFH), angiosarcoma, pleomorphic rhabdomyosarcoma, chondrosarcoma and MPNST. It is assumed that a disruption of normal p53 function plays a key role in the development of these nonspecific chromosomal aberrations. In contrast, p53 inactivation is rarely to be found in sarcomas with discrete genetic alterations. Two distinct pathways are implicated in the development and generation of complex genetic alterations in sarcomas: impaired joining of non-homologous ends (NHEJ) and telomere dysfunction (5). Several studies implied that the haploinsufficiency of NHEJ component DNA ligase IV (lig4) is promoting the development of soft tissue sarcomas. Loss of a single lig4 allele is considered to result in NHEJ activity reduction sufficient to allow the emergence of chromosomal aberrations that could drive sarcomagenesis (45). Telomeres comprise the nucleoprotein complexes that coat the ends of eukaryotic chromosomes. They are sustained by the reverse transcriptase telomerase, which maintains the telomerase length by adding hexanucleotide repeats to existing telomeres. The lack of telomerase activity, frequently as a function of age and successive cell divisions, leads to progressive telomere shortening and ultimately to chromosomal instability through end-to-end fusions. Provided the presence of a functional p53/RB pathway, these cells should typically undergo apoptosis as a protective

mechanism against severe genetic instability. Abrogation of these pathways is instrumental to tumor cell survival and the selection of a more aggressive tumor phenotype (5). Ultimately, reactivation of telomerase and telomere maintenance is to be observed, which promotes tumor cell survival and immortality. In sarcoma, there is another telomerase-independent mechanism discovered, termed alternative lengthening of telomeres (ALT), which is mechanistically still poorly understood (46). Several studies have associated ALT with chromosomal instability in osteosarcomas (47), which further supports the role of telomere dysfunction in the development of non-discrete complex genetic alterations. It has been observed that p53 mutation may be a common early event in sarcomas with complex non-discrete gene alterations. Triggered by telomeric dysfunction and NHEJ, the inactivation of p53 is postulated to be a cellular defense mechanism against malign alteration (5). P53 deficiency has been shown to play a role in tumorigenesis by promoting a process called fusion-bridge breakage (BFB), which leads to the formation of complex nonreciprocal translocations – a classical cytogenetic feature of human carcinomas. Several studies have shown a high frequency of BFBs in human sarcoma specimens, lacking simple tumor-specific aberrations. In contrast, this could not be noted in any of the specimens carrying fusion gene mutations (48). The following Table 3 presents known chromosomal alterations with a suspected role in Sarcomagenesis.

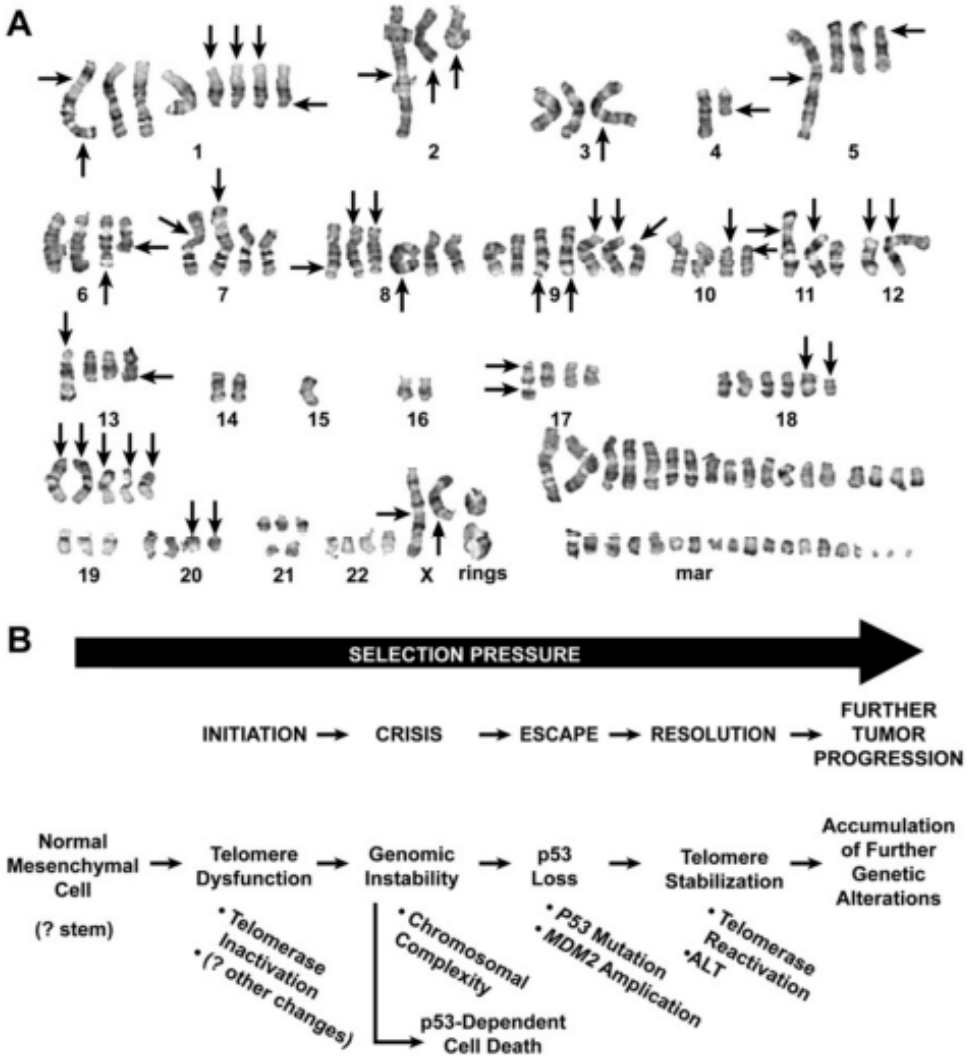


Table 3: Chromosomal alterations in Sarcomagenesis, cited from (5)

Environmental factors that are associated with the development of sarcoma include exposure to radiation and multiple organic and inorganic substances. Radiation exposure has been recognized since the 1920s to be able to induce sarcomagenesis. The published reports from that time have shown that workers manufacturing radium watch dials had a significantly higher sarcoma incidence compared to the normal population (49). Further, it has been shown that patients in whom radiotherapy was used to treat a non-sarcomatoid primary tumor (for example lymphoma, breast, testicular, prostate or lung cancer) have a significantly increased risk of secondarily developing sarcoma (50) (51) (52). According to estimates based on a series of patients operated on for sarcoma, post-radiogenic sarcomas account for approximately between 0.5 and 5.5% of all sarcomas

(53) (54). On the other hand, large population-based studies of long-term outcomes for patients after radiotherapy for various reasons, show a significantly lower incidence, in the range of 0.03 to 0.8% (55) (56). There appears to be a dose-response correlation between the dose of radiation and the incidence of sarcoma development. For example, a dose less than 10 Gy is considered to be associated with a very low risk (57). It has been shown, that sarcoma typically arises in the radiation field margins, suggesting that the mutagenic effect may be maximal at the periphery, where scatter radiation could reach a sufficient dose for inducing mutation, although insufficient to destroy the mutated cells. (58) A delayed onset of several years between radiotherapy and subsequent sarcoma development has been observed. Initially, an interval of a minimum of 5 years was suspected (59). However, more recent data have shown a much shorter latency period to be possible. (53) (60) Radiotherapy-associated sarcomas have been observed to be in most cases aggressive high-grade malignancies. The most common radiation-induced histologic sarcoma subtypes are extraskeletal osteosarcoma (21%), MFH (16%,) and (lymph)angiosarcoma (15%) (51).

Exposure to certain inorganic as well as organic chemicals has shown a strong association with sarcoma occurrence. For example, PVC, thorotrast (thorium dioxide), inorganic arsenic and androgenic-anabolic steroids are strongly associated with the development of hepatic angiosarcoma (61). In the following Table 4, we listed the most prevalent etiological factors, which could potentially lead to sarcomagenesis.

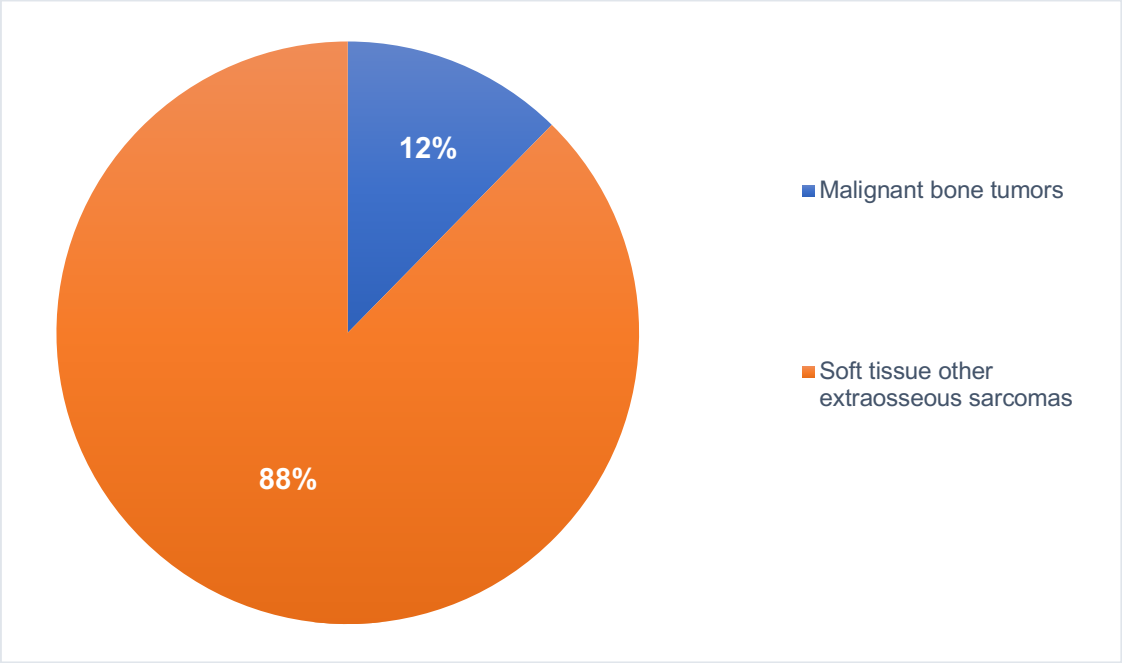
Potential sarcoma etiologies
Host related
Immune suppression
AIDS
Transplantation
Chronic irritation of tissues
Chronic inflammation
Foreign body

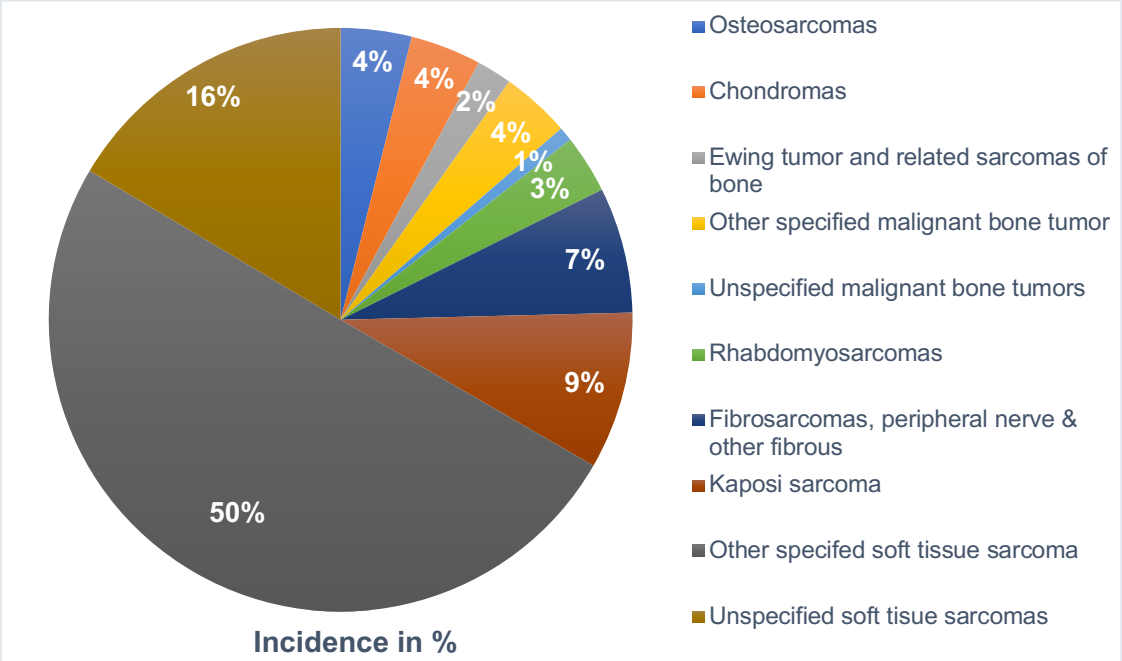
Genetic alternations
Genetic syndromes
Discrete genetic alternations-fusion genes
Complex nonspecific genetic alternations

Table 4: Potential sarcoma etiologies, cited from (5)

1.1.3. Epidemiology

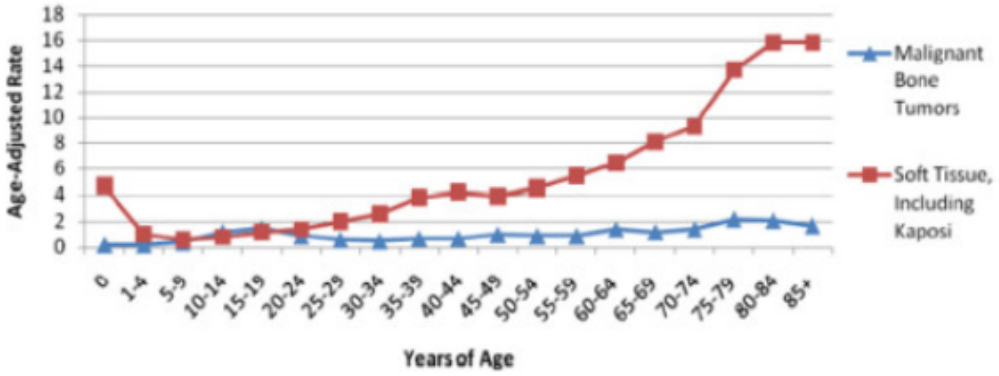
Age is an important contributing factor to sarcoma occurrence. According to current statistics, sarcomas constitute about 15% of all pediatric and less than 1% of all solid malignancies in the adult population (61) worldwide. In SEER (Surveillance, Epidemiology and End Results) data shows that the vast majority affect soft tissues (80%), whereas only one-fifth affects the bones (61). Among the malignant bone tumors, osteosarcomas and chondrosarcomas were the most frequently diagnosed, accounting for over half of all bone malignancies (62). In graphic 1, we present the official sarcoma incidence.





Graphic 1: Sarcoma incidence, cited from (63)

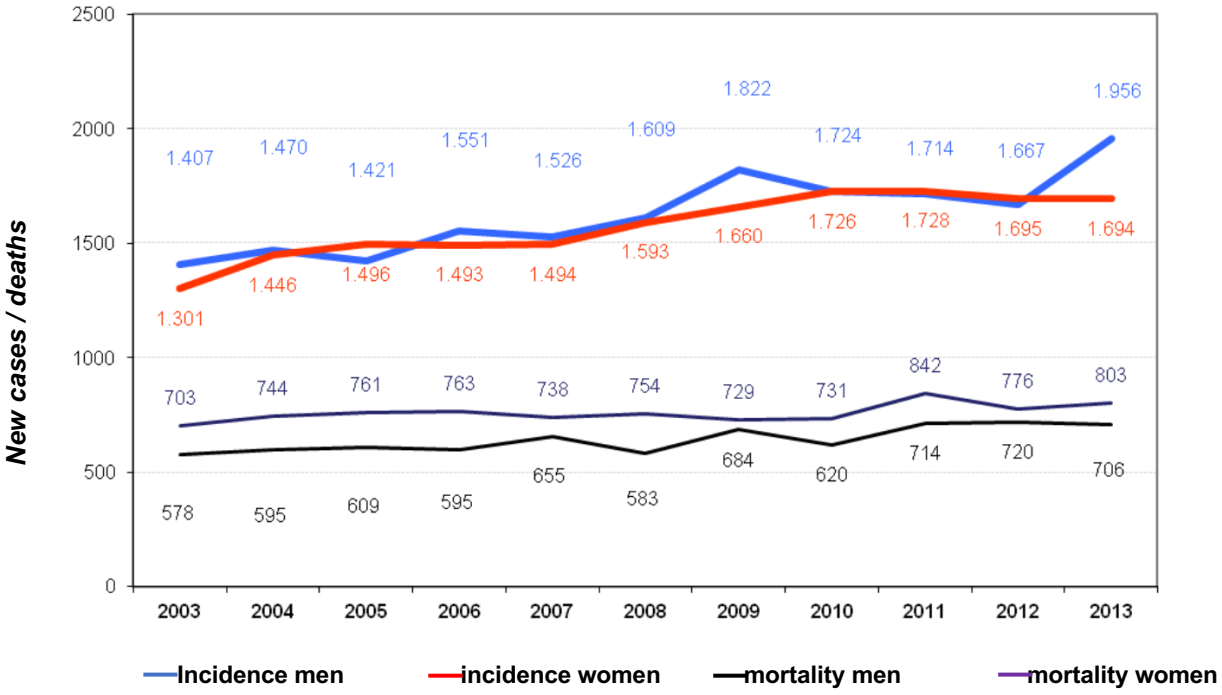
Regarding age distribution, an increase in the rate of STS occurs in babies and young children until the age of 5 (64). The lowest incidence is observed in young adults, with slowly and steadily increasing until the age of 50 (64). Above the age of 50, the incidence of STS exhibits a considerable rise. Malignant bone tumors, on the other hand, show a rather stable rate of incidence across all age groups (64) with noticeable increases in rates can be observed in adolescents and young adults, mostly due to osteosarcoma and Ewing's sarcoma (64) as well as in people in their 70s and 80s (62), as shown in graphic 2.



Graphic 2: Age-adjusted sarcoma rates, cited from (63)

Despite their rarity and heterogeneity, in the United States of America and Europe, guidelines for the management of sarcomas have been developed by the National Comprehensive Cancer Network and by the European Society of Medical Oncology respectively (64) (65). In high-income countries, these guidelines have been accepted broadly. It has been observed that the recommended multidisciplinary approaches, which have led to great improvements in oncologic and functional outcomes, find only limited applicability in lower-income countries, mainly due to resource constraints (66). More than two-thirds of the world´s population lives in low-income countries, which have seen a surge in cancer burden from a mere 15% in 1970 to 56% in 2008. In 2020, the estimated total number of new malignancies would increase by 73% in low-income countries compared with a 29% increase in high-income countries (67). A further striking difference would be the cancer mortality to incidence ratio, which is 0.66 in low-income areas and almost double the ratio in high-income countries (0.38) (68). This discrepancy is probably caused by inequitable distribution as well as a lack of healthcare access and expertise. Lack of established public health policies or its inadequate integration, cultural misbelief and illiteracy lead to delayed presentation to medical attention, which adversely affects the rate of limb salvage and overall survival of patients with sarcoma. A multi-pronged approach is required to tackle these complex issues (66).

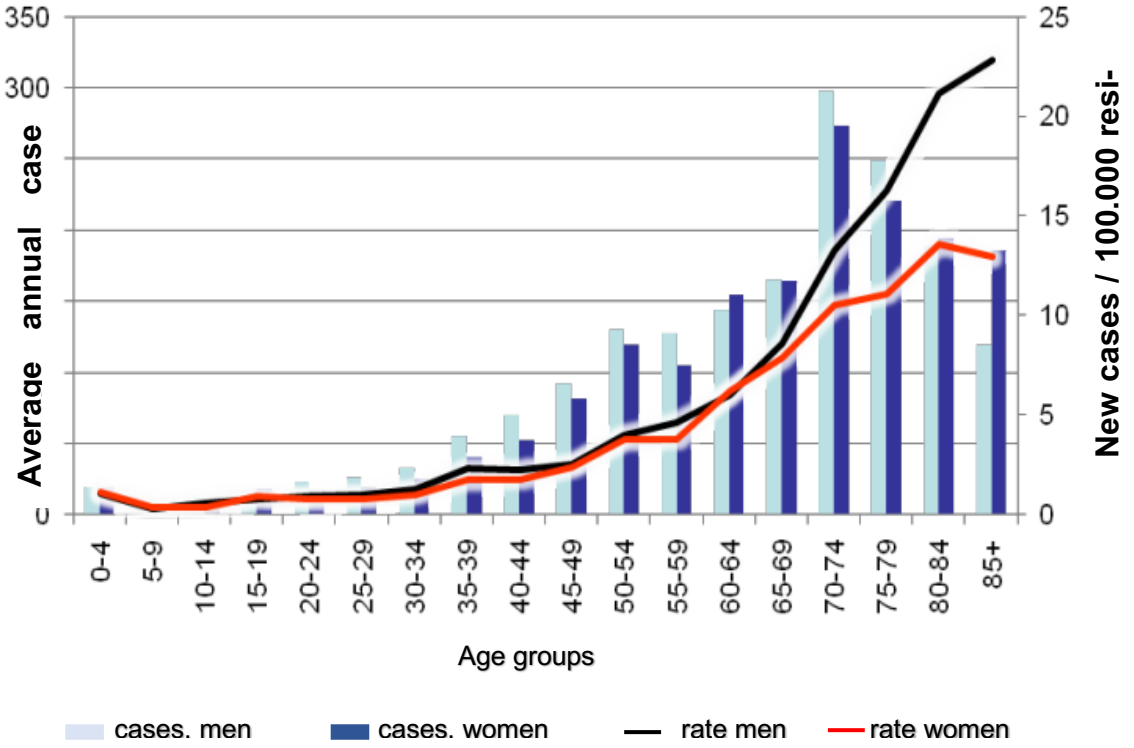
According to the latest available statistics, in 2013 in Germany, approximately 3,650 new cases of soft tissue sarcomas occurred (69). In comparison to statistics from 2004, the incidence has increased by an average of 2.9% per year in men and 3.9% per year in women over the past decade (2004-2013). The age-standardized disease rate in Germany in 2013 was 3.6/100,000 for men and 2.7/100,000 for women (70). Over the past 10 years, the rate has increased by an average of 1.5% for women and 1.2% for men. The differences in the development over time between the probability of disease (rates) and the registered case numbers can be explained by the change in the age structure of the population (69).



Graphic 3: Sarcoma, incidence/mortality curves, cited from (63)

The federal cause-of-death statistics show a total of 1,509 deaths for soft tissue sarcomas in 2013 (men 706, women 803). The number of deaths has increased by an average of 2.2% per year in men and 1% per year in women over the past decade (2004-2013). The age-standardized mortality rate was 1.3/100,000 for men and 1.1/100,000 for women, and mortality rates have been nearly constant for both sexes over the past decade (men averaged +0.1%, women averaged +0.6% per year) (69).

The disease rates for men and women are remarkably similar up to about the age of 70. After that, the likelihood of disease increases significantly more for men than for women and is almost twice as high for men in the highest age group. In the lowest age group (under 5 years), about 11 per 1000000 children develop the disease. After that, the probability of contracting the disease decreases again. Among 5 to 10-year-olds, it is less than 4 per 1,000,000 but increases steadily from then on. Although the highest rates of disease are in those over 80 years of age, most cases of the disease in both sexes occur in the 70 to 80 age group. This can be explained by the greater population in this age group (69).



Graphic 4: Sarcoma annual average case numbers stratified by age groups and gender, cited from (69).

1.1.4. Classification

Sarcomas are typically divided into two major groups: bone sarcoma and soft tissue sarcoma, both of which are further divided into multiple subtypes, based on histological, clinical and molecular-genetic characteristics (1).

Due to the vast heterogeneity in morphologic, immunohistochemical and molecular-genetic characteristics of mesenchymal neoplasms, reaching an integrated pathologic diagnosis, while particularly challenging, represents a key step in clinical decision-making. A step toward standardizing pathologic diagnostics was made through the new 2020 World Health Organization (WHO) classification of soft tissue tumors, in which many new entities have been introduced, based on their distinct biological behavior, genetics and morphology. With the recent advances and a better understanding of molecular genetics, several novel recurrent genetic alterations have been incorporated in this edition. These may serve as reliable diagnostic and prognostic markers for various soft tissue tumors (70).

The 2020 WHO classification describes soft tissue tumors under eleven categories. Based on the biological behavior, these are further subcategorized into benign (do not recur after resection), intermediate – locally aggressive (locally infiltrative, have a high rate of recurrence but do not metastasize), intermediate – rarely metastasizing (metastasis in less than 2% cases) and malignant (high risk of metastasis). Seven years after the previous version, the fifth edition of this WHO classification was published in 2020 and represents a consensus amongst experts comprising a multidisciplinary soft tissue tumor board including pathologists, oncologists, geneticists, radiologists and surgeons. The availability of extensive new molecular and genetic data has led to the introduction of new entities and the reorganization of some previously existing ones. The hallmark of the new WHO revision is the inclusion of multiple novel genetic alterations and their surrogate immunohistochemical (IHC) markers for various entities (71). This has enabled the reclassification and prognostication of existing tumor entities (like solitary fibrous tumors). Furthermore, many of the newly described genetic rearrangements are potential therapeutic targets and may lead to the optimization of chemotherapy regimens in the future.

The following Table 5 presents the aforementioned WHO 2020 Sarcoma classification.

Category	**Locally aggressive**	**Rarely metastasizing**	**Malignant**
Adipocytic	Atypical Lipomatous Tumor		Liposarcoma • Well-differentiated • Dedifferentiated • Myxoid • Pleomorphic • Myxoid pleomorphic
Fibroblastic and Myofibroblastic	Solitary Fibrous Tumor	Dermatofibrosarcoma Protuberans	Solitary Fibrous Tumor
	Fibromatosis	Solitary Fibrous Tumor	Fibrosarcoma
	• Palmar/plantar	Inflammatory Myofibroblastic Tumor	Myxofibrosarcoma
	• Desmoid Type	Myofibroblastic Sarcoma	Low-grade fibromyxoid sarcoma

	Lipofibromatosis	Superficial CD34 Positive Fibroblastic Tumor	Sclerosing epithelioid fibrosarcoma
	Giant Cell Fibroblastoma	Myxoinflammatory Fibroblastic Sarcoma	
		Infantile Fibrosarcoma	
So-called Fibrohistiocytic Tumors	Plexiform Fibrohistiocytic Tumor		Malignant Tenosynovial Giant Cell Tumor
	Giant Cell Tumor of Soft Parts		
Vascular Tumors	Kaposiform Hemangioendothelioma	Retiform Hemangioendothelioma	Epithelioid Hemangioendothelioma
		Papillary Intralymphatic Angioendothelioma	Epithelioid Hemangioendothelioma with YAP1-TFE3 Fusion
		Composite Hemangioendothelioma	Angiosarcoma
		Kaposi Sarcoma	
		Pseudomyxogenic Hemangioendothelioma	
Pericytic Tumors			Malignant Glomus Tumor
Smooth Muscle Tumors	Smooth Muscle Tumor of Uncertain Malignant Potential		Leiomyosarcoma
			Inflammatory Leiomyosarcoma
Skeletal Muscle Tumors			Rhabdomyosarcoma • Embryonal • Alveolar • Pleomorphic • Spindle cell
			Ectomesenchymoma
Chondro-Osseous Tumors			Extraskeletal Osteosarcoma
Peripheral Nerve Sheath Tumors			Malignant Peripheral Nerve Sheath Tumors
			Melanotic Malignant Nerve Sheath Tumor
			Malignant Granular Cell Tumor
			Malignant Perineurinoma

Tumors of Uncertain Differentiation	Epithelioid Angiomyolipoma	Atypical Fibroxanthoma	NTRK Rearranged Spindle Cell Neoplasm
	Hemosiderotic Fibrolipomatous Tumor	Angiomatoid Fibrous Histiocytoma	Synovial Sarcoma
		Ossifying Fibromyxoid Tumor	Epithelioid Sarcoma
		Myoepithelioma	Alveolar Soft Part Sarcoma
			Clear Cell Sarcoma
			Extraskeletal Myxoid Chondrosarcoma
			Desmoplastic Small Round Cell Tumor
			Rhabdoid Tumor
			Malignant Perivascular Epithelioid Tumor
			Intimal Sarcoma
			Malignant Ossifying Fibromyxoid Tumor
			Undifferentiated Sarcoma
			Undifferentiated Spindle Cell Sarcoma
			Undifferentiated Pleomorphic Sarcoma
Undifferentiated Small Round Cell Sarcoma of bone and soft tissues			Ewing´s Sarcoma
			Round cell sarcoma with ESWR1-non ETS fusion
			CIC rearranged sarcomas
			Sarcoma with BCOR genetic alteration

Table 5: 2020 WHO Sarcoma classification, cited from (70)*, Alteration: column "Benign tumors" removed due to irrelevance to the topic.*

1.1.5. Diagnostics

If soft tissue sarcoma is suspected, imaging diagnostics of local spread is performed before the biopsy (69). The method of choice is magnetic resonance imaging (MRI) with contrast medium. In case of contraindications for MRI or mainly osseous involvement, computed tomography (CT), conventional X-ray examinations and sonography can be performed. In individual cases, a complementary PET-CT examination may be useful (69).

The key step in identifying and classifying the sarcoma is the biopsy. A biopsy ought to be performed after imaging diagnostics of local spread, not before (69). The aim is to obtain sufficient representative tumor tissue for histopathological, immunohistochemical and molecular-pathological classification. According to guidelines, such procedures should be conducted in sarcoma reference centers and/or within reference networks sharing multidisciplinary expertise and treating a relevant number of patients annually (66). Such centers are usually involved in ongoing clinical trials, which may lead to patients' enrolment and offering potentially a novel therapy option (66). Sarcoma reference centers and reference networks need to meet certain defined quality criteria (66). These criteria may exhibit a certain country-related variability, are, however, usually based on common traits, such as: multidisciplinarity (for example, weekly tumor boards), the volume of patients and appropriate facilities for proper application of clinical practice guidelines, recording and publication of outcomes (66) (72).

Possible biopsy procedures include an open incisional biopsy or image-guided punch biopsy. In oncology centers, diagnostic confidence of punch biopsy is 97%. Accordingly, punch biopsy is hardly inferior to incisional biopsy, but should be performed in interdisciplinary consultation with the subsequently appointed surgeon, radiation therapist and pathologist (73) (74). Fine needle aspiration biopsy is mostly inadequate in sarcoma diagnosis and therefore justifiable only in a few selected cases and only in specialized centers (69). For small (< 3 cm) and superficially (cutaneous, subcutaneous) localized soft tissue tumors, primary resection may be considered if no functional deficit is to be expected (69).

Lastly, diagnostics of a systemic spread is performed after histological confirmation of the diagnosis utilizing a CT scan of the thorax, abdomen/pelvis. In individual cases, a whole-body MRI examination may be useful.

This histological and molecular heterogeneity makes sarcomas particularly difficult to diagnose, leading to the debate surrounding the sufficiency of histological diagnosis versus the need for ancillary molecular-genetic diagnostics as well as the timing of these ancillary procedures. Treatment has proven equally challenging, and research findings in one subtype often do not translate to others. These

limitations are magnified within the context that sarcomas are among the rarest of cancer diagnoses, making research and trials more difficult (66)(75). Nonetheless, according to ESMO (European Society for Medical Oncology) clinical practice guidelines for diagnosis, treatment and follow-up, each sarcoma case should be provided with malignancy grading and staging (66) (72). The grading should be provided in every case (in which feasible) and should be based on established systems, as it has immense impact on the prognosis and treatment. One of the most commonly used grading systems is the ”Fédération nationale des Centres de lutte contre le cancer” (FNCLCC) system, which distinguishes three malignancy grades based on differentiation, necrosis and mitotic rate (66)(72). According to the new guidelines, the mitotic rate should be provided, whenever possible, independently (66) and an effort to improve the reliability of mitotic count should be made (66). Due to major therapy-related changes of the tumor tissue, grading cannot be assigned after preoperative medical treatment, (72).

	Tumor differentiation
Score 1	Sarcomas closely resembling normal adult mesenchymal tissue (e.g., well-differentiated liposarcoma)
Score 2	Sarcomas for which histologic typing is certain (e.g., myxoid liposarcoma)
Score 3	Embryonal and undifferentiated sarcomas, sarcomas of doubtful type, and synovial sarcomas
	Mitotic Count
Score 1	0–9 mitoses per 10 HPF
Score 2	10–19 mitoses per 10 HPF
Score 3	≥ 20 mitoses per 10 HPF
	Tumor Necrosis
Score 0	No necrosis
Score 1	< 50% tumor necrosis
Score 2	≥ 50% tumor necrosis

Table 6: FNCLCC grading system, cited from (76)

In addition to the histopathological degree of differentiation (grading), as explained above, tumor size and tumor localization (superficial vs. deep-seated tumors) are further prognostically relevant. These three prognostic factors form the basis of the staging of the UICC (Union for International Cancer Control) and AJCC (American Joint Committee on Cancer) respectively (69). The Cancer Staging Manual of the AJCC has recently been revised and updated to its eighth edition (77).

A major characteristic of TNM classification in bone and soft tissue sarcomas is that histopathological grade (G) is included as a factor in staging. By contrast, in the TNM system for other cancers, the stage is basically determined by only three factors: T factor based on the depth of tumor infiltration and the greatest diameter of the tumor, N factor of lymph node metastasis and M factor of distant metastasis. In the World Health Organization classification (70), the histologic types of bone and soft tissue sarcomas vary considerably, and the biological properties of individual tumors differ widely as well. Therefore, it is difficult to reflect the prognosis with only three factors of TNM in all tissue types of sarcomas. However, even if the tissue type is different, the biological property could be similar if the pathologic grade of the sarcoma is the same. Therefore, with the G factor, a simple staging classification becomes possible for various tissue types of sarcomas. In addition, lymph node metastasis is extremely rare in bone and soft tissue sarcomas, which is why the N factor is rarely used. If the G factor is not considered, the stage would have to be determined by only two factors, i.e., T and M. As such, in addition to the usual three TNM factors, the staging system (such as UICC or AJCC) for bone and soft tissue sarcomas necessarily includes the factor of histological grade (G) (77). The following Tables 7, 8 and 9 provide AJCC prognostic stage groups for sarcoma concerning different primary tumor localizations.

Stage	Primary tumor (T)	Regional lymph node (N)	Distant metastasis (M)	Histologic grade (G)
IA	T1	N0	M0	G1 or GX
IB	T2 or T3	N0	M0	G1 or GX
IIA	T1	N0	M0	G2 or G3
IIB	T2	N0	M0	G2 or G3
III	T3	N0	M0	G2 or G3
IVA	Any T	N0	M1a	Any G
IVB	Any T	N1	Any M	Any G
	Any T	Any N	M1b	Any G

Table 7: AJCC prognostic stage groups for bone sarcoma in the appendicular skeleton, trunk, skull and facial bones, table taken from (77)

Stage	Primary tumor (T)	Regional lymph node (N)	Distant metastasis (M)	Histologic grade (G)
IA	T1	N0	M0	G1, GX
IB	T2, T3, T4	N0	M0	G1, GX
II	T1	N0	M0	G2, G3
IIIA	T2	N0	M0	G2, G3
IIIB	T3, T4	N0	M0	G2, G3
IV	Any T	N1	M0	Any G
	Any T	Any N	M1	Any G

Table 8: AJCC prognostic stage groups for STS in the trunk and extremity, table taken from (77)

Stage	Primary tumor (T)	Regional lymph node (N)	Distant metastasis (M)	Histologic grade (G)
IA	T1	N0	M0	G1, GX
IB	T2, T3, T4	N0	M0	G1, GX
II	T1	N0	M0	G2, G3
IIIA	T2	N0	M0	G2, G3
IIIB	T3, T4	N0	M0	G2, G3
	Any T	N1	M0	Any G
IV	Any T	Any N	M1	Any G

Table 9: AJCC prognostic stage groups for STS of the retroperitoneum, table taken from (77)

1.1.6. Therapy

Sarcoma therapy, considering massive heterogeneity, is very complex. An optimal treatment strategy requires interdisciplinary cooperation, ideally from the point of diagnosis (69). It has two goals: locoregional tumor control and prevention/therapy of distant metastasis. The treatment strategy is determined by tumor stage, prognostic factors such as histology, grading, size and location, and patient-specific factors. It includes several modalities (69):

Surgical therapy is the basis of local tumor control. The defined goal is a resection of the soft tissue sarcoma in healthy tissue, so-called R0 resection. Depending on histology, size, and location, adjuvant radiotherapy follows for highly malignant tumors. Marginal (R1 resections) or intralesional resections (R2 resections) should not be attempted from the oncological standpoint, as they usually do not achieve the goal of local tumor control even when adjuvant therapy options are considered.

If R0 resection is achievable in stages I-III after completion of staging without a mutilating procedure, surgical therapy is primarily indicated in adulthood (69). Otherwise, neoadjuvant therapy options (e.g., systemic chemotherapy +/- hyperthermia, radiotherapy and isolated hyperthermic limb perfusion) should be considered in the treatment planning in an interdisciplinary manner (69). Marginal tumor resections (R1 resection) along the pseudotumor capsule are associated with a significantly increased risk of local recurrence (69). This pseudotumor capsule is usually the active growth front of the soft tissue sarcoma and not its actual boundary. Histologically, vital tumor cells could be detected in the peritumoral edema (78). This aspect seems to be responsible for the high local reoccurrence rate. An oncologically safe metric resection distance has not yet been defined (69).

Radiotherapy plays a significant role in the multimodal therapy concept. It allows lowering the incidence of a loss of function or mutilation significantly by reducing the extension of radical surgical measures needed for local tumor control. For example, limb-preserving surgery, whereby local control can be achieved in up to 90% of cases with the aid of radiotherapy (69). In this way, radical surgical measures, such as amputation or compartment resection, usually associated with a loss of function or mutilation, can be avoided (69). Postoperative radiotherapy is a standard procedure. Preoperative and intraoperative radiation, radiation therapy alone, and hyperthermia may also be used as part of the primary/recurrent therapy strategy (69).

Chemotherapy. In general, chemotherapy for sarcoma can be divided into neoadjuvant, adjuvant and palliative.

Regarding neoadjuvant chemotherapy for sarcoma, there is currently no unanimous position – it is a decision based on sarcoma type, localization and stage (69). It is increasingly used with aim to limit the loss of function after wide margin surgical excision with the ultimate goal of improving patient survival (79). Patient selection for such treatments is expected to be improved by the eighth edition of AJCC´s TNM staging system, as it tailors T-stage categories based on primary tumor site and considers a prognostic nomogram, which also includes soft tissue

sarcoma histology and other patient and tumor features not directly included in the TNM staging (79).

In advanced IIB and III stage sarcomas, in which R0 resection with sufficient safety margin cannot be reliably achieved, preoperative/neoadjuvant therapy procedures should be considered (69). Preoperative chemotherapy alone may lead to objective remission in up to approximately 30% of patients but has not had a clearly defined role (69) and the decision regarding the appropriate therapy for locally advanced and borderline resectable sarcomas should be coordinated on an interdisciplinary basis with/in a sarcoma center (69). In addition to radiotherapy alone, preoperative/neoadjuvant therapeutic procedures include approaches such as combined radiochemotherapy or chemotherapy with regional hyperthermia, and if necessary, the use of isolated limb perfusion (ILP) with TNF-alpha/melphalan (69). This therapeutic procedure should be considered for locally advanced sarcomas of extremities which cannot be resected in a healthy state and/or cannot be resected in a way that preserves the extremities (80). After ILP and adequate resection, 5-year recurrence-free survival can be achieved in 78% of patients. Here, compared to a combination of resection and adjuvant radiotherapy, limb perfusion can achieve a comparable oncologic outcome with the frequent omission of radiation (81).

Also, in the recurrence situation and after pre-radiation in the initial therapy, local tumor control can be improved, a resection in healthy tissue can be made possible and a potentially mutilating operation can be avoided. As a palliative measure in locally irresectable tumors and existing metastasis, ILP may also be indicated in individual cases (82).

In case of metastatic or irresectable disease, the standard first-line therapy to date is Doxorubicin (Adriamycin, ADM) monotherapy (83). For patients with rapidly progressive, symptomatic disease or locally advanced STS (soft tissue sarcoma), combination therapy with Doxorubicin/Ifosfamide (ADM/IFS) versus ADM monotherapy should be considered because of the higher probability of remission (26% vs. 13%) and longer progression-free survival (7.4 vs. 4.6 months) in sev-

eral STS entities (84) (85), and in leiomyosarcoma in combination with Dacarbazine (DTIC). However, overall survival is not improved by ADM/IFS combination therapy compared with sequential monotherapy (2-year survival 31% vs. 28%) (69). Provided that achieving arrest of tumor progression is the primary focus of therapeutic efforts, sequential monotherapy is a reasonable approach with fewer side effects. Thus, to date, monotherapy with Doxorubicin at a dose of 70-80 mg/m² represents the first-line therapy of choice for the majority of patients. An alternative to ADM would be Gemcitabine/Docetaxel combination (86).

For the second-line therapy, Ifosfamide at a dose of approximately 9-12 g/m² (over 3-5 days) can be considered (87) (88) (89). Trabectedin is approved as the second/third-line therapy after failure of Doxorubicin +/- Ifosfamide, with most trial experience with this agent for leiomyo- and liposarcomas (90) (91). Trabectedin is superior to monotherapy with Dacarbazine in this setting. Trabectedin may also be effective in other entities, including synovial sarcoma (92) (93).

Another therapeutic option as a possible second/third-line therapy is Pazopanib (94), which is, however, not approved for liposarcomas (95). The cytostatic drug Eribulin is approved in liposarcoma patients as second-line therapy after anthracyclines or in case of contraindications to anthracyclines. It leads to prolongation of overall survival compared with Dacarbazine (hazard ratio 0.51; median 7.2 months) (92), (96). For Dacarbazine, response rates of 8-17% have been described in older studies after the failure of Doxorubicin/Ifosfamide. Gemcitabine is also an established ('off-label') treatment option (97) (98), possibly in combination with Docetaxel, which was superior to Gemcitabine alone in one study (99). A combination of Gemcitabine and Dacarbazine was shown to be superior to therapy with Dacarbazine in another phase II trial (100).

1.2. DNA Sequencing

1.2.1. DNA Sequencing, overview and history

According to definition, DNA sequencing is a process of determining the nucleic acid sequence, i.e. the order of nucleotides in DNA (101), including any method or technology used to determine the sequence of the four canonical nucleotide bases adenine, guanine, cytosine, and thymine. Due to its versatility, it has become indispensable in a myriad of basic biological and applied fields of research (medicine, forensics, microbiology, virology, biotechnology, biological systematics, to name just a few examples). It brought about a rapid acceleration of research and widening of our knowledge (102).

Comparing healthy and mutated DNA sequences can yield indispensable information in the diagnostics of different diseases, including various malignancies (103), or allow for characterization of individual antibody repertoire (104). Such possibilities allow it to be used as a tool to guide and inform clinical decisions in patient care more accurately (105). A quick way to sequence DNA allows for faster and more individualized medical care, as well as more organisms to be identified, researched and cataloged. The speed and efficiency attained by modern DNA sequencing technology enabled the examination of complete DNA sequences or genomes of numerous types and species of life, including the human genome (102).

Historically, DNA sequencing intimately followed the discovery of DNA structure and function by James Watson and Francis Crick in 1953 based on crystallized X-ray structures studied by Rosalind Franklin (106). The pioneering work of Frederick Sanger, who discovered the sequence of all the amino acids in insulin by 1955 (107), laid the foundation for protein sequencing, which in turn inspired the hypothesis that the arrangement of nucleotides in DNA determined the sequence of amino acids in proteins, which eventually led to the discovery of the protein function by Francis Crick (1958). In 1970, at Cornell University in USA, the first method for determining DNA sequences was developed by Ray Wu that involved a location-specific primer extension strategy with synthetic location-specific primers (108). This was the basis for developing much more rapid DNA sequencing

methods in subsequent years, such as DNA sequencing with chain-terminating inhibitors (Sanger sequencing) by Frederick Sanger at the MRC Centre, Cambridge UK (109) or DNA sequencing by chemical degradation (Maxam-Gilbert sequencing) by Walter Gilbert and Allan Maxam at Harvard (110). The first sequencing of a complete genome was conducted on a bacteriophage φX174 in 1977 (111) and subsequently on EBV in 1984. Prior to that, no genetic profile of the viruses was known. The next major step came in the early 1980s through the development of a non-radioactive method for transferring DNA molecules in sequencing reaction mixtures onto an immobilizing matrix during electrophoresis by Herbert Pohl and co-workers (112). The commercialization of a DNA sequencer followed. Examples are “Direct-Blotting-Electrophoresis-System GATC 1500” by GATC Biotech, which was intensively used in the framework of the EU genome-sequencing program or Applied Biosystems’, which brought to market the first fully automated sequencing machine (113). By 2001, using shotgun sequencing methods, a sequence of the human genome was drafted. Shotgun sequencing, defined as a sequencing method designed for the analysis of DNA sequences longer than 1000 base pairs, up to and including entire chromosomes, is an especially important step in the development of sequencing technology. Using this method, a target DNA has to be broken into random fragments, which are subsequently individually sequenced using the chain termination method. As a last step, the sequences can be reassembled based on their overlapping regions (114).

With further technological advances, new methods for DNA sequencing emerged, named collectively “second-generation” or “next-generation” sequencing (NGS) to distinguish them from earlier methods. The next generation method`s main characteristic is high scalability, allowing the sequencing of an entire genome simultaneously, which is accomplished by fragmenting the genome into short nucleotide sequences, sampling for a random fragment, and sequencing multiple of those at the same time (giving it a name “massively parallel” sequencing) using one of a myriad technologies such as pyrosequencing (115), colony sequencing (116) (used currently in Illumina’s Hi-Seq genome sequencers) or massively par-

allel signature sequencing (MPSS) (117). Growing demand for low-cost affordable sequencing allowed for the technological development of high-throughput sequencing using parallelizing, allowing for a concurrent production of thousands or millions of sequences. Such technologies encompass sequencing methods like next-generation “short-read” and third-generation “long-read” sequencing, which can be applied to sequencing of whole exomes or genomes, genome resequencing, epigenome characterization, transcriptome profiling (RNA-Seq), DNA-protein interactions (ChIP-sequencing) and many more (118). This technological development allowed for sequencing of an entire human genome in as little as one day, which was, until recently, unimaginable. Some of the current corporate leaders in the development of high-throughput sequencing products are Illumina, Qiagen and ThermoFisher Scientific (119).

The aforementioned advances in the next-generation sequencing (NGS) technologies allowed for a swift, accurate, and increasingly affordable sequencing of nucleic acids. Last decades show large-scale discovery efforts that have examined genomics of different types of malignancies in, until now, unprecedented detail (120) (122), which led to the discovery and mapping of genomic landscapes of various tumors including novel genetic drivers of disease, large-scale genomic alterations, which brought about a new molecular understanding of intratumoral heterogeneity and tumor evolution (121) (122). Furthermore, NGS has proved itself as an exceedingly valuable tool in management of certain malignancies, due to the ability to improve prognosis and patient management as well as to allow stratification and therapy selection based on clinically actionable driver mutations or mechanisms of drug resistance, ultimately impacting decision-making treatment algorithms (123). Detecting mutations, insertions, deletions, copy number alterations, gene fusions, structural rearrangements as well as alternatively spliced isoforms with swiftness and ease has transformed the complementary diagnostic landscape, prompting the Food and Drug Administration (FDA)`s to approve NGS-based multigene panel tests for cancer-related genes (122) (124).

NGS allows for determining not only individual genetic alterations, but it can also be used to characterize global genomic features, which may be used as predictor

of a clinical response. Those include tumor mutational burden (TMB), microsatellite instability (MSI) and DNA damage repair scores (122).

Tumor Mutational burden (TMB), defined as the total number of somatic coding mutations pro megabase of tumor DNA (125), has emerged as a potential biomarker for response to immune checkpoint inhibitors, primarily in NSCLC (Non-small cell lung cancer) (122) (126) (127). Mounting evidence suggest utility across increasing number of malignancies, including melanoma (128) and urothelial carcinoma (129). NGS panels allow for a quick quantification of TMB, however methods for TMB quantification and reporting are in need of a wide scale standardization, which would improve the broader adoption of this diagnostic method as a clinical biomarker for immunotherapy response (122)(125).

Microsatellite Instability (MSI) is defined as variation in the length of microsatellite sequences in the genome (130), associated with defects in DNA mismatch repair genes, accumulation of frameshift mutations and malignancies with a distinctive genetic and epigenetic profile (130). Microsatellites are short (1–6 bp), repetitive sequences in a genome, which are known to be able to lengthen or shrink during DNA replication (116). In a physiologic state, MSI is repaired by the MMR (Mismatch repair) machinery. However, pathological states, such as cancer, are associated with MMR defects and subsequently detectable levels of MSI. According to levels of MSI, malignancies can be classified as microsatellite stable (MSS), and instable (MSI). The microsatellite instable tumors can be further categorized as low-MSI and high-MSI, depending on the proportion of markers regarded as evidence of MSI (116). Even though MSI is routinely assessed using standard polymerase chain reaction (PCR)-based assessment of specific DNA markers, it can be analyzed using genome-wide NGS-based methods as well. Like TMB, MSI is mostly regarded as predictor of response to immune checkpoint inhibitors in a tumor-agnostic manner, resulting in the FDA´s recent approval of the application of anti-PD1 antibody Pembrolizumab for all MSI-high solid tumors, agnostic of tumor type or the anatomical site (131).

Damage repair scores can also be identified and quantified with NGS. These are calculated based on specific DNA damage repair signatures (134), such as

'BRCAness', which are defined as characteristic genomic traits typically occurring within deficient homologous recombination repair mechanisms (HRD) often associated with the loss of BRCA1/2 (132). Damage repair scores are particularly shown to be of importance in breast and ovarian cancers as a prediction marker for patients likely to benefit from polyadenosine diphosphate-ribose polymerase (PARP) inhibitor therapy (122) (133) (134).

1.2.2. DNA sequencing in sarcoma

Sarcomas, a very rare heterogeneous group of mesenchymal derived malignancies, exhibit characteristically a considerable heterogeneity present at the molecular level, observed not only between different histological subtypes but also within the same subtype as well as within the same entity. This constellation makes accurate diagnosis and 'one-size-fits-all' therapy algorithms rather challenging (135). The last decade brought about several large-scale studies aimed to examine cancer-related genes in sarcomas using NGS panels. For example, Jour et al. sequenced 194 cancer-related genes in 25 soft tissue sarcomas (122) (136) and Groisberg et al. performed a more extensive NGS-based analysis of 102 patients across multiple sarcoma subtypes (122) (137). In both studies, approximately 60% of cases were described to harbor potentially actionable mutations with available clinical trials (122). Although informative, such data about the genetic landscape of sarcomas does not yield clarity on how many identified mutations are to be seen as the primary drivers of the disease, due to NGS diagnostic unfortunately not being able to inform if an individual mutation represents a primary or a secondary mutation. Secondary mutations are acquired later in disease development (138) and may contribute to advanced disease, but they might not represent a key driver of early cancer growth, therefore, targeting them, may have a rather limited impact on the course of the disease (139) (122). In conclusion, NGS may be useful in identifying sarcoma patients with actionable mutations, which may lead to enrolment into prospective trials of novel agents, however, an NGS-based diagnostic to detect targetable drivers should be evaluated on individual basis for every sarcoma patient referred to a tertiary center following expert pathology review (139).

Vyse et al. (122) have conducted a review of several new studies that have applied NGS based prediction markers such as TMB, MSI and DNA damage repair scores in sarcomas with no known driver mutations (139) (122) aiming to highlight opportunities and challenges of introducing NGS-based analyses into the routine clinical management of sarcoma patients. The studies in the review, depicted in table 10, have shown mixed results regarding the usage of TMB as a sole biomarker for immune checkpoint inhibitor response in sarcoma (122). For example, the SARC028 trial, a phase II study of 80 advanced STS and bone sarcoma patients (140), aimed to evaluate the treatment with the immune checkpoint inhibitor Pembrolizumab, showing 18% of the patients (7 out of 40) to have an objective response (122), notably with the highest incidence in undifferentiated pleomorphic sarcoma subgroup (4 of 10), significantly lower in dedifferentiated liposarcoma (2 out of 10) and synovial sarcoma (1 out of 10) (122) and no detected responses in the leiomyosarcoma study subgroup (122). Another phase II trial aiming to assess the effects of monotherapy with Nivolumab, a PD1 blocking antibody, in uterine leiomyosarcoma patients similarly demonstrated no clinical benefit in 12 patients (122) (141). The limited cohort sizes in these trials do not allow for drawing definitive conclusions about subtype-specific benefits, however, the results provide insight in the complexity of prediction biomarkers (122) (139). Also, in sarcomas with defined genetic drivers, the use of TMB as a biomarker for immunotherapy may be limited (122) (142). An analysis of The Cancer Genome Atlas Research (TCGA) database found a low overall TMB (average 1.06 mutations/ Mb) across 206 soft tissue sarcoma cases, which further suggests that using TMB as a sole biomarker may be insufficient in sarcoma patients (122) (143) and that further markers may be needed (122). A recent study examined gene expression data and tumor microenvironmental traits in 608 soft tissue sarcoma (122) (144), describing an immune-high “class E” subtype, which is characterized by the presence of B-cell lineage genes and associated with tertiary lymphoid structures. As a next step these molecularly defined subgroups were applied to the SARC028 trial database, showing a significantly higher objective response rate to Pembrolizumab in high-class E subtype patients compared to

any other subgroup (122). The aforementioned findings would indicate that including TLS (Tertiary lymphoid structures) and gene expression signatures may help identify sarcoma patients that are more likely to benefit from treatment with immune checkpoint inhibitors and thus imply that such an intervention could improve the predictive power and robustness of immune checkpoint inhibitor response biomarkers (122). Another marker that could potentially play a role in predicting an immune checkpoint inhibitor response in sarcoma is mismatch repair defects (145) (122). Doyle et al. explored 447 genes for the frequency of mismatch repair defects in 304 sarcoma cases across multiple subtypes and found this feature to be associated with response to immune checkpoint inhibitor therapy (122) (145). In total, a rather low incidence (2.3%) of sarcomas were described to be mismatch repair-deficient (MMR-D). Compared to mismatch repair-proficient sarcomas (4.6 mutations/Mb), MMR-D sarcomas had a significantly higher median TMB (16 mutations/Mb). However, TMB in MMR-D sarcomas was still generally lower in comparison to carcinomas with MMR-D (28 mutations/Mb) (122). Therefore, further studies with larger cohort sizes are needed to further illuminate whether TMB can be used as a sole prediction marker for response to Pembrolizumab in MMR-D sarcoma patients (122).

Historically, the evidence for the presence of MSI-high signatures in sarcomas has been rather contradictory, with early IHC-based studies reporting a range of 0.9–25% MSI-positive cases in soft tissue sarcoma cohorts (139) (122). A recent PCR-based study of 71 STS patients including multiple subtypes detected 5 MSI markers in combination with IHC protein expression analysis of the MMR proteins and identified all 71 cases as MSS, suggesting a rather limited utility of MSI in unselected sarcoma study cohorts (146) (122). In conclusion, a focused MSI screening in specific sarcoma subtypes in which MMR-D is expected to be more prevalent was postulated to be a more effective approach (145) (122). However, this hypothesis remains to be evaluated in more studies (122).

Further NGS based explorations of the genomic landscape of sarcomas has revealed that certain sarcoma subtypes tend to exhibit hallmarks similar to those observed in cancers with a deficiency in BRCA1/2 genes (147) (148) (122), foremostly breast and ovarian cancer. Tumors that exhibit these features of

'BRCAness' have hallmarks that include defects in HRD genes, structural rearrangements and specific mutational signatures associated with errors in double-strand break repair (149) (122). Such hallmarks are clinically used as a marker of a sensitivity to PARP inhibitors, offering a possible new targeted therapy option for those patients. Multiple ongoing clinical trials aim to evaluate the use of PARP inhibitors in sarcoma patients (150) (151) as well as translational studies striving to determine the association of enriched for 'BRCAness' hallmarks and a beneficial therapeutic response (122). Depending on the results of these studies, HRD may show potential as a prospective biomarker in sarcoma, unleashing further NGS-based prospective clinical trials of PARP inhibitors in sarcomas (122).

Key study(s)	Cancer subtypes, *n* pts	Biomarker analysis	Key results
He *et al.*	SS ($n=21$)	TMB using WES data	1/21 pts with high TMB (212 muts/Mb)
Abeshouse *et al.*	Multiple sarcomas ($n=206$)	TMB using WES/WGS data	Low overall Median TMB (1.06 muts/ Mb)
SARC028 Petitprez *et al.*	40 STS LMS ($n=10$) UPS ($n=10$) Liposarcoma ($n=10$) SS ($n=10$)	Transcriptomic gene expression analysis	ORR to pembrolizumab: 50% in 'Class E' immune-high group
Doyle *et al.*	Multiple Sarcomas ($n=304$)	TMB using WES data Sequencing of MMR genes	2.3% sarcomas MMR-D MMR-D sarcomas had higher TMB (16 muts/ Mb) 1/3 pts MMR-D pts treated with pembrolizumab had SD
Florou *et al.*	AS ($n=7$)	TMB using WES/Foundation CDx assay	1 CR exceptional responder to anti-CTLA-4, low TMB (0.09 muts/Mb)
Painter *et al.*	AS ($n=47$)	TMB using WES data	Low overall median TMB (3.3 muts/Mb) 9 HNFS pts with high median TMB (20.7 muts/Mb) 2/3 HNFS pts treated with anti-PD1 had exceptional response
Campanella *et al.*	Multiple STS subtypes ($n=71$)	MSI using PCR and IHC	All 71 cases were MSS
Kovac *et al.*	OS ($n=31$)	BRCAness hallmarks (single base substitutions, LOH, genomic instability) using WES data	>80% of OS had BRCAness hallmarks
Chudasama *et al.*	LMS ($n=49$)	BRCAness hallmarks (HRR gene deletions, structural rearrangements) using WES data	>90% of LMS had BRCAness hallmarks
International Sarcoma Kindred Study	Multiple sarcomas ($n=1162$)	Germline DNA sequencing	1 in 6 patients' families matched hereditary cancer criteria 1 in 15 patients had actionable germline variants

Table 10: Overview of prominent sarcoma studies, cited from (139)

Demonstrating vast potential for diagnostics and precision medicine in sarcoma, further evaluation of the utility of aforementioned genomic-wide features such as TMB or MSI as predictive biomarkers for patient stratification is required (122). Even though becoming significantly more affordable in the last couple of decades, routine and wide-spread use of NGS-based diagnostic remains a neither viable nor cost-effective option for many parts of the world and is mostly considered for sarcoma patients with limited therapy options and usually poor outcomes, such as patients with chemotherapy-refractory, non-resectable or metastatic disease (122). NGS based diagnostic may offer a significantly improved patient stratification, foremostly in sarcoma subtypes exhibiting the greatest genomic complexity and heterogeneity. However, our understanding of predictive biomarkers in sarcoma finds itself still in its infancy, as observed phenomena, such as absence of measurable clinical response in patients with positive predictive biomarkers (142) or a relevant response in those exhibiting no predictive markers (153), remain to be explained (122). As previously postulated, combining current available predictive biomarkers with emerging and novel ones (such as sarcomas TLS scores (144) for example) may be necessary in order to ensure more accurate and robust predictive power in sarcoma patients (122).

2. Objectives

The goal of this study was to retrospectively examine how different molecular-genetic characteristics of sarcomas could have an impact on the clinical course and outcome of sarcoma patients in a real-life cohort through identifying prognostic risk groups and evaluating applied targeted therapies as part of precision medicine based on examined molecular-genetic characteristics.

As previously already well established, sarcomas are exceedingly rare and, on several levels, heterogeneous malignancies with limited therapy options and an unfavorable prognosis in the advanced stage. Studying sarcomas is further made more difficult, challenging and frequently only practically possible in large centers, where a relevant study cohort could be generated.

Since University Hospital Tübingen constitutes such a certified center being specialized on sarcomas, we conducted a retrospective study of patients from our site diagnosed with multiple different sarcoma entities who received a sequencing of the respective tumor genomes.

In this study we aimed at examining data generated through this molecular-genetic analysis: (i) the frequency and distribution of potentially predictive genetic markers, such as tumor mutational burden (TMB), microsatellite instability (MSI) and homologous recombination deficiency (HRD); (ii) the frequency and distribution of fusion genes, (iii) the ratio of clinically relevant fusion genes to fusion genes without known clinical relevance, (iv) the distribution of clinically relevant fusion genes; (v) the frequency and distribution of copy number alterations (CNAs) such as duplications, amplification, homozygous and heterozygous deletions and loss of heterozygosity as well as (vi) the frequency of genomic instability and distribution of CNAs in individual genes; (vii) frequency and distribution of germline mutations in individual genes; (viii) frequency and distribution of somatic mutations, (ix) frequency of clinically relevant somatic mutations with distribution in individual genes.

Through analyzing this data our goal was to examine the impact of molecular-genetic diagnostics on the expansion of limited therapeutic options in sarcoma treatment through the application of novel therapies based on “next-generation” sequencing (NGS) data as well as to examine the impact of these therapies on clinical outcomes in patients and therefore, reexamining the significance and impact of molecular-genetic diagnostics in a real-world setting in sarcoma patients.

3. Materials and methods

3.1. Background and ethics

In order to examine how different molecular-genetic characteristics of sarcoma influence the prognosis and clinical course of the disease, we included a total of 58 patients of our sarcoma center (Comprehensive Cancer Center Tubingen-Stuttgart) in this retrospective study. The work presented here was prepared based on data provided by the Department of Internal Medicine VIII - Medical Oncology and Pneumology of the University Hospital Tübingen. The ethical permission for this study was granted by the University Tübingen research ethics committee (vote number 886/2020BO2).

3.2. Data protection

For the retrospective data collection, diagnosis, disease and treatment histories and disease-specific examination, findings are recorded in pseudonymized form. The doctors involved in the study are responsible for the pseudonymization of the data. No other person except the participating doctors and scientific staff has access to the data necessary to identify the patients. In order to be able to follow the patients' progress, patient names and number codes are stored by the doctors responsible for data protection. In the event of publication, no patient-related data is published. It would not be possible to trace the data back from the publication. Personal data will be stored at the Department of Internal Medicine VIII - Medical Oncology and Pneumology of the University Hospital Tübingen until the end of examinations and a potential publication. All electronically stored personal data will be deleted after 10 years. Paper printouts that carry patient data are professionally destroyed after the completion of examinations. Only the attending physicians and scientific staff involved have access to the patient data and the re-identification list. After the publication of the results, the data is stored for the prescribed period of 10 years, after which all patient data is deleted. Paper printouts are professionally destroyed and disposed of. Patient-related data is

only stored in pseudonymized, i.e., coded form. Outsiders cannot draw any conclusions about the identity of the patients. Patient-specific data (names and dates of birth) are only processed at University Hospital Tübingen. All employees who have access to this data are subject to medical confidentiality.

3.3. Composition of the study

3.3.1. Study cohort size, inclusion and exclusion criteria

Our research project is a retrospective analysis of 58 sarcoma patients, diagnosed in the time period between 01.01.2018 and 31.12.2021.

Inclusion criteria are (all of the criteria are mandatory):

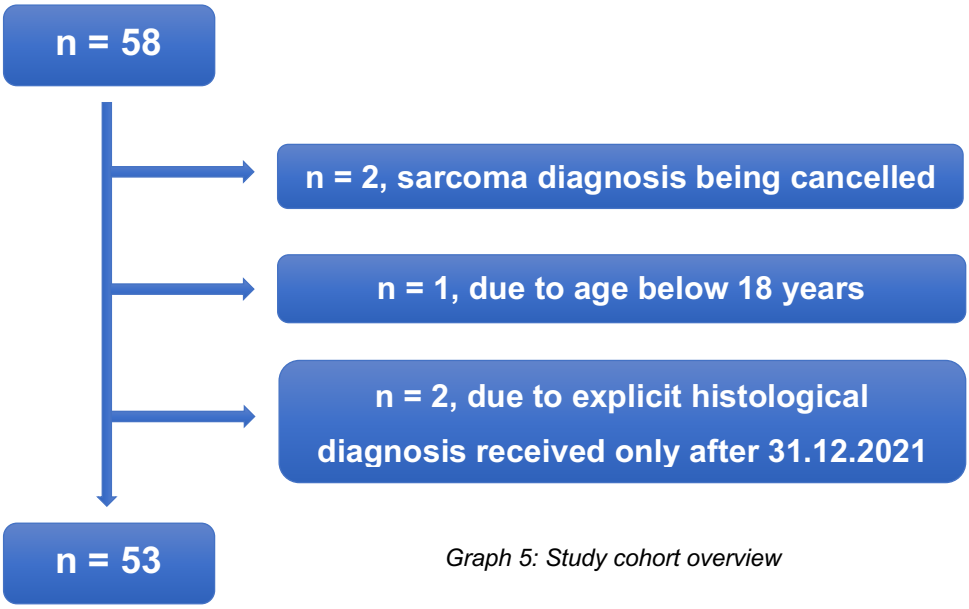
- age above 18 years,
- confirmed sarcoma diagnosis in the defined time span and
- molecular-genetic tumor sample diagnostics.

Exclusion criteria are (one criterion is sufficient to exclude):

- age below 18 years,
- different tumor entity histologically other than sarcoma, or
- the absence of molecular-genetic tumor sample diagnostics.

Out of the initial 58 patients, a total of 5 patients had to be excluded from the study due to the following reasons: 1 patient was younger than 18 years of age, 2 patients received the diagnosis only after 31.12.2021, and 2 patients had no more sarcoma diagnosis after the reevaluation of the histopathology by a reference pathologist.

Figure 5 provides an overview of the recruitment of the study cohort.



Graph 5: Study cohort overview

3.3.2. Patient data

In our study cohort, we analyzed demographic data of included patients, such as age and gender using the SAP program to gather data from the patient charts. Further, we examined relevant clinical data such as clinical outcomes, overall survival, primary tumor site, tumor classification using TNM and UICC classification systems, comorbidities, BMI, radiologic imaging exams, endoscopic and sonography exams, tumor board interdisciplinary statements as well as operative treatment, radiotherapy and chemotherapy using a myriad of clinical programs such as SAP, Meona, Chemocompile, PACS, Viewpoint, Ultima as well as central archives of our University Hospital. For every patient, we analyzed laboratory markers including neutrophil count, lymphocyte count and LDH using the Lauris program. The normal neutrophil count range for men above the age of 18 years is defined in our laboratory as 1800-7000 /µl (per microliter) and 2100-7700 /µl for women above 18 years of age. The normal lymphocyte count range for men above the age of 18 years is defined in our laboratory as 1100-3200 /µl (per microliter) and 1200-3500 /µl for women above 18 years of age. According to our software, LDH (lactate dehydrogenase) enzyme is present in the cells of most tissues. Elevated plasma LDH levels are a non-specific indicator of cell death and occur in myocardial infarction, pulmonary embolism, liver disease, muscle activity, skeletal muscle disease, hemolysis, kidney disease and tumors (154). The upper limit of the normal range for adults is 250 U/l (units per liter). The data

regarding tumor histology, histological grading and immunohistochemistry was also obtained from the SAP program. Molecular-genetic diagnostics of every patient in our study were performed using HiSeq/NovaSeq tumor genome sequencing method by CeGaT GmbH, Center for Genomics and Transcriptomics and Praxis Für Humangenetik Tübingen.

Laboratory values, normal range for adults	
Neutrophil count	
Male	1800-7000 /µl
Female	2100-7700 /µl
Lymphocyte count	
Male	1100-3200 /µl
Female	1200-3500 /µl
LDH	
Male and female, all gender	< 250 U/l

Table 11: Laboratory values, normal ranges, cited from Lauris software and (155)

3.3.3. Used programs and statistical methods

For the analysis of our data and for the creation of this manuscript we used the following software: MS Word, MS Excel, Adobe acrobat reader, GraphPad Prism Version 9.5.0 (for graphs), SPSS statistics (IBM) Version 29.

3.3.4. Literature search

For the introductory part regarding sarcoma, including definition, history, etiology, epidemiology, diagnostics and therapy we performed a literature search using primarily PubMed, but due to the sparsity of the literature relying on several other government-based and well-established internet sites as well (such as National

Institute for Cancer Research, Cancer.gov, etc.). Search terms included keywords and phrases such as common names of soft tissue and bone tumors, the names of sarcoma subtypes as well as common phrases in medicine, such as “etiology”, “history”, “epidemiology”, “diagnostics” and “therapy”. For the introductory part regarding DNA sequencing, a literature search was performed using primarily PubMed. Search terms included keywords and phrases such as “NGS”, “genetic profile” and “target therapies”. The majority of cited papers are limited to a rather small patient study cohort sizes due to the sheer rarity of the sarcoma. A relevant number of cited papers are limited to a certain sarcoma (sub)type. In the discussion part, an overview of the relevant current literature was presented using primarily PubMed as a search engine using the aforementioned keywords and common phrases in medicine.

4. Results

4.1. Description of the study cohort

4.1.1. Patient characteristics: age, gender, OS and BMI

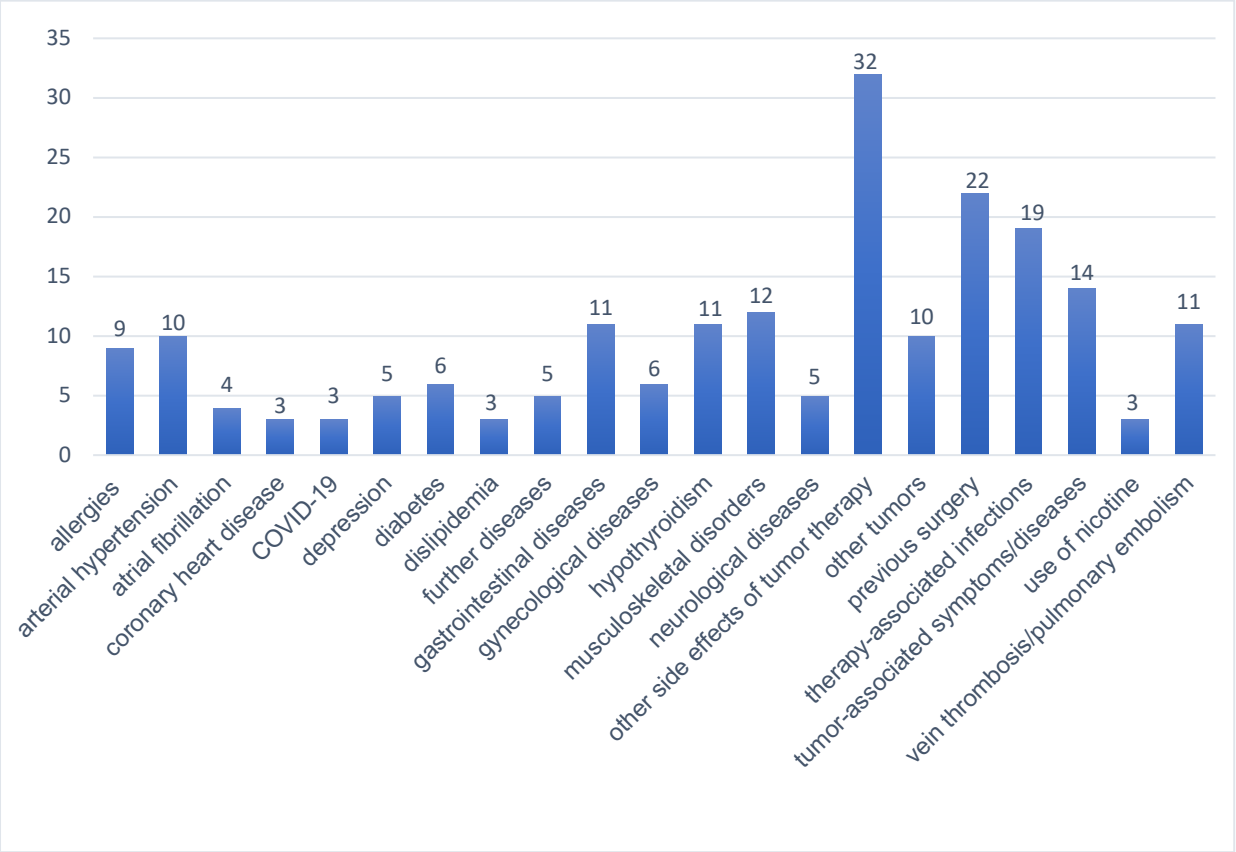
Our study cohort initially included 58 patients, of which 53 matched the enrolment criteria, i.e., every enrolled patient had to be older than 18 years of age, had to have a histopathologically confirmed sarcoma diagnosis and every tumor sample had to have received an NGS DNA sequencing. Out of the enrolled 53 patients, 35 were female (66.04%) and 18 were male (33.96%). The mean age at the time of diagnosis is 44.4 years (STDEV.s 14.46) and 49 (STDEV.s 14.63) at the time of our analysis. The median age was 43 years at the time of diagnosis and 51 years at the time of our analysis. In our patient study cohort, 24 patients (45.28%) were still living at the time of the analysis and 29 (54.72%) are deceased. Mean overall survival (OS) was 55.89 months (STDEV.s 54.33) and the median OS was 39.5 months. The mean body mass index (BMI) of our study cohort was 23.84 kg/m^2 (STDEV.s 4.86) and the median BMI was 22.8 kg/m^2. Data regarding BMI is only available in 51 patients.

Patient characteristics	
Gender	
Male	**18 (34%)**
Female	**35 (66%)**
Mean age (years)	
At first diagnosis	**44.4**
At the time of analysis	**49**
Outcome	
Alive	**24 (45%)**
Dead	**29 (55%)**
Mean overall survival	**56 months**
Mean BMI	**23.8 kg/m^2**

Table 12: Patient characteristics

4.1.2. Comorbidities

In our study cohort, we analyzed the comorbidities. Ranked by the frequency of occurrence the secondary diagnoses are musculoskeletal disorders (including femoral fracture, fracture of the lower leg, benign tumor of femur, fracture of the upper arm, gonarthrosis, osteoporosis, scoliosis, rupture of rotator cuff, hip surgery, hip dysplasia), gastrointestinal disorders (including hemorrhoids, dysphagia, primary sclerosing cholangitis, ulcerative colitis, reflux esophagitis, colon polyp, type A gastritis, Budd-Chiari-syndrome), deep vein thrombosis / pulmonary embolism and hypothyroidism in 11 patients each (20.75%), arterial hypertension and other tumors (including NSCLC, breast cancer, Hodgkin lymphoma, therapy-induced AML (Acute Myeloid Leukemia), follicular lymphoma, melanoma, MGUS (Monoclonal gammopathy of undetermined significance) and testicular teratoma) in 10 patients each (18.97%), allergies (including allergic rhinitis, Metamizole, bronchial asthma, contrast agent, Codeine, Aspirin, Ibuprofen, Penicillin, Ciprofloxacin, preservatives, Nickel, atopic dermatitis, Vibramycin, Streptomycin, Bacitracin, Ampicillin) in 9 patients (16.98%), diabetes mellitus (including type 2, type 3/ gestational diabetes) and gynecological diseases (including uterine myoma, extrauterine gravidity, tubo-ovarian abscess, endometriosis) in 6 patients (11.32%) each, depression and neurological diseases (including cognitive developmental delay, chronic pain syndrome, myoclonus, epilepsy, migraine) in 5 patients (9.54%) each, atrial fibrillation in 4 patients (7.55%), dyslipidemia, nicotine abuse, COVID-19-infection and coronary heart disease in 3 patients (5.66%) each. Further, secondary diagnoses including rheumatoid arthritis, traumatic vertebral artery dissection with brainstem ischemia, clipping of a right internal carotid artery aneurysm, insult of the middle cerebral artery due to persistent foramen ovale, lysis, mechanical recanalization and PFO closure, splenic infarction is observed in 5 patients (9.43%).



Graph 6: Patients comorbidities distribution

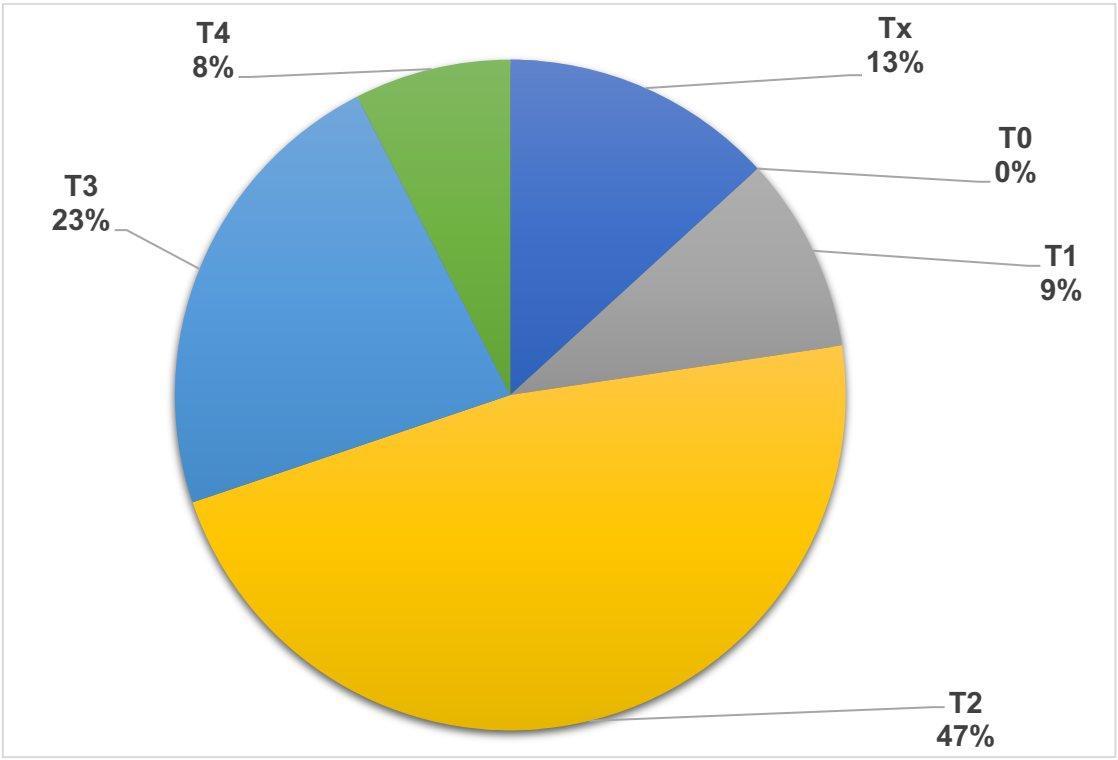
In 12 patients, a previous surgical therapy is observed in the patient history (including hysterectomy, adnexectomy, appendectomy, tonsillectomy, cholecystectomy, surgery for inguinal hernia, surgery for phimosis, surgery for varicocele, leg vein surgery, cesarean section, surgery for a rupture of the tympanic membrane). Regarding tumor- and therapy-associated secondary diagnoses, side effects of tumor therapy (including pulmonary hypertension, cachexia, cardiomyopathy, anemia, thrombopenia, tricytopenia, chronic renal failure, hearing impairment, polyneuropathy, hand-foot-syndrome, urogenital fistula, pneumonitis, Ifosfamide-induced encephalitis, cerebral radionecrosis, pneumothorax, secondary sclerosing cholangitis, postoperative phrenic nerve paralysis, postoperative long-term ventilation, dysphagia) are documented in 32 patients (60.38%), therapy-associated infections (including pneumonia, peritonsillar abscess, recurrent cholangitis, pyelonephritis, pseudomembranous colitis, port infection, VRE colonization, Herpes simplex reactivation, thrush, neutropenic sepsis, osteomyelitis, epididymitis, panaritium digitum I right side) in 19 patients (35.38%) and tumor-associated

symptoms or diseases (including malignant pleural effusion, bile duct stenosis, duodenal stenosis, portal hypertension, 2x mechanical ileus, adrenocortical insufficiency, SIADH, epilepsy, mitral stenosis, intracerebral hemorrhage, sacral wound with multiple life-threatening bleeding events) in 14 patients (26.42%).

4.2. Description of the tumor characteristics

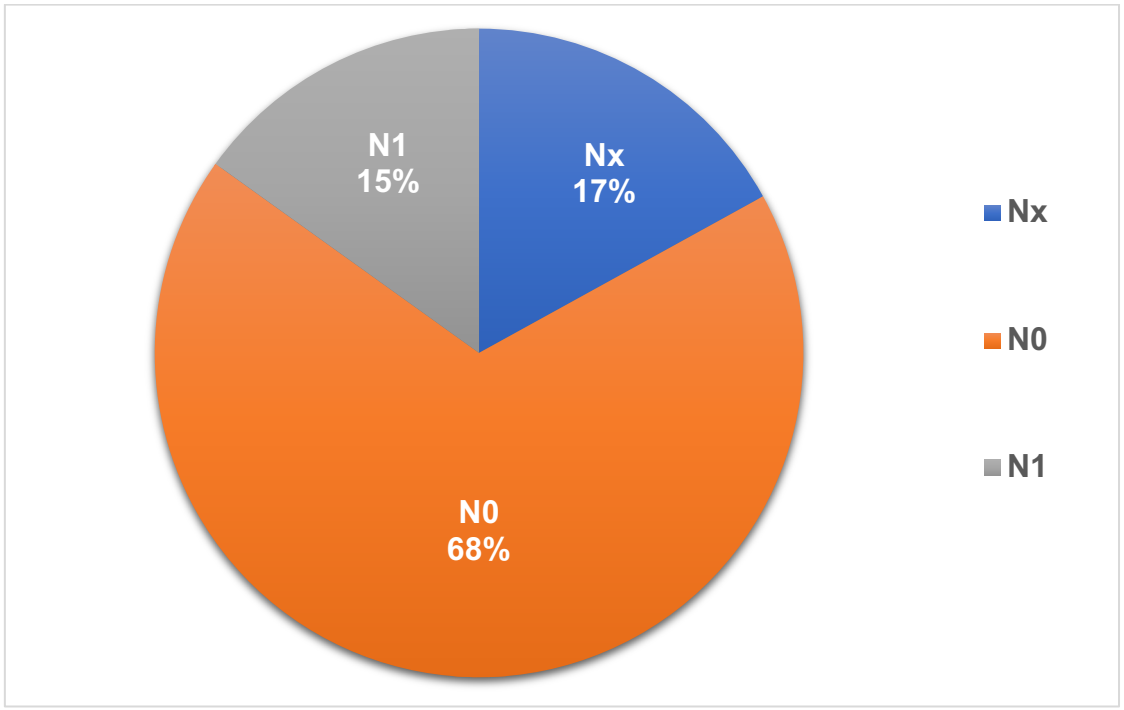
4.2.1. Staging and grading

Every patient received staging and grading at the time of diagnosis. According to TMN classification, our patient structure is as follows: Tx stage in 7 patients (13.21%), stage T0 was not recorded in any patient, T1 stage was observed in 5 patients (9.43%), T2 stage in 25 patients (47.17%), T3 in 12 patients (22.64%) and T4 in 4 patients (7.55%).



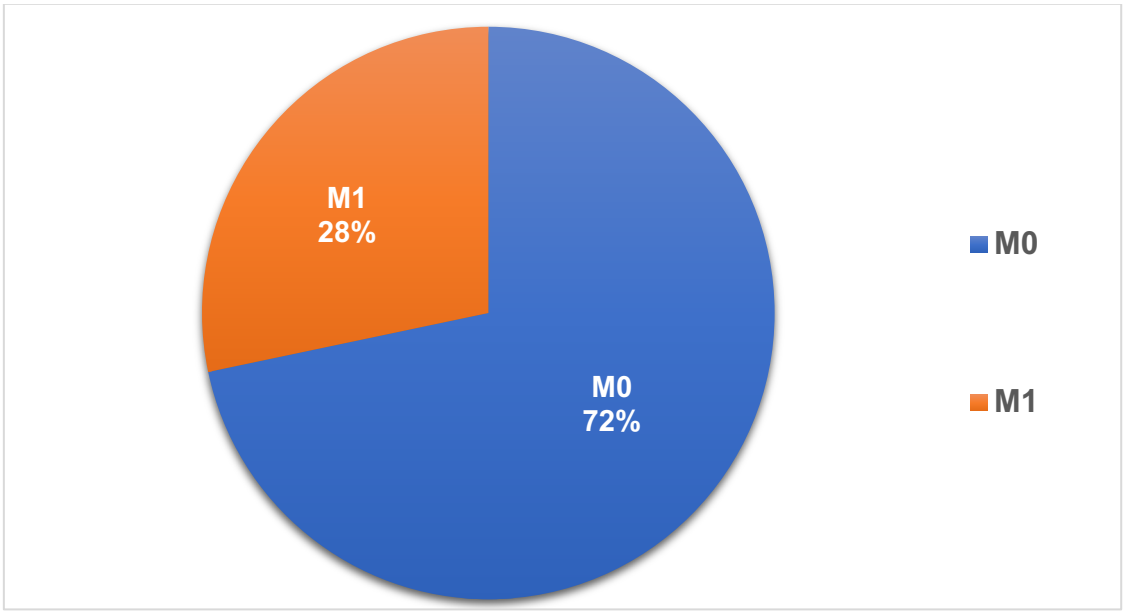
Graph 7: Tumor staging, TMN classification T stadium distribution

Nodal status is unknown (Nx) in 9 patients (16.98%), status N0 is observed in 36 patients (67.92%) and N1 in 8 patients (15.09%).



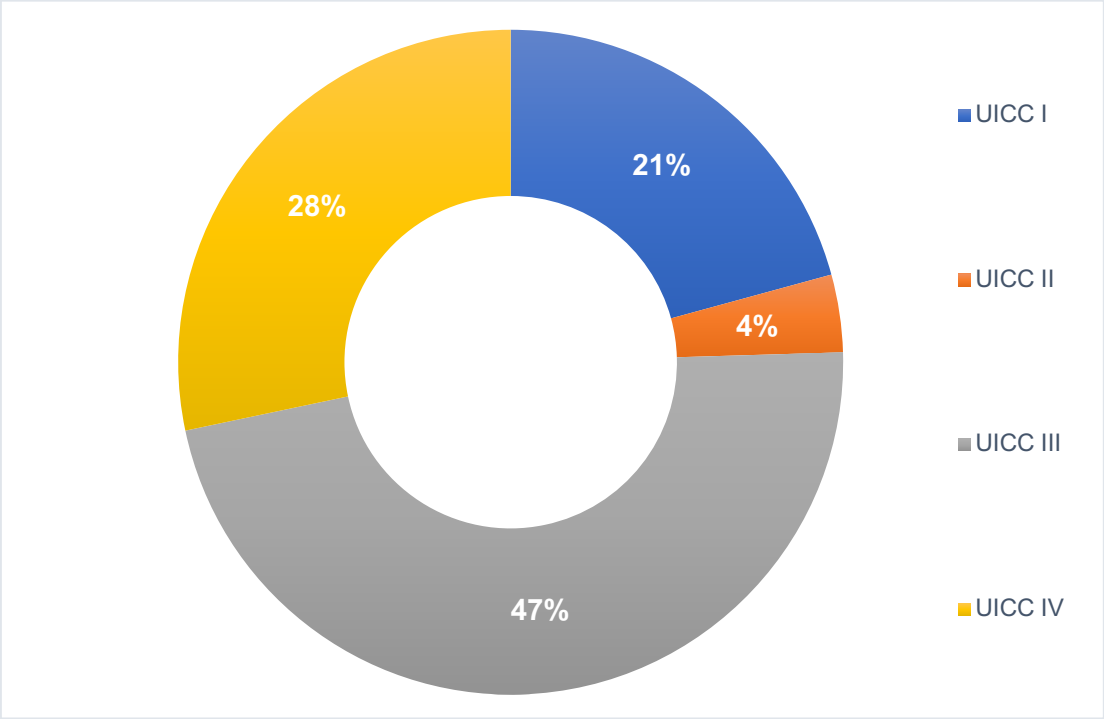
Graph 8: Tumor staging, TMN classification N stadium distribution

At the time of diagnosis, no metastases (M0) are found in 38 patients (71.7%) and stadium M1 is observed in 15 patients (28.3%).



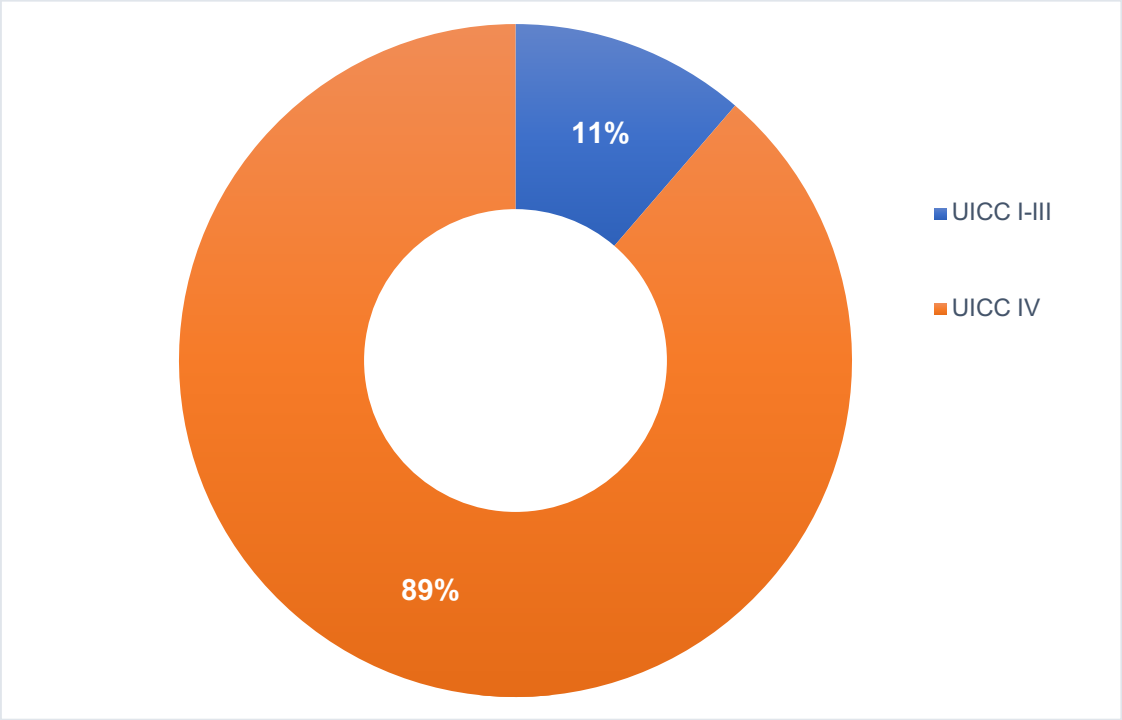
Graph 9: Tumor staging, TMN classification M stadium distribution

According to the UICC classification, stadium I is observed in 11 patients (20.75%) at the time of the diagnosis, stadium II in 2 patients (3.77%), stadium III in 25 patients (47.17%) and stadium IV in 15 patients (28.2%).



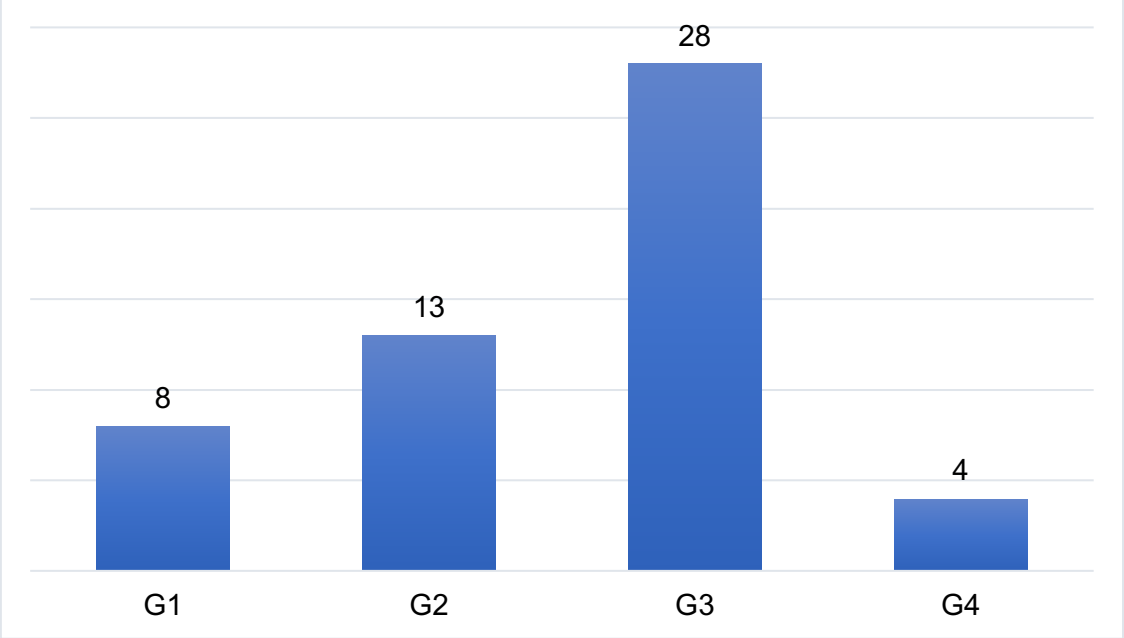
Graph 10: Tumor staging, UICC classification at diagnosis

In contrast, at the time of DNA sequencing M0 stadium is found in 6 patients (11.32%) and metastatic disease (M1) in 47 patients (88.68%), therefore, UICC IV stadium.



Graph 11: Tumor staging, UICC classification at sequencing

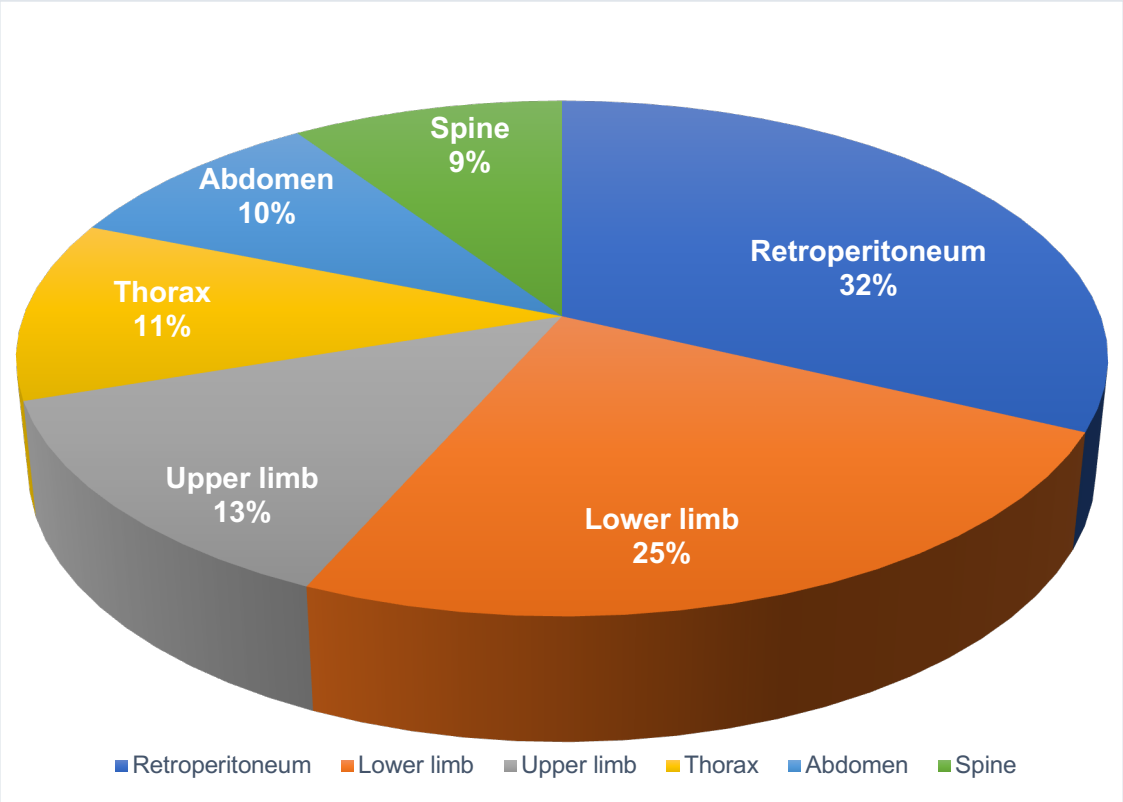
Histological grading showed the following distribution: Grade G1 in 8 patients (15.09%), G2 in 13 patients (24.53%), G3 in 28 patients (52.83%) and G4 in 4 patients (7.55%).



Graph 12: Tumor grading distribution

4.2.2. Primary tumor sites

In our study cohort, we examined tumor location distribution. Primary tumor sites, ranked by frequency, are retroperitoneum (incl. small pelvis, 17 patients, 32.08%), lower limb (13 patients, 24.53%), upper limb (7 patients, 13.21%), thorax (6 patients, 11.32%), abdomen (5 patients, 9.43%) and spine (5 patients, 9.43%).

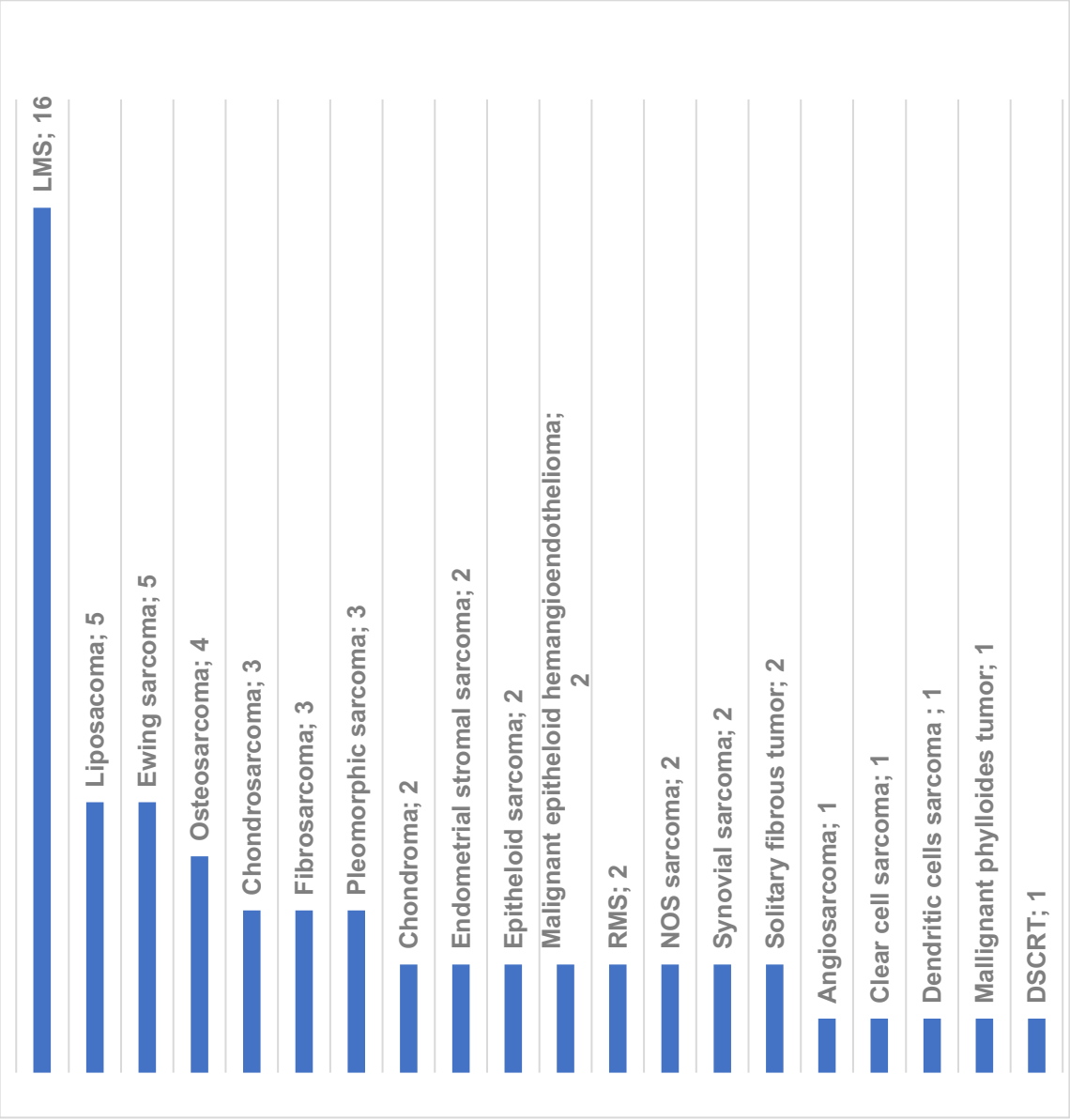


Graph 13: Primary tumor sites distribution

4.2.3. Histology

Every patient in our study cohort received a histopathological examination of the tumor sample, which was an inclusion criterion. The histological entities showed a wide margin of diversity and are ranked by frequency of occurrence: leiomyosarcoma (LMS) in 16 patients (16.98%), liposarcoma and Ewing/Ewing-like sarcoma in 5 patients each (9.43%), osteosarcoma in 4 patients (7.55%), chondrosarcoma, fibrosarcoma and pleomorphic sarcoma in 3 patients each (5.66%),

chondroma, endometrial stromal sarcoma, epithelioid sarcoma, malignant epithelioid hemangioendothelioma, rhabdomyosarcoma (RMS), not otherwise specified (NOS) sarcoma, synovial sarcoma and solitary fibrous tumor in 2 patients each (3.77%), angiosarcoma, clear cell sarcoma, dendritic cells sarcoma, malignant phyllodes tumor and desmoplastic small round cell tumor (DSRCT) in 1 patient each (1.89%).



Graph 14: Histopathology distribution

4.3. Diagnostics

4.3.1. Laboratory values

At the time of DNA sequencing mean neutrophil count was 4155.7/µl (normal: 2100-7700/µl, STDEV.s 2097.05) and the median neutrophil count was 3910/µl. The mean lymphocyte count was 950.7/µl (normal: 1200-3500/µl, STDEV.s 557.43) and the median lymphocyte count was 875/µl. LDH mean value was 307.72 U/l (normal up to 250 U/l, STDEV.s 378.8) and the median LDH value was 196.5 U/l. The laboratory data were available in 50 patients.

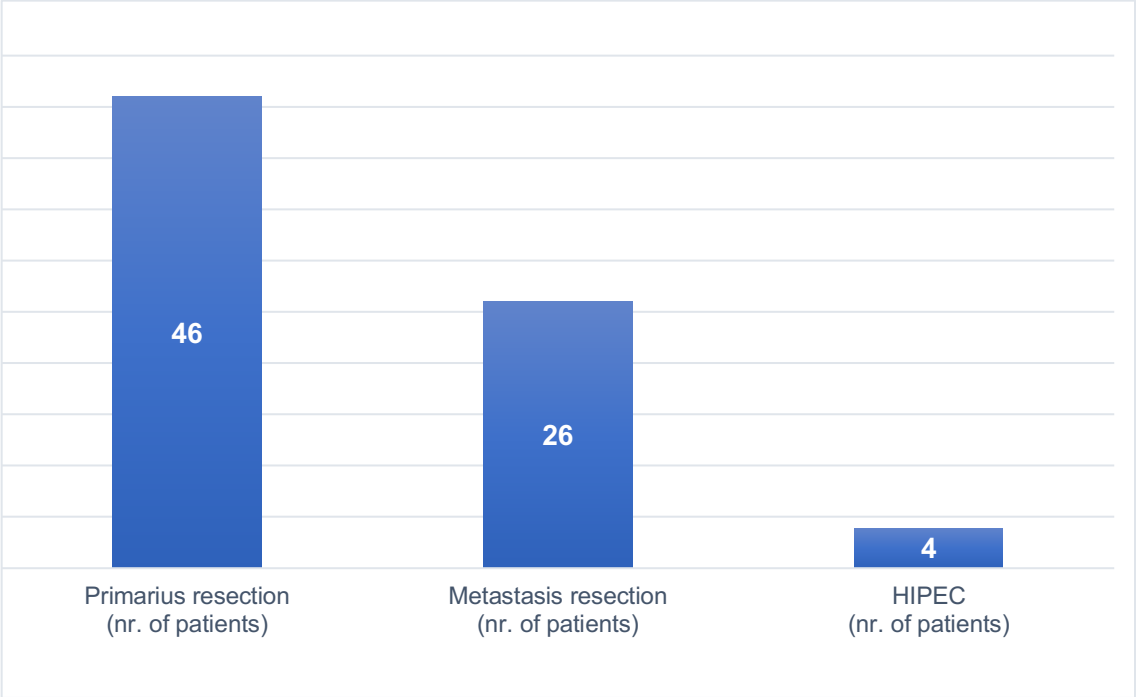
*Mean laboratory values (*at the time of analysis*)*	
Neutrophil count	**4,156/µl**
Lymphocyte count	**951/µl**
LDH	**197 U/l**

Table 13: Patients, mean laboratory values

4.4. Therapy

4.4.1. Surgery

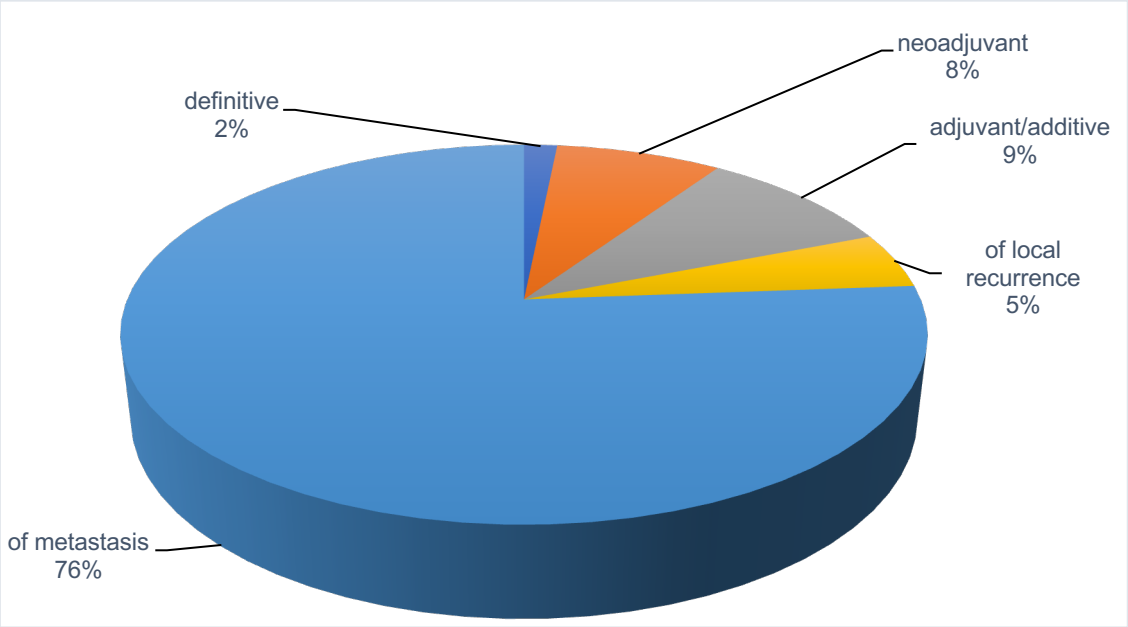
Regarding therapeutic interventions, a total of 47 patients (88.67%) received surgical therapy (a total of 124 operations) in general with a mean value of 2.34 operations per patient (STDEV.s 2.05) and a median value of 2 operations per patient. 46 patients received an operation on the primary tumor (86.79%), and 26 patients received an operation on the secondary metastatic deposits (26%). Further, 4 patients (11.43%) received hyperthermic intraperitoneal chemotherapy (HIPEC) in addition to the surgical therapy.



Graph 15: Surgical treatments distribution

4.4.2. Radiotherapy

A total of 63 radiotherapies were applied. Definitive radiotherapy was implemented in 1 patient (1.89%, a patient with chordoma). In a neoadjuvant setting, 5 patients received radiotherapy (9.43%). Of those cases, 2 patients additionally received an implementation of Ifosfamide sensitizing and hyperthermic therapy and further 2 patients Ifosfamide sensitizing only. Adjuvant radiotherapy was performed in 6 patients (11.32%). Radiotherapy of a local recurrence was administered in 3 patients (5.66%) for the tumor localized in the retroperitoneum, thorax wall and left thigh. Further 48 radiotherapies (76.19% of all radiotherapies) of the secondary metastatic deposits were performed on metastasis localized in sternum, lungs, liver, mediastinum, thoracic spine vertebra, right upper arm, left flank, cervical spine vertebra, pelvis, cranial bones, intraspinal, cerebrum (WBRT-whole brain radiotherapy and SBRT-stereotactic body radiotherapy), epigastrium, left femur, left femur neck, os ileum, distal humerus right, os sacrum, lumbar spine vertebra, sacral spine vertebra, proximal femur on both sides, skin, pancreas, nuchal, right shoulder, right knee, abdomen, multiple costae, retrosternal, paravertebral, right upper thoracic aperture, left scapula, left humerus.

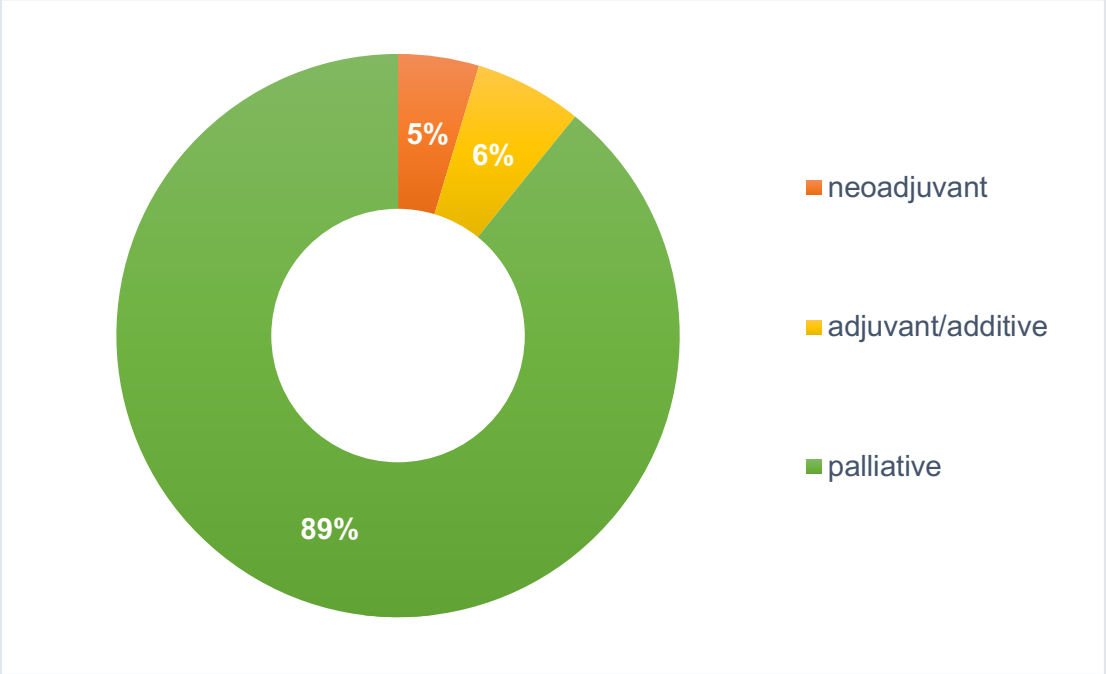


Graph 16: Radiotherapeutic treatments distribution

4.4.3. Chemotherapy

In the course of our study, a total of 194 chemotherapies or chemotherapy protocols were administered. In a neoadjuvant setting, a total of 9 chemotherapies or chemotherapy protocols (4.6% of total chemotherapies or chemotherapy protocols) was documented, including 4 patients receiving Doxorubicin/Ifosfamide (44% of neoadjuvant chemotherapies or chemotherapy protocols), 1 case of Epirubicin/Cyclophosphamide (0.11%), 1 case of VIDE protocol (including Vincristine, Ifosfamide, Doxorubicin and Etoposide, 0.11%), 2 cases of EURO-BOSS protocol (including Cisplatin, Ifosfamide and Doxorubicin, 0.22%) and 1 case of EURAMOS protocol (Methotrexate, Doxorubicin and Cisplatin, 0.11%). In an adjuvant or additive setting a total of 12 chemotherapies or chemotherapy protocols (6.1% of total chemotherapies or chemotherapy protocols) were administered, including 1 case of Doxorubicin (8.3% of adjuvant/additive chemotherapies or chemotherapy protocols), 4 cases of Doxorubicin/Ifosfamide (33.3%), 1 case of Nivolumab (8.3%), 2 cases of EURO-BOSS protocol (including Cisplatin, Doxorubicin and Ifosfamide, 16.6%), 1 case of VIDE protocol (including Vincristine, Ifosfamide, Doxorubicin and Etoposide, 8.3%), 1 case of Letrozole (8.3%), 1 case

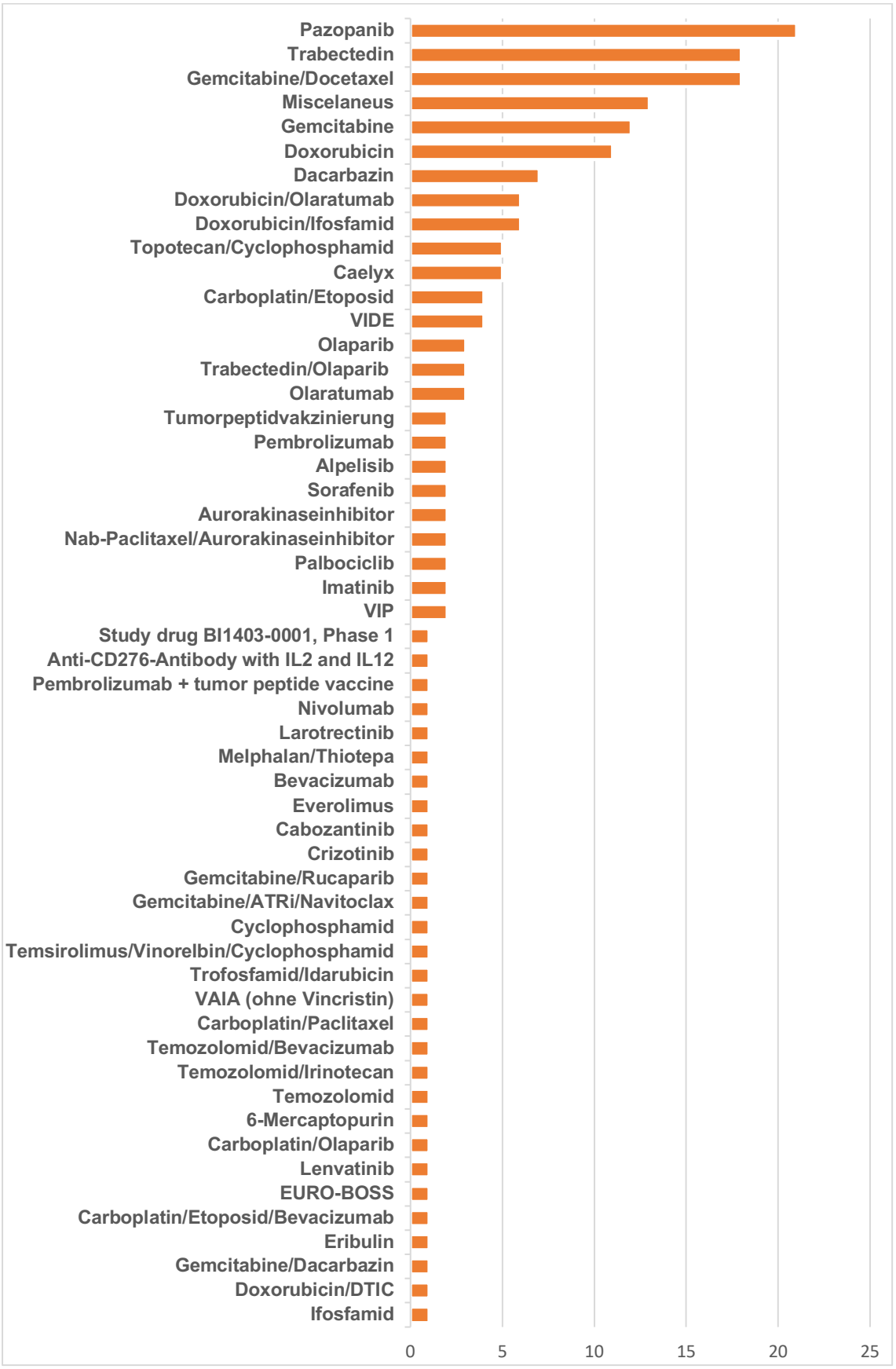
of EURAMOS-1 protocol (including Methotrexate, Doxorubicin, Cisplatin and Mifamurtide, 8.3%) and 1 case of Cisplatin/Paclitaxel (8.3%).



Graph 17: Chemotherapeutic treatment distribution

In palliative setting a total of 173 chemotherapies or chemotherapy protocols (89% of total chemotherapies or chemotherapy protocols) were administered, including following therapy protocols or substances ranked by frequency of occurrence: 21 cases of Pazopanib (12.1% of palliative chemotherapies or chemotherapy protocols), 18 cases (10.4%) each of Trabectedin and Gemcitabine/Docetaxel , 12 cases (6.9%) of Gemcitabine, 11 cases (6.3%) of Doxorubicin, 7 cases (4%) of Dacarbazine , 6 cases (3.4%) each of Doxorubicin/Ifosfamide and Doxorubicin/Olaratumab, 5 cases (2.8%) each of Caelyx (liposomal Doxorubicin) and Topotecan/Cyclophosphamide, 4 cases (2.3%) each of VIDE protocol (including Vincristine, Ifosfamide, Doxorubicin and Etoposide) and Carboplatin/Etoposide, 3 cases (1.7%) each of Olaratumab, Trabectedin/Olaparib (as a part of TOP-ART-study) and Olaparib, 2 cases (1.1%) each of VIP protocol (including Cisplatin, Etoposide and Ifosfamide), Imatinib, Aurora-kinase-inhibitor, Palbociclib, Nab-Paclitaxel/Aurora-kinase-inhibitor, Sorafenib, Alpelisib, Pembrolizumab and tumor-peptide-vaccination as well as 1 case (0.5%) each of Ifosfamide, Doxorubicin/Dacarbazine, Gemcitabine/Dacarbazine, Eribulin, Carboplatin/Etoposide/

Bevacizumab, EURO-BOSS protocol (including Cisplatin, Doxorubicin, Ifosfamide), Lenvatinib, Carboplatin/Olaparib, 6-Mercaptopurin, Temozolomide, Temozolomide/Irinotecan, Temozolomide/Bevacizumab, Carboplatin/Paclitaxel, modified VAIA protocol (including Vincristine, Adriamycin, Ifosfamide, Actinomycin-D – without Vincristine), Trofosfamide/Idarubicin, Temsirolimus/Vinorelbine/Cyclophosphamide, Cyclophosphamide, Gemcitabine/ATR-inhibitor/Navitoclax, Gemcitabine/Rucaparib, Crizotinib, Cabozantinib, Everolimus, Bevacizumab, Melphalan/Thiotepam, Larotrectinib, Nivolumab, Pembrolizumab/tumor-peptide-vaccination, immunotherapy with CD276 antibody with IL2 and IL12 intratumorally and subcutaneously administered and study drug BI1403-0001 (phase I study).



Graph 18: Chemotherapies distribution

A total of 11 further miscellaneous therapeutic interventions were documented. Those encompass, ranked by frequency of occurrence, 4 cases (36.3% of miscellaneous therapies) of complementary medical therapies, 2 cases (18.1%) each of autologous stem cell transplant and radiofrequency ablation and 1 case (9.1%) each of whole body hyperthermia (parallel to Gemcitabine/Docetaxel), CXCR4-targeted radioligand therapy with GBqYg0-Pentixather, high-intensity focused ultrasound (HIFU) therapy of pancreatic metastasis, embolization of pulmonary metastasis and laser evaporation of visceral peritoneum.

4.5. Tumor genome sequencing

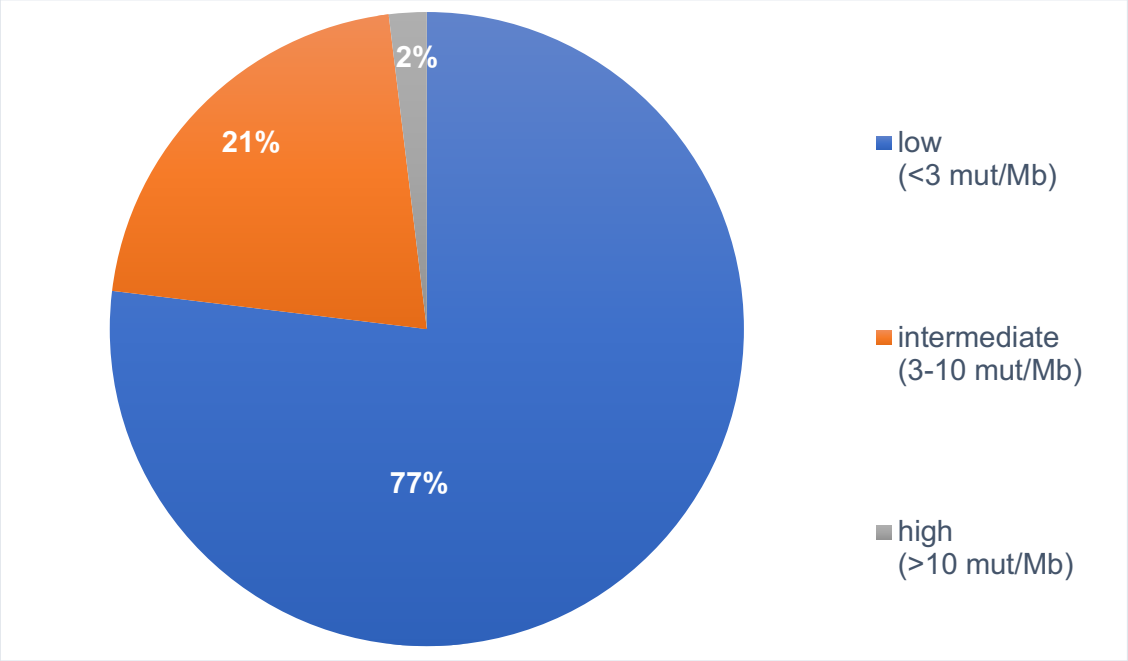
4.5.1. Tumor samples characteristics

Every patient in our study cohort had received a DNA sequencing of tumor tissue. The mean temporal distance from diagnosis to DNA sequencing is 46.75 months (STDEV.s 52.5) with a median value of 28 months. Our samples show a mean tumor content of 72.67% (STDEV.s 21,19) with a median value of 80% tumor content. A total of 17 samples (32.08%) came from the primary tumor and a total of 36 samples (67.92%) were obtained from metastatic secondary lesions. The localization of biopsied secondary lesions, ranked by frequency of occurrence, was the following: soft tissue (10 samples, 27.78%), pleura/lung (9 samples, 25%), bone (5 samples, 13.89%), lymph node (6 samples, 16.67%), liver (3 samples, 8.33%), pancreas, duodenum and peritoneum (1 sample each, 2.78%).

4.5.2. Tumor mutational burden

As a part of the molecular-genetic examination of the tumor samples, an analysis of tumor mutational burden (TMB) was performed. A mean TMB value of 2.3 (STDEV.s 3.48) and a median value of 1.5 was observed. We defined a low TMB as less than 3 mut/Mb, an intermediate as TMB between 3 and 10 mut/Mb and a high TMB as above 10 mut/Mb. In the case of 40 samples (76.92%) a low TMB was observed, 11 samples (21.15%) have shown an intermediate TMB and in the

case of 1 sample (1.92%), a high TMB was noted. The data was available for 52 patients. The patient with the highest TMB had a value of 24,7 mut/Mb.



Graph 19: Tumor mutational burden distribution

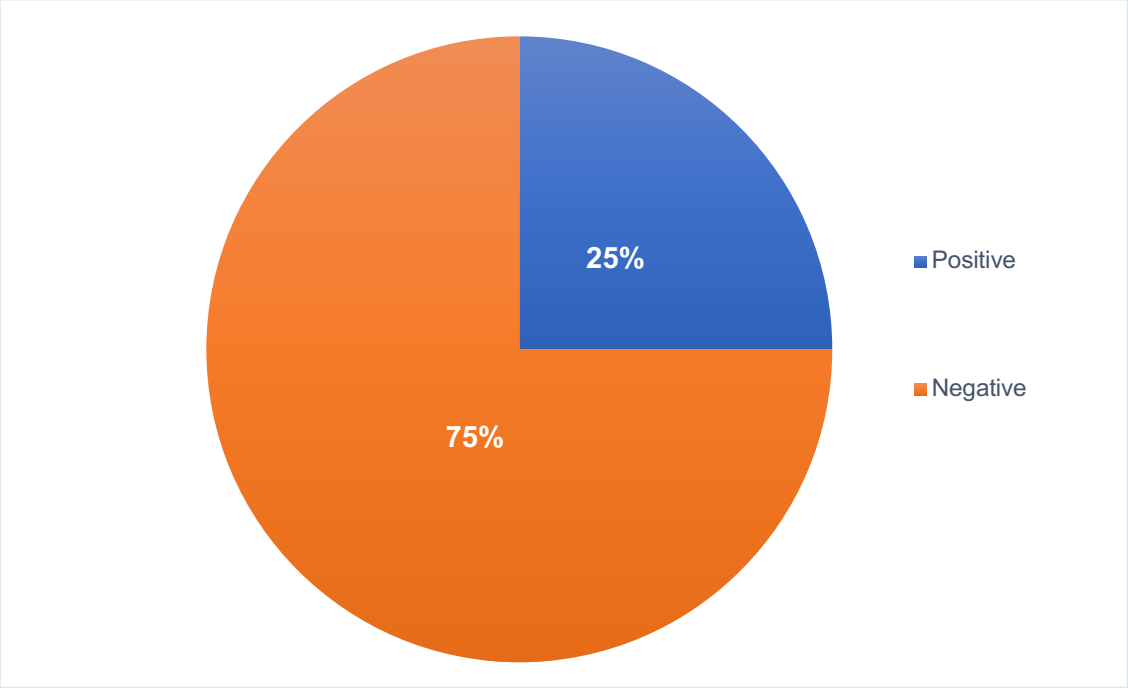
4.5.3. Microsatellite instability

Regarding microsatellite instability (MSI), all 52 examined samples (100%) have shown microsatellite stability. In 1 patient the analysis of microsatellite stability was not performed.

Graph 20: Microsatellite status distribution

4.5.4. Homologous recombination deficiency

Homologous recombination deficiency (HRD) was shown to be positive in 13 samples (25%) with a mean HRD score of 32.28 (STDEV.s 22.44) and a median value of 24.5. The data for HRD analysis was available in 52 out of 53 patients and HRD score was determined in 32 patients.

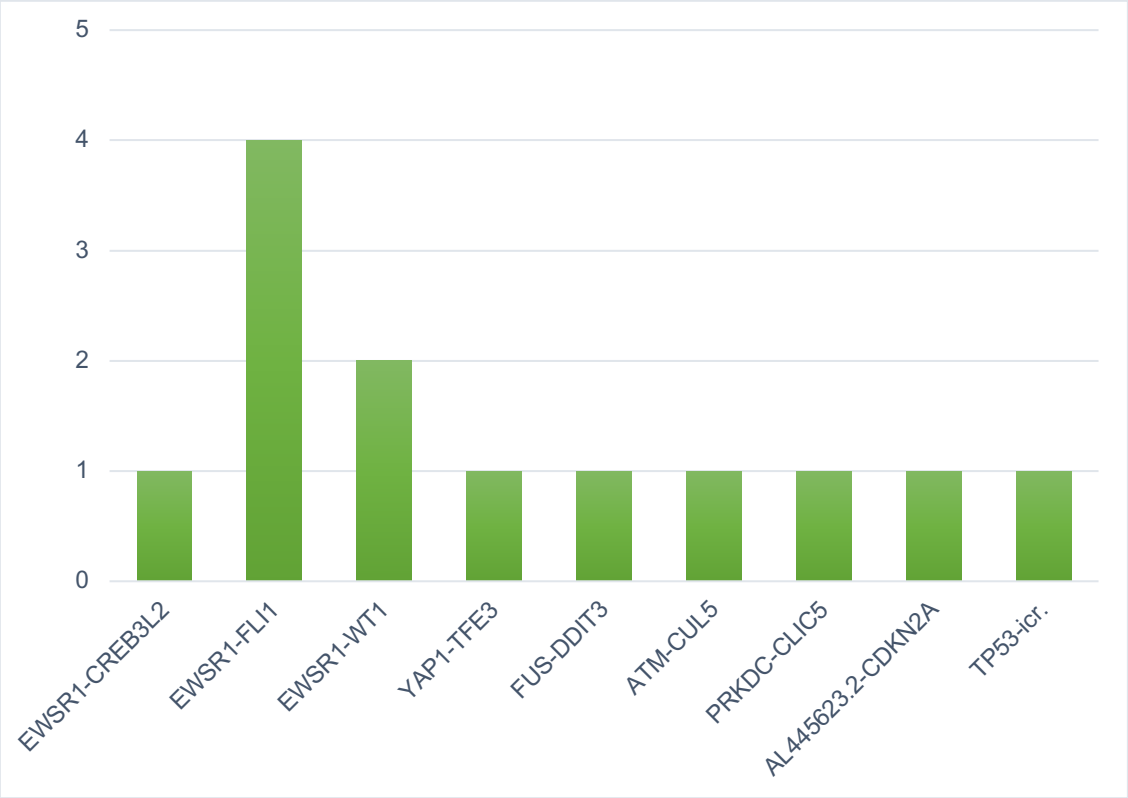


Graph 21: Homologous recombination deficiency status distribution

4.5.5. Fusion genes analysis

Our study cohort received an analysis of fusion genes. Out of 52 patients for whom the data was available, 16 patients (30.77%) have had the presence of fusion genes detected. Of those patients with detected fusion genes, the mean number of fusion genes per patient was shown to be 1.375 (STDEV.s 0.885) and a median number of fusion genes per patient 1, with 1 patient (6.25%) each showing the presence of 4, 3 and 2 different fusion genes and remaining 13 patients (81.25%) exhibiting a presence of a single fusion gene. In our analysis, a total of 22 fusion genes were detected. Of those, 13 genes (59.09%) are known to have a clinical therapy relevance and in the remaining 9 fusion genes (40.9%) a clinical relevance was not known at the time of our study. Following clinically relevant

fusion genes were found, ranked by the frequency of occurrence: 4 cases of EWSER1/FLI1 (30.7% of clinically relevant fusion genes), 2 cases (15.3%) of EWSR1-WT1 and 1 case (7.7%) each of EWSR1-CREB3L2, FUS-DDIT3, YAP1-TFE3, ATM-CUL5, PRKDC-CLIC5, AL445623.2-CDKN2A and TP53-icr. The genes EWSER1/FLI1, EWSR1-WT1, EWSR1-CREB3L2 and FUS-DDIT3 are fusion genes that have an activating function. In contrast, the fusion genes ATM-CUL5, PRKDC-CLIC5, AL445623.2-CDKN2A and TP53-icr are known to have an inactivating function. In the fusion gene YAP1-TFE3, an altered function was detected. Out of fusion genes with no known clinical relevance, 1 case each (11.1%) of the following was found: RP11-408A13.2-NFIB, breakpoint EWSR1 (chr22:29683174, intron 7/chr13:37911311), EWSR1-CREM, FN1-COL1A1, CAND1-HMGA2, CPSF6-FILIP1, FRS2-SPTA1 (translocation), HMCN1-HMGA2 (translocation), ETV1-MEOX2.

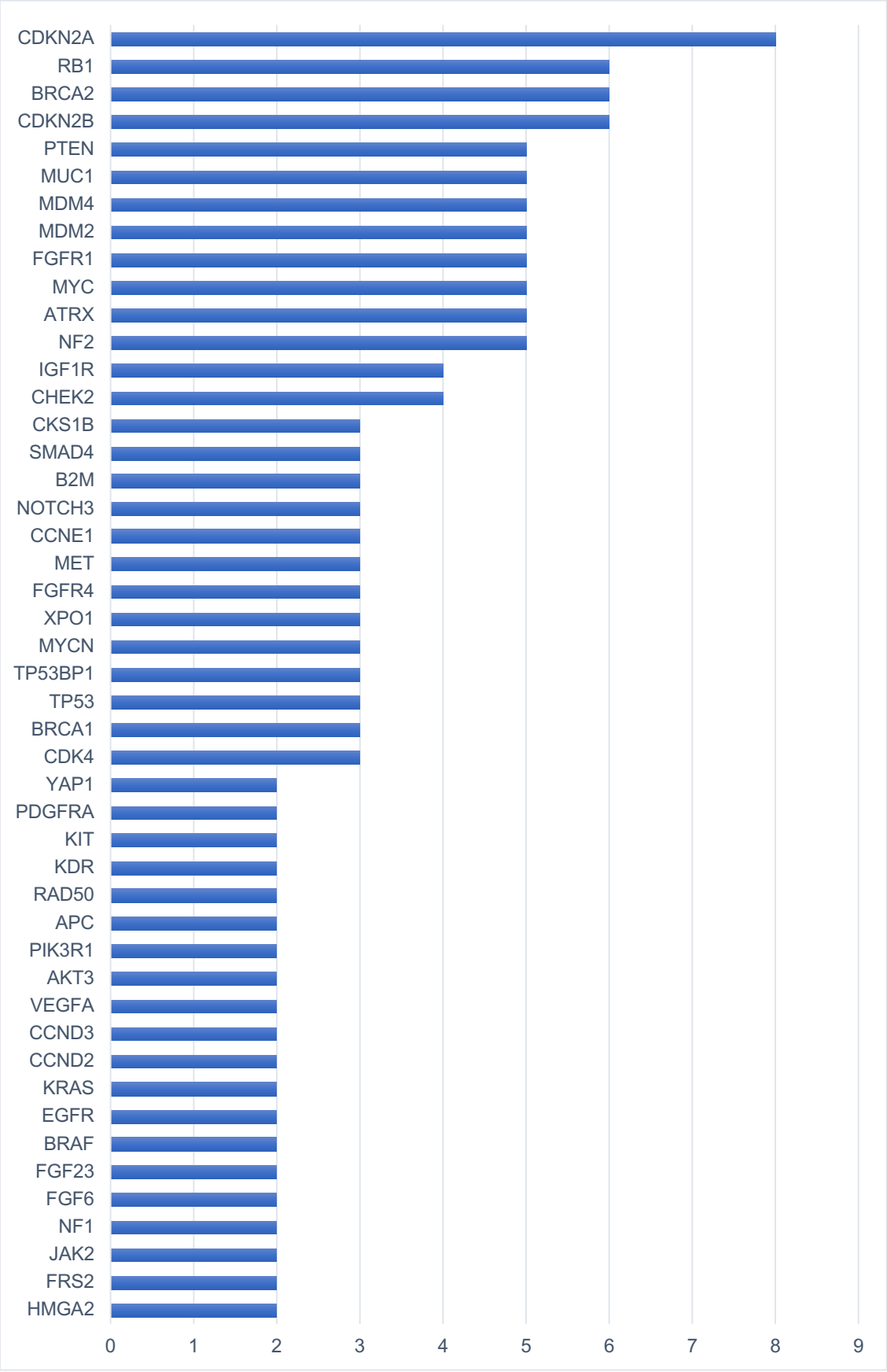


Graph 22: Therapy-relevant fusion genes distribution

4.5.6. Copy number alterations

In our study, every examined tumor sample received an analysis of copy number alterations (CNAs) – such as duplications, amplifications, heterozygous or homozygous deletions and loss of heterozygosity. These variations are frequently leading to genomic instability. In our study cohort, a total of 38 cases (71.7% of the samples) of genomic instability were detected.

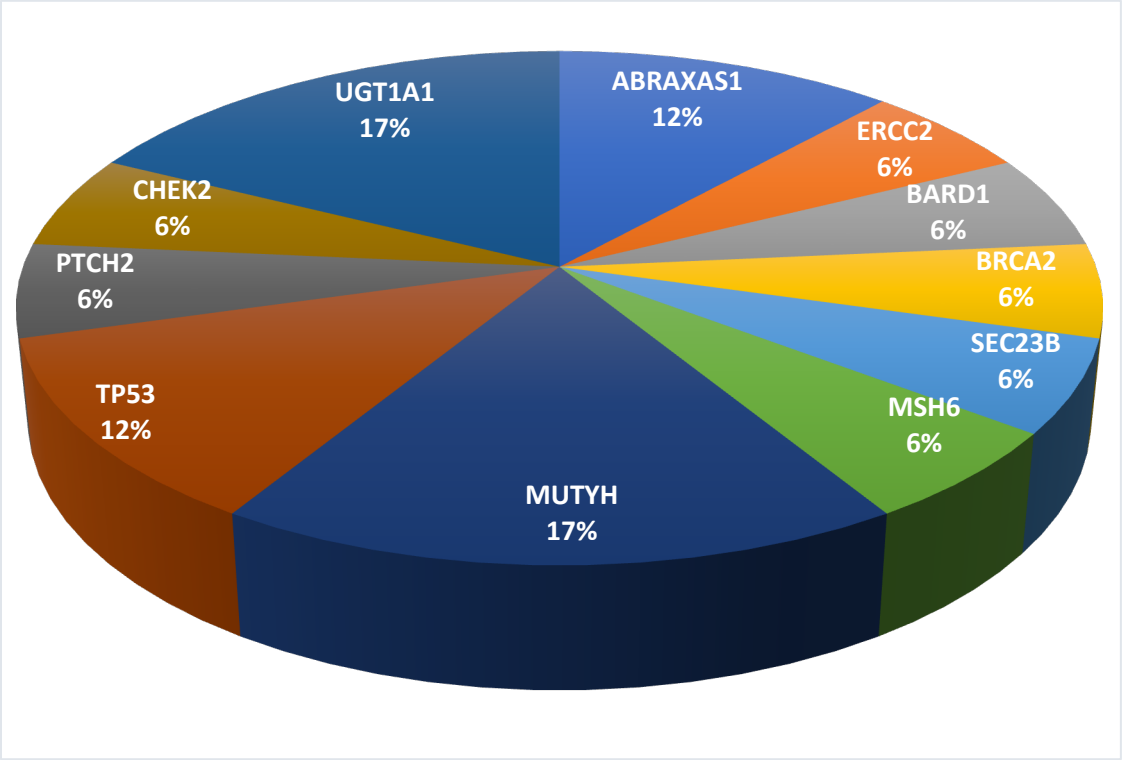
The following genes were affected, ranked by frequency of occurrence: 8 cases (21% of the CNAs) of CDKN2A, 6 cases (15.7%) each of CDKN2B, BRCA2 and RB1, 5 cases (13.1%) each of ATRX, MYC, FGFR1, MDM2, MDM4, MUC1, as well as PTEN, and NF2; 4 cases (10.5%) of CHEK2, and IGFR1R; 3 cases (7.8%) of CDK4, BRCA1, TP53, TP53BP1, MYCN, XPO1, FGFR 4, MET, CCNE1, NOTCH3, B2M, SMAD4, and CKS1B; 2 cases (5.2%) of HMGA2, FRS2, JAK2, NF1, FGF6 and FGF23, BRAF and KRAS, EGFR, CCDN2 and CCD3, VEGFA, AKT3, PIK3R1, RAD50, KDR and YAP1; 1 case (2.6%) of CDK5, CDK6, MTAP, FGF3, FGF4 and FGF19, CCND1, NOTCH1, EWSR1 and RNA FAT1, FANCA, ABCB 1, RAC1, AKT 2, PARP1, PALB2, PIK3CA, TERT, VHL, EMSY and RSF1, ERBB2, YES1, ATS1, ROS1, SPINK1.



Graph 23: Most prevalent copy number alterations distribution

4.5.7. Germline mutations

Our study cohort received a molecular-genetic analysis of the germline mutations: In 15 patients (28.3%) a total of 17 germline mutations were found, all of which (100%) were clinically relevant. Per patient with detected germline mutations a mean of 1.13 mutation (STDEV.s 0.35) and a median of 1 mutation were observed. Germline mutations were found in the following genes, ranked by the frequency of occurrence: 3 cases (17.6% of all germline mutations) each of germline mutation in MUTYH and UGT1A1 genes; 2 cases (11.7%) each of germline mutation in ABRAXAS1 and TP53 gene; 1 case (5.8%) each of germline mutation in ERCC2, BARD1 (BRCA1-associated RING Domain 1), BRCA2, SEC23B, MSH6, PTCH2, and CHEK2 genes.



Graph 24: Germline mutations distribution

4.5.8. Somatic mutations

Besides germline mutations, our study cohort received a molecular-genetic analysis of somatic mutations, which were divided into clinically relevant and irrelevant ones. Clinically relevant mutations were detected in 47 patients (88.68% of the patients). A mean somatic mutation number per patient with detected clinically relevant mutations of 3.15 was observed. A total of 148 clinically relevant somatic mutations in the following genes were found, ranked by the frequency of occurrence: 15 cases (10.1% of all clinically relevant somatic mutations) in the TP53 gene; 10 cases (6.5%) in the CDKN2A gene; 9 cases (6.1%) in CDKN2B gene; 8 cases (5.4%) each in CDK4 and RB1 gene; 7 cases (4.7%) in MDM2 gene; 6 cases (4%) in ATRX gene; 5 cases (3.3%) each in PTEN and TERT genes; 4 cases (2.7%) each in MYC and PDGFRA genes; 3 cases (2%) each in FGF6, KIT, KDR, and PIK3CA genes; 2 cases (1.3%) each in CCND2, CCND3, FGF23, FRS2, HMGA2, KRAS, NF1, TET2 and VEGFA genes as well as 1 case (0.6%) each in AKT2, ARID1A and ARID2, BIRC2, BIRC3, BRAF, BRCA2, CDKN2C, CHEK2, CTNNB1, DOT1L, FANCI, FAT1, IDH1 and IDH2, INPPL1, JUN, LZTR1, MAP2K1, MED12, MET, MTAP, NF2, NRAS, NTRK1, PIK3R1, PTCH1 and PTCH2, SETD2, SF3B1, SMARCB1, USP9X and YAP1 genes.

A total of 212 somatic mutations with no known clinical relevance was detected in following genes, ranked alphabetically: ABCG2, ABL1, AJUBA, AKT1, AMER1, ANKRD26, APC, APOBEC3B, AR, ATP1A1, ATRX, BCL9, BCL11B, BCOR, BCR, BRCA1, BRCA2, BRD4, B2M, CBLB, CDH2, CDH5, CDH11, CDKN1A, CFTR, CHD1, CHD4, COL1A1, CRLF2, CSMD1, CTCF, CTRC2, CYP2B6, CYP2C9, DDR1, DDX11, DICER1, DNMT3A, DPYD, EGFR, EMSY, EPHA2, EPHA3, EPHA4, ERBB3, ERCC3, ERCC5, ERRFI1, ESR2, ETV6, EZH1, E2F3, FGF23, FGFR1, FLI1, FLT3, GATA6, GLI3, GNAS, GNA11, GRM3, HCK, HDAC6, HLA-DPA1, HLA-DRA, IDH2, IKZF1, INPPL1, IRS2, JAK1, JAK3, KMD5A, KDM6A, KDR, KLF2, KMT2A, KMT2C, KMT2D, LATS1, LATS2, LMO1, LRP1B, LRRK2, MAF, MDM2, MDM4, MED12, MN1, MSH4, MSH6, MSR1, MST1R, MTOR, MUC1, MUTYH, MYH9, MYH11, NCOR1, NFE2L2, NFKB2, NIN, NKX2-1, NOTCH3, NPM1, NQO1, NTRK2, NTRK3, PALB2, PARP4, PDF,

PIGA, PIK3CA, PIK3C2B, PKHD1, PML, PREX2, PRKCA, PRKD1, PRKN, PSIP1, PSMB2, PSMB9, PTK7, PTPN12, PTPRD, PTPRT, RABL3, RAC1-ABCB1 Inversion, RAD54B, RBM10, RB1, ROS1, RPTOR, RSF1, RYR1, SKP2, SLC19A1, SLC26A3, SLX4, SPEN, SPINK1, SPTA1, STAT3, TAP1, TENT5C, TERT, TERT-MRM2 Translocation, TET2, TFE3, TGFB1, TMPRSS5-FANCA Translocation, TNFAIP3, TNFRSF8, TOP1, TOP2A TP53, TP53BP1, TRAF5, TRAF7, TRRAP, USP34-CTNNA2 Inversion, YAP1 and ZFHX3.

4.5.9. Impact of NGS on OS and PFS

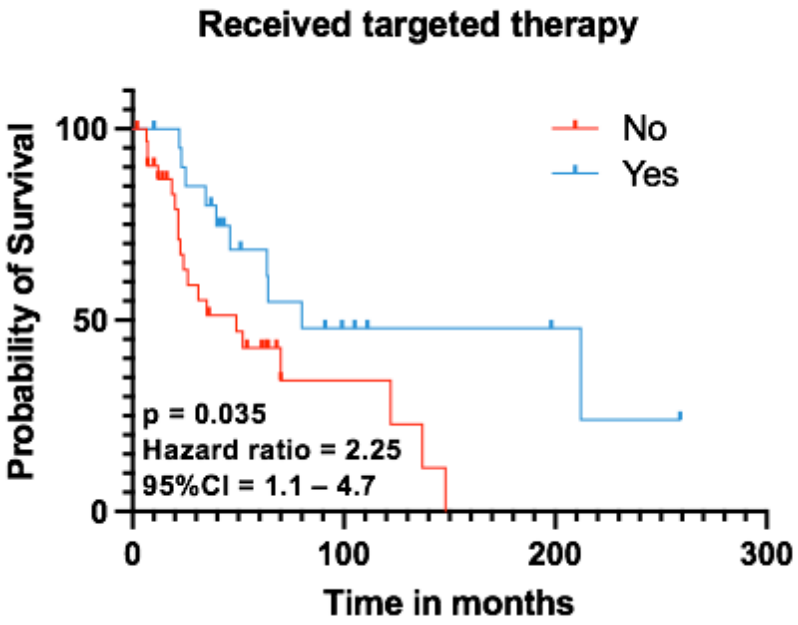
In our cohort, we could observe that the NGS sequencing led to the detection of therapeutic targets as well as the consecutive application of targeted therapies. Following Table 14 shows the used targeted therapies and the respective line of therapy. In some cases, the targeted therapies were combined with cytostatic chemotherapeutic agents (not listed here):

Patient number	Targeted Therapy	Therapy line
Patient 3	Lenvatinib (TKI)	3rd
Patient 6	Imatinib (TKI)	7th
	Olaparib (PARP-Inhibitor)	8th
	Palbociclib (CDK4/6-Inhibitor)	12th
Patient 9	ATR-Inhibitor + Navitoclax (bcl2-Inhibitor)	3rd
	Rucaparib (PARP-Inhibitor)	7th
Patient 11	Tumor peptide vaccination	7th
Patient 13	Crizotinib (TKI)	12th
Patient 16	Olaparib (PARP-Inhibitor)	12th
	Aurora kinase-Inhibitor	13th
Patient 17	Nivolumab (CPI)	3rd Therapy line, adjuvant after two operations
Patient 21	Olaparib (PARP-Inhibitor)	4th
Patient 22	Cabozantinib (TKI)	4th
	Olaparib (PARP-Inhibitor)	5th

Patient 25	Aurora kinase-Inhibitor	7th
Patient 26	Temsirolimus (mTOR-Inhibitor)	5th
	Palbociclib (CDK4/6-Inhibitor	10th
Patient 29	Nivolumab (CPI)	3rd
Patient 31	Alpelisib (PIK3CA-Inhibitor)	14th
Patient 32	Pembrolizumab (CPI)	5th
	Olaparib (PARP-Inhibitor)	6th
Patient 34	Everolimus (mTOR-Inhibitor)	12th
Patient 36	Sorafenib (TKI)	6th
Patient 38	Pembrolizumab (CPI) + Tumor peptide vaccination	13th
Patient 42	Tumor peptide vaccination	21st
	Aurorakinase-Inhibitor	22nd
Patient 43	Larotrectinib (TRK-Inhibitor)	4th
Patient 47	Aurorakinase-Inhibitor	11th
	Anti-CD276-Antibody+ IL2 + IL12 intra-tumoral (Immunotherapy)	12th
Patient 49	Imatinib (TKI)	2nd
	Olaparib (PARP-Inhibitor)	4th
	Sorafenib (TKI)	5th
	Alpelisib (PIK3CA-Inhibitor)	8th

Table 14: Applied targeted therapies

Patients receiving a targeted therapy based on the NGS sequencing results showed significant prolonged survival of 43 months compared to patients not receiving targeted therapies, OS 33 months, (p=0.035, HR=2.25, 95%CI:1.1-4.7).



Graph 25: Targeted therapies and outcomes.
Blue line: patients with targeted therapy.
Red line: no targeted therapy

5. Discussion

Considering the rarity and remarkable heterogeneity of sarcomas at the histological, molecular and genetic levels, the aim of our thesis was to retrospectively examine the genetic profile of a real-life cohort of 53 sarcoma patients in our sarcoma center using a large NGS 720 gene panel.

Despite all advances in modern cancer treatment, sarcoma patients presenting with metastasis still have limited therapeutic options and show an unfavorable prognosis. According to clinical guidelines, first-line therapy for advanced disease includes Doxorubicin, either as a single agent or in combination with Ifosfamide (155), resulting in a rather limited median progression-free survival (PFS) ranging between 4.5 and 6 months (155). Second-line treatment is increasingly differentiated toward particular histological subtypes encompassing the use of classical chemotherapeutics including Trabectedin, Gemcitabine, Eribulin and taxanes (155), as well as Pazopanib, a multi-targeted receptor tyrosine kinase inhibitor (156) (157). Unfortunately, the median PFS for most second-line therapies remains dismally low – below 5 months (155) (158), thus further highlighting the dire need for novel predictive and therapeutic options in sarcoma.

Since genetic profiling enables not only the identification of prognostic but also therapy-relevant alterations in heterogeneous diseases – large-scale genetic analysis has become an indispensable part of modern anti-cancer treatment. As prognostic therapy markers, we analyzed tumor mutational burden, microsatellite status, homologous recombination deficiency, fusion genes, copy number alterations and germline as well as somatic mutations and compared the outcomes in the patients receiving NGS-based target therapy to those without NGS-based target therapy. A comparison to the often-scarce existing literature on the topic in the following pages seeks to draw conclusions about the significance of the molecular genetic diagnostic and the impact on the outcomes of sarcoma patients.

5.1. Study cohort

Our study´s real-life sarcoma cohort included a total of 53 patients which is in terms of the number of enrolled patients comparable to several big existing sarcoma studies such as 40 patients with soft tissue sarcoma in SARC028 (144), 47 patients Painter et al. (159) and 49 patients Chudasama et al (147), but smaller in scope when compared to the sample size of 71 patient in Campanella et al. (146) or 304 Patients in Dyle et al. (145) and larger compared to the sample size of 7 patients in Florou et al. (153), 31 patients in Kovac et al. (148) or 21 patients in He et al. (142).

The Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma (160) study is one of the by far largest NGS sarcoma studies in scope and included 7494 sarcoma patients. Besides adult, it included the pediatric population as well. A very well-known challenge in sarcoma research is achieving a significant study cohort size due to the sheer rarity of the disease. Furthermore, structuring the study cohort and individual groups within it can also be challenging due to the heterogeneity of the disease. We enrolled patients with all sarcoma subtypes with the goal in mind to analyze the significance of molecular profiling in sarcomas in general and the clinical implication of the common molecular-genetic features between different sarcoma subtypes. However, according to recent literature, this poses a certain limitation in itself, due to significant intertumoral diversity between individual sarcoma subtypes, as described in multiple studies (145) (144) (146) (160), which further complicates the development of precision-based therapeutics. Some studies tried to solve this problem by recruiting only patients with a certain sarcoma subtype (153) (161) (148) (147) in an attempt to examine the effects of certain interventions on this strictly defined study cohort. Alternatively, other studies, such as the Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma (160) study, aimed to identify highly recurrent and type-specific alterations with a potential to impact diagnosis and treatment decisions (160). Despite a massive study population, even in this study were represented not more than 44 different histological sarcoma subtypes, which reflects a fraction of the sarcoma subtypes diversity.

Relative to the cohort size, one of the strengths of our study is the representation of the relatively rare sarcoma subtypes in a real-life cohort. The weaknesses of our study are aligned with typical disadvantages of a retrospective analysis, such as, for example, not being able to determine causality but only association based on the available data.

The mean age at the time of diagnosis for all the sarcoma patients (bone as well as STS) was 44.4 years. Based on current literature provided by the NCHS and SEER databases, the mean age at diagnosis for soft tissue sarcomas and malignant bone tumors was 58 and 40 years of age, respectively (63)(162), which is comparable to our findings.

5.2. Tumor genome sequencing

5.2.1. Tumor mutational burden

In analyzing TMB, whole exome sequencing remains the gold standard diagnostic method. However, TMB is also shown to be reliably extrapolated from NGS-based multigene panels (125), as done in our study. We applied the following TMB ranges, which are defined as: low TMB as less than 3 mutations/Mb, intermediate TMB as a value between 3 and 10/Mb, a high TMB as a value above 10 Mb. However, in several other studies on the same topic, somewhat differing TMB ranges were observed (163). We propose a unified TMB range system for sarcoma studies as a step towards simplification and standardization of future studies. A detected median TMB value of 1.5 mutations/Mb in our study is higher compared to Abeshouse et al. (143), who found a median of 1.06 mutations/Mb, but lower compared to Painter et al. (159) who found an overall median TMB of 3.3 mutations/Mb. In our study, only 1 out of 52 patients (1.92%) has shown a high TMB which is comparable to He et al. (142) who also detected only 1 out of 21 patients (4.7%) with a high TMB in their study cohort. The highest TMB in our study was 24,7 mut/Mb and was observed in a patient with MSH-6 germline mutation. The sweeping Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma study found a median TMB of 2.4 mut/Mb

(160), which is higher than our results, However, a high TMB-score was detected in 2.9% of the patients (160), which is comparable to 2% in our study.

Large initial studies involving multiple types of malignant tumors (such as KEY-NOTE-158 (164)) tried to establish TMB as a novel and useful predictive biomarker for response to Pembrolizumab monotherapy in patients with previously treated recurrent or metastatic advanced solid tumors. Despite a rather limited array of cancer types included in the study, across 790 cases, patients exhibiting a high TMB status (defined in this study as more than 10 mutations per megabase) demonstrated a significantly higher response rate of 29% to Pembrolizumab compared to 6% in patients exhibiting a non-high TMB status (defined in this study as less than 10 mutations per megabase) (164). Although underlining the significant potential of TMB as a predictive biomarker for immune checkpoint inhibitor therapy, this study fails to demonstrate if a clear association remains when stratified in specific cancer types such as sarcomas, highlighting a need for further studies (139). One such trial was The SARC028 (140), a two-study cohort, single-arm, open-label, phase 2 study, which examined the overall response on anti-PD-1-antibody Pembrolizumab in 86 patients with metastatic or surgically non-resectable locally advanced sarcoma, treated with up to three previous lines of systemic anticancer therapy (140). This trial has shown an objective response in only 7 out of 40 (18%) patients. However, when stratified in different sarcoma subtypes, notable differences in response were detected: the undifferentiated pleomorphic sarcoma (UPS) group exhibited the highest objective response (in 4 out of 10 UPS patients; 40% OR), followed by patients with dedifferentiated liposarcoma (DDLPS) (2 out of 10 DDLPS patients; 20% OR) and synovial sarcoma (SS) (1 out of 10 SS patients; 10% OR) (122). In the leiomyosarcoma (LMS) group no responses were detectable (122). Further, a phase II trial (NCT02428192) aimed to assess Nivolumab, a PD1 blocking antibody, as a monotherapy in uterine LMS (ULMS) patients and similarly demonstrated no clinical benefit in 12 patients (165) (122). Unfortunately, no TMB analysis was conducted in these trials, limiting the further deductions. Due to rather limited patient study cohort sizes, drawing definitive conclusions about potential subtype-specific benefits and a connection between certain histopathological subtypes and favorable

checkpoint inhibition response proves challenging. However, the dire need for robust predictive biomarkers to prospectively identify sarcoma patients that would likely benefit from immune checkpoint inhibitor therapy as well as for exploring further biomarker-driven approaches is becoming increasingly clear. To further underline this point, it was shown that the presence of other confounding factors (such as known defined genetic drivers) may further limit the use of TMB as a sole marker for CPI immunotherapy. For example, The Cancer Genome Atlas Research (TCGA) detected in a recently published report a low overall TMB (averaging 1.06 mutations/ Mb) across 206 soft tissue sarcoma cases, which is, on one side, comparable to our own results and suggests, on the other side, that TMB alone as a biomarker may be insufficient in sarcomas (143). In search of a complementary marker to increase robustness and predictive power of TMB, Petitprez et al. analyzed gene expression data and tumor microenvironmental features across 608 soft tissue sarcomas, identifying 5 distinct molecular subtypes associated with the enrichment of specific subsets of immune-related genes (144) (122). The immune-high subtype, christened “class E”, exhibited presence of B-cell lineage genes and an association with tertiary lymphoid structures (TLS) (144) (122). Using these molecularly-defined subgroups to stratify patients from the SARC028 trial (140), the class E patients have been shown to exhibit a significantly higher OR rate (ORR; 50%) to Pembrolizumab compared to any other subgroup (144) (122). This suggests that including TLS and gene expression signatures may increase sensitivity in predicting sarcoma patients that may benefit from treatment with immune checkpoint inhibitors. Further evaluation of the integration of TMB with TLS scoring and other immune-based gene expression signatures (as described by Petitprez et al. (144)) may improve their predictive power and robustness as a biomarker of immune checkpoint inhibitor response (122).

In our study a checkpoint inhibitor therapy was administered a total of 5 times: 1 case of Nivolumab in an adjuvant setting in a patient with an intermediate TMB (5.6 mutations/Mb) and metastasized interdigitating dendritic cell sarcoma (IDCS, an extremely rare neoplasm that mainly arises from the lymphoid tissues of the immune system (166)) with a complete remission as a result. In a patient with a

metastasized small blue round cell tumor of Ewing sarcoma/PNET group and an intermediate TMB (5.6 mutations/Mb), a checkpoint inhibition with Pembrolizumab had to be discontinued after 3 therapy cycles due to tumor progression. Further, the above-mentioned patient with metastasized extraskeletal chondrosarcoma and a high TMB score (24.7 mutations/Mb) has responded well to the CPI immunotherapy with Nivolumab. Alas, due to severe side effects, this therapy had to be stopped after 10 therapy cycles. Due to the small sample size and the rarity of the patients with a high or very high TMB in our study cohort, it is difficult to draw definite conclusions regarding the predictive power of TMB as a biomarker for the checkpoint inhibition response. Unfortunately, it was not possible to retrospectively examine immune-based gene expression signatures additionally and correlate those to the effects of the immunotherapy, which is in line with typical limitations of a retrospective study. As Yang et al. noticed in their study about predictive markers (167), the immune context of the tumor microenvironment (TME) is critical for effective immunotherapy, but DNA-based biomarkers for the immune-sensitive TME and the identification of immune checkpoint inhibitor responders are nonetheless under-explored. Further studies are needed here.

5.2.2. Microsatellite instability

Despite PCR-based detection of MSI markers remaining the gold standard (168), multiple NGS-based methods exhibit accurate and reliable MSI signature detection (130) utilizing either genome-wide sequencing or sequencing of smaller gene subsets. PCR-based methods brought about advantages regarding throughput, sensitivity and simultaneous analysis of samples for additional genomic signatures (122). We employed these advantages in our study. As previously discussed, an MSI-high signature associated with MMR defects may be able to predict response to immune checkpoint inhibitors, as described in several key trials (131) (169), which has subsequently led the FDA to approve MSI as a predictive biomarker for checkpoint inhibitor therapy response agnostic of tumor type or anatomical site (131).

As previously detailed in the introductory part, the evidence for the presence of MSI-high signatures in sarcomas has been rather unpromising, with most of the studies reporting a rather small incidence of sarcomas exhibiting microsatellite instability (146) (122). A recent study of 71 STS patients by Campanella et al. (146), detected MSS status in every patient, suggesting that MSI may have a rather limited utility in sarcoma patients. This data is unfortunately comparable to our own findings: out of 52 patients in our study cohort, none have expressed microsatellite instability casting further doubt in the role of MSI as prediction marker in sarcoma. However, further investigation is required to illuminate whether a high MSI signature is a reliable predictor of response to checkpoint inhibitors in sarcomas and if MSI can be used either as a sole parameter or in combination with other parameters.

5.2.3. Homologous recombination repair deficiency

Another promising predictive biomarker for novel therapy options in several tumor entities is homologous recombination repair deficiency (HRD). It is defined as an unweighted sum of three independent DNA-based measures of genomic instability in a tumor: loss of heterozygosity (LOH (173)), telomeric allelic imbalance (TAI (174)), and large-scale transitions (LST (175)). This marker is strongly associated with a sensitivity to treatment with PARP inhibitors (176). Furthermore, several large studies (167) linked HRD-high genotypes with neoantigenesis in multiple malignancies, including bladder cancer, breast cancer, head and neck squamous carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, ovarian cancer and sarcoma. The analysis of the TCGA database illuminated that tumors with high HRD scores are more likely to exhibit increased leukocyte infiltration and lymphocyte fraction and demonstrate an immune-sensitive microenvironment (167), suggesting the HRD-high genotype as a potential predictor of immune checkpoint inhibitor therapy response (167). However, in this regard, HRD seems to be insufficient as a sole marker (177) and may be inferior to immuno-tumor microenvironment (iTME) (177) and therefore is mostly clinically used as a marker for PARP-inhibitor therapy response (176).

The existing literature shows the highest frequency of HRD genotypes in ovarian and breast cancer, followed by pancreatic and prostate cancer (178). Several large pan-cancer HRD studies are rather difficult to interpret as sarcomas were not considered and analyzed as a separate group, since they comprise a very heterogeneous family of more than 100 distinct histological subtypes arising on a wide variety of primary locations. Several studies in recent years identified HRD-ness traits in soft tissue and bone sarcoma (148) (179) (180).

A clear cut-off value for HRD in sarcoma is not universally defined. In other malignancies with the “BRCAness” phenotype, a cut-off value of 42 for the use of PARP inhibitors is well established (181). Although the HRD score remains insufficiently explored, several comprehensive sarcoma studies with HRD in focus (155) have used 32 as an optimal cut-off value for sarcoma, so we abided by this value as a cut-off in stratifying our patients as well aiming for establishing a unanimous value system and cut-off values in future studies. Furthermore, the basis for this cut-off value found reinforcement in a significant correlation with high PARP-inhibitors sensitivity in patient-derived ex-vivo-sarcoma models (155).

One of the largest sarcoma studies with HRD in focus (155) describes striking sarcoma subtype related differences in the genomic instability signatures and HRD scores, with undifferentiated pleomorphic sarcoma (UPS), osteosarcoma (OS), myxofibrosarcoma (MFS) and uterine leiomyosarcoma (ULMS) exhibiting the highest HRD scores, followed by malignant peripheral nerve sheath tumor (MPNST), extra-uterine leiomyosarcoma (LMS) and dedifferentiated liposarcoma (DDLPS) (155). A very comprehensive Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma study (160) also found a variation in HRD genetic signatures frequency between different sarcoma subtypes, with uterine leiomyosarcoma (ULMS) exhibiting the most frequent HRD markers (160). However, overall, only 2.5% (184/7494) of samples harbored pathogenic alterations in homologous recombination repair pathways (such as BRCA1/2, PALB2, RAD51, and its paralogs RAD51B, RAD51D, RAD52, RAD54L), which is significantly lower compared to our results. A study of 83 uterine leiomyosarcomas (182) found an HRD signature in 25% of the samples coinciding with our

finding. Another sweeping molecular-genetic-based study of 52426 tumor samples (183) of multiple cancer types found pathologic alterations associated with HRD in 9.3 % of sarcoma. However, no further stratification in sarcoma subtypes was performed. A multi-cancer study of 501 samples (184) observed the presence of HRD genetic patterns in as much as 65% of the 60 sarcoma samples. Unfortunately, further quantification (for example through calculation of the HRD score) or data on the structure of the sarcoma study subpopulation were not available. In the study of 351 Chinese sarcoma patients, 13.7% were found to have positive HRD molecular-genetic patterns, which is lower compared to our findings.

In our real-life cohort, we could observe a positive HRD score in 13 patients (25%) with a mean HRD score of 32.28. This significant variability between the studies (2.5% – 65%) could be explained through a different proportion of various sarcoma subtypes in the compared studies. Furthermore, we postulate that higher HRD frequency in our findings may have a foundation in the fact that leiomyosarcoma is the sarcoma subtype with the highest prevalence in our cohort, which was shown in the aforementioned studies to have a higher frequency of HRD compared to other sarcoma subtypes.

Based on the positive HRD score, we could introduce a PARP-inhibitor to 6 of our patients. As presented in Table 12, patient 6 with a metastasized chordoma and an HRD score of 22 received a PARP-inhibitor therapy with Olaparib in the 8^{th} therapy line (as a part of TOP-ART study) over 11 months until tumor progression. Patient 16 with rhabdomyosarcoma and an HRD score of 71 received Olaparib together with carboplatin as the 7^{th} therapy line and discontinued after 3 months due to tumor progression. Patient 21 with metastasized phyllodes tumor received Olaparib in the 8^{th} therapy line (as a part of the TOP-ART study) over 2 months until tumor progression. Furthermore, patient 22 with osteoblastic osteosarcoma and an HRD score of 47 received Olaparib as the 4^{th} therapy line, which was discontinued after 7 months due to the progressive tumor dynamic. Another case was a patient 32 with a small blue round cell tumor of the Ewing sarcoma / PNET family having an HRD score of 38. The therapy with Olaparib was intro-

duced as the 4th therapy line and was continued for 3 months until a tumor progression was detected. Finally, patient 49 with metastasized chordoma and without a determinable HRD score received Olaparib in the 4th therapy line with Trabectedin (as a part of the TOP-ART study) over 5 months until tumor progression was determined. The described entities that were treated with PARP-inhibitor therapy are rather atypical in light of existing literature. We could observe that the PFS under this therapy correlated with HRD-score.

5.2.4. Fusion genes analysis

Fusion genes are common mutations induced by chromosomal aberrations. They are causally associated with sarcomas (185) and most of them are strongly associated with a particular histological (sub)type, serving as an ideal molecular diagnostic marker (186) and, as such, heavily impacting diagnostic and therapy decisions. Out of 142 reported fusions, more than half exhibit recurrence in the same histologic subtype (186). Furthermore, some of the chimeric proteins have been shown to constitute excellent treatment targets, giving rise to much needed novel target therapy options (for example for fusions that activate protein kinases, such as ALK and ROS1, or growth factors, such as PDGFB) (186).

A current literature search yields only a few studies analyzing the fusion gene frequency in a sarcoma patient cohort. Besides several ongoing studies (for example NCT03375437), a Japanese study of 55 sarcoma patients detected fusion genes in 29 (55%) (185) cases with a total of 47 fusion genes. The cohort consisted of 30 spindle cell sarcoma and 25 round cell sarcoma patients, which would explain the high fusion gene frequency. As a part of this work, the researchers found a potentially treatment-relevant novel mutation (185), which shows an exciting potential in this line of research. In our study population, we detected the presence of 22 already established fusion genes in 30.77% of the patients, with 59.09% of those genes having a known clinical relevance at this time and EWSR1-FLI1 and EWSR1-WT1 being the most frequent. The lower incidence in our study compared to the aforementioned Japanese study could be explained

by a significantly greater sarcoma subtypes heterogeneity in our real-life study cohort.

As the NGS technologies continue to improve technically and to become more affordable and user-friendly, they will gradually replace old-fashioned methods for detecting gene fusions not only in research projects but also in the clinical setting. Hence, the number of known gene fusions in sarcoma can be expected to show the same dramatic increase within the next few years (186).

5.2.5. Copy number alteration

Exploring the landscape of copy number alterations (CNAs) is a novel and promising research field. However, due to the aforementioned challenges, there have been only several such studies with sarcomas in focus. A CNA is defined as any deviation from the 2n copy number state of a region in the genome (187) and is considered as one of the core mechanisms of sarcomagenesis (188). CNAs arising post-zygotically in a somatic cell are referred to as somatic CNAs or SCNAs. In contrast, CNAs that occur in the germline, and are therefore inheritable, are labeled as copy number variants or CNVs (187). A massive pan-cancer study of 853218 SCNAs across 10729 tumor samples concluded that ovarian carcinomas and sarcomas carry, on average, the highest burden of SCNAs, followed by uterine carcinosarcoma (189). Unfortunately, a more detailed exploration of the sarcoma samples with analysis of the frequency of individual mutations or in specific subtypes was not published.

The Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas (190) from Cancer Genome Atlas Research Network is one of the most thorough papers on this subject to date, involving 206 adult STS patients and a vast database. This study has shown that only a few genes, such as TP53, ATRX and RB1, were found to be highly recurrently mutated across sarcoma types (190). The pan-sarcoma analysis has shown that soft tissue sarcomas harbor frequent copy number alterations (190). SCNAs were observed to frequently affect the MDM2-p53 and the p16-CDK4-RB1 pathway. Overall, the complex karyotype sarcomas were characterized by more frequent SCNAs compared to most

other tumor types in the TCGA database (190). Of the sarcoma subtypes, dedifferentiated liposarcoma (DDLPS) has shown the highest frequency of SCNAs, due to its highly recurrent focal amplifications at 12q13~15. In contrast, synovial sarcoma (SS) displayed very few SCNAs or mutations (190). Further, it was observed across the examined sarcoma types that deletions were more prominent than amplification, and relevant mutations in tumor suppressors were substantially more frequent than those in oncogenes: MDM2, CDK4, JUN, and TERT amplifications in DDLPS; MYOCD amplification, PTEN mutations/deletions, and AKT, IGF1R, and MTOR pathway activation in LMS; and VGLL3 amplification and Hippo pathway activation in UPS/MFS (190).

Our data is comparable to the aforementioned large genome-driven sarcoma and pan-cancer studies. We could detect a total of 38 CNAs (71.7% of the samples) in our study cohort. In our real-life heterogeneous sarcoma cohort, we have observed the highest sCNA frequency in CDKN2A/B, BRCA2, ATRX, MYC, RB1, ATRX and MDM2/4 genes – the genes that have a key role in cell cycle regulation, chromatin remodeling or are tumor suppressors. Variations in gene frequency and proportion of the detected CNAs between the studies may be explained through different proportions of the examined sarcoma subtypes. For example, liposarcomas were significantly less represented in our study cohort in comparison to the aforementioned comparable studies (for example (190)). Therefore, the frequency of the MDM2 gene affection is somewhat lower in our paper. MDM2 is a known suppressor of genome guardian p53, and its genetic amplification is a hallmark of liposarcoma (191). Due to the detection of MDM2 amplification, we could include our liposarcoma patient in an appropriate BI1403-001 phase 1 study with an MDM2 inhibitor. At the end of our observation period, the patient still had a favorable response to this precision therapy. Further, a patient with chondrosarcoma and detected CNAs in CDKN2A/B genes received as NGS-based treatment in the 5th therapy line Palbociclib with excellent results and clinical tolerability. Furthermore, a patient with metastasized desmoplastic small round blue cell tumor received targeted therapy with Palbociclib in 9th for three months, which had to be discontinued due to tumor progression.

The CNAs reflect the significant genomic instability and should be considered an important part of understanding tumor biology and tumorigenesis itself, as well as finding further therapy targets. Suffice to say, this is another promising line of research. However, more studies are needed before a clear clinical application can be derived.

5.2.6. Germline mutations

Even though a minority of sarcoma have been associated with hereditary cancer syndromes such as LFS, RB or NF (all of which are thoroughly discussed in the introduction), most of the sarcoma cases appear to be sporadic in occurrence (5). However, little existing literature addresses the role of genetic susceptibility in sporadic sarcoma (192). A study of 66 Asian patients with a sporadic sarcoma has shown an incidence of 13.6% of a pathogenic germline mutation in 10 cancer-associated genes including ATM, BRCA2, ERCC4, FANCC, FANCE, FANCI, MSH6, POLE, SDHA and TP53. As described in the study, the most frequently affected genes are involved in the DNA damage repair pathway (192). A large international study of 1162 sarcoma patients (193) of the International Sarcoma Kindred Study (ISKS) detected pathogenic germline variants in as much as 55% of the patients, with the highest mutation frequency observed in TP53, ATM, ATR, and BRCA2 genes. However, a significant number of patients in this study have had a known or at least anamnestic strongly implied family history of malignancies, sometimes multiple cancers as well (193).

In our heterogeneous sarcoma cohort, we have detected at least one germline mutation in almost a third of our patients (28.3%), all of which were clinically relevant. As far as we know, none of our patients has a clear positive family cancer anamnesis. Nonetheless, our cohort has exhibited more than a twofold higher frequency compared to the aforementioned Asian study (192), which may be explained by a greater sarcoma subtype heterogeneity among our patients. The most prevalent germline mutations in our cohort were MUTYH, UGT1A1, TP53 and ABRAXAS1, which is comparable to the sparse existing literature on this topic.

The sum of the aforementioned findings suggests that genetic predisposition plays a larger role than expected in sporadic sarcoma occurrence, which implies that young sarcoma patients may be carriers of inherited mutations in cancer genes and should be considered for genetic testing, regardless of family history. Furthermore, the prevalence of germline mutations in DNA damage repair genes would suggest that therapeutic strategies exploiting the vulnerabilities resulting from impaired DNA repair may be promising areas for translational research.

5.2.7. Somatic mutations

A somatic mutation is per definition an alteration in DNA that occurs after conception (76). Some of those are considered as driving mutations due to being able to drive tumorigenesis and to confer on cells in a somatic tissue certain selective advantages leading up to tumor cells surviving and eventually spreading (194). Seeking to understand driver mutations and the underlying cellular pathology is considered to be crucial for the further understanding of the process of tumorigenesis and the development of target therapies.

The Cancer Genome Atlas (TCGA) Research Network reported a recent pan-sarcoma analysis of 206 adult STSs representing six major subtypes (195), in which only a few genes (TP53, ATRX, RB1) were shown to be highly recurrently mutated across common sarcoma types (except for SS). Further, the authors denote specific genomic and transcriptomic alterations and also defines molecular subtypes, which are associated with patient outcome (195). In 80 LMS (53 STLMS and 27 ULMS), 50 DDLPS, 44 UPS, 17 MFS, 10 SS, and 5 MPNST included in this study, the significantly mutated genes across the entire cohort were observed to be TP53, ATRX and RB1. The incidence of the mutations has shown a wide margin of variability between different subtypes, with TP53 mutations, for example, being most prevalent in LMS. Further, a study of 102 sarcoma patients observed, that the most commonly affected genes were TP53 (31.4%), CDK4 (23.5%), MDM2 (21.6%), RB1 (18.6%), and CDKN2A/B (13.7%) (196). Several other studies analyzed somatic mutation in a particular sarcoma subtype cohorts showing a distinct subtype-associated profile (197) (198) (199) (200).

In our real-life pan-sarcoma cohort, we could observe a total of 148 clinically relevant somatic mutations. Comparably to the scarce existing literature, we have detected the highest incidence in TP53 (10.1%), CDKN2A (6.5%), CDKN2B (6.1%), CDK4 (5.4%), RB1 (5.4%), MDM2 (4.7%) and ATRX (4%) genes.

5.2.8. Impact of NGS on outcome

The ultimate goal of personalized medicine is to be able to integrate clinical, genomic, transcriptomic, and epigenomic data to increase the accuracy of diagnosis and prognosis, and to identify the most effective therapy for treatment (200). Besides understanding cancer biology and the incidence of different molecular-genetic markers in sarcoma, our immediate goal as clinicians was finding actionable genetic targets in our patients and assessing the effectiveness of our therapy approach based on those targets through analyzing overall and progress-free survival.

Several studies analyzed the impact of the target therapies/personalized oncology on the outcomes. As a side-effect of the NGS approach, an observational study with 395 patients from 32 centers of the French Sarcoma Group/Reference Network in Pathology of Sarcomas has shown that the inclusion of genomic analyses led to the re-classification of 13% of sarcoma cases and would have resulted in changes to the clinical treatment pathway or prognosis in 11% of cases, demonstrating the importance of including molecular and computational tools for classification and risk-stratification of sarcomas (201). In the aforementioned study (196) of 102 sarcoma patients, sixteen percent of the cohort had received a targeted therapy, more than 50% of whom had a stable disease at the end of the observation period (196). The authors of this study drew the conclusion, that incorporating NGS into sarcoma management may allow for more precise diagnosis and sub-classification, as well as personalized matching of patients to targeted therapies such as those available in basket clinical trials (196). An ongoing clinical MULTISARC trial (ClinicalTrials.gov No. NCT03784014) should provide the first glimpse into the successes and potential pitfalls of personalized medicine in sarcoma. Based on a retrospective survey of genomic alterations that could be

therapeutically actionable in STS patients (202), MULTISARC is a two-arm, randomized trial aiming to prospectively evaluate their potential as predictive biomarkers for response to therapy. STS patients will be randomized to receive standard therapy or undergo genomic profiling for suitability for therapy with 16 different agents. This trial should be completed by 2025.

Our data are comparable. Based on the NGS results, 39.6% of our patients received personalized anti-tumor therapy. The implementation of the targeted therapies may have led to improved outcomes. Median overall survival (OS) for patients with an NGS-based treatment was 43 months compared with 33 months in patients without targeted therapies.

5.3. Conclusion

As described, our cohort was a real-life and - by nature - very heterogeneous group of patients, who already received, in most cases, several lines of conventional therapy.

Employing this cohort, we specifically intended to examine the impact of the molecular genetic personalized precision oncology therapy approach on the outcomes and to compare our results to the sparse existing literature on this topic.

We would like to point out, that almost a third of our patients have had distant metastasizes at the time of the diagnosis, but close to 90% at the time of molecular genetic diagnostics (on average 46.8 months later). To the best of our knowledge, there are currently no clear recommendations for the timing when to undertake the very first NGS analysis. We would like to postulate that the optimal time point may be at the end of the first-line therapy, because of the somewhat longer time span until the final results as well as a molecular tumor board recommendation, considering that current literature describes a PFS of around 4 months only in the second line therapy.

Based on our NGS data, we could initiate a targeted therapy protocol in more than one-third of our patients and observed a significantly longer OS in the group that received a personalized therapy based on prior NGS analysis.

Certainly, the limitations that abound in a retrospective observational study such as ours must be considered. Compared to existing literature, we consider our cohort size to be rather adequate, even though there was an extensive variety of sarcoma subtypes. In the cases, where a subtype comprises only one patient, any subtype-specific conclusion is impossible. This is an unfortunate consequence of researching a disease with such enormous heterogeneity. However, we strived to examine our hypothesis rather as subtype-agnostic, which created a distinct advantage in showing that molecular genetic patterns are observed in diverse subtypes, providing a myriad of therapeutic targets and a novel therapeutic potential.

In conclusion, our study suggests that personalized targeted therapies based on a large panel analyzing 720 genes might lead to improved clinical outcomes in sarcoma patients and that patients with such a rare and heterogeneous neoplasm like sarcoma may especially benefit from an NGS-based precision oncology therapeutic approach.

6. Summary

Sarcomas are rare tumors, known for considerable heterogeneity at the histological, molecular and genetic levels. Despite all advances in modern cancer treatment, sarcoma patients in advanced stages still have limited therapeutic options and an unfavorable prognosis. Since genetic profiling enables not only the identification of prognostic but also therapy-relevant alterations in heterogeneous diseases, large-scale genetic analysis has become an indispensable part of modern anti-cancer treatment. In this study, we retrospectively analyzed the genetic profile of a real-life cohort of 53 sarcoma patients using a state-of-the-art 720-gene panel.

Reflecting the heterogeneous nature of sarcoma, several histopathological subtypes were analyzed, with leiomyosarcoma (17 %) being the most prevalent. The mean patient age at the time of analysis was 49 years. The average time period from primary diagnosis to genetic analysis was 46.8 months. Overall survival was 55.9 months on average. Every patient received a tumor genome sequencing with a large-scale 720 gene panel. We observed a low TMB in 76.9% of the patients. None of the patients was identified as microsatellite unstable. 25% of the patients had a homologous recombination deficiency (HRD). In 30.8% a fusion gene was detected, with EWSR1-FLI1 and EWSR1-WT1 being the most frequent. A total of 38 copy number alterations (CNAs) were found, reflecting significant genomic instability. In 15 patients germline mutations were found, all of them treatment relevant, with mutation in the MUTYH gene being the most frequent. Therapy-relevant somatic mutations were found in 47 patients (3.2 mutations/patient). The most prevalently involved genes were TP53, CDKN2A-C, CDK4, RB1 and ATRX.

Based on the next-generation sequencing (NGS) results, 39.6% of patients received a personalized anti-tumor therapy. Median overall survival (OS) for patients with an NGS-based treatment was 43 vs. 33 months in patients without targeted therapies.

Our NGS data obtained from a heterogeneous cohort of 53 metastasized sarcoma patients suggest that personalized therapies instructed by 720 gene panel sequencing might lead to improved clinical outcomes for sarcoma patients.

7. Zusammenfassung

Sarkome sind seltene Tumore, die sich durch eine erhebliche Heterogenität auf histologischer, molekularer und genetischer Ebene auszeichnen. Trotz aller Fortschritte in der modernen Krebsbehandlung haben Sarkom-Patienten im fortgeschrittenen Stadium weiterhin begrenzte therapeutische Möglichkeiten und eine ungünstige Prognose. Da die Untersuchung des genetischen Profils nicht nur die Identifizierung prognostischer, sondern auch therapierelevanter Veränderungen bei heterogenen Erkrankungen ermöglicht, sind genetische Analysen ein unverzichtbarer Bestandteil der modernen Krebsbehandlung geworden.

In dieser Studie analysierten wir retrospektiv das genetische Profil einer real-life Kohorte von 53 Sarkom-Patienten anhand eines 720-Gen-Panels.

In Anbetracht der Heterogenität von Sarkomen wurden mehrere histopathologische Subtypen analysiert, wobei das Leiomyosarkom (17 %) am häufigsten vorkam. Das Durchschnittsalter der Patienten zum Zeitpunkt der Analyse betrug 49 Jahre. Die durchschnittliche Zeitspanne von der Erstdiagnose bis zur genetischen Analyse betrug 46,8 Monate. Das Gesamtüberleben betrug im Durchschnitt 55,9 Monate.

Jeder Patient erhielt eine Tumorgenomsequenzierung mit einem 720-Gene-Panel. Bei 76,9% der Patienten wurde ein niedriger TMB-Wert festgestellt. Keiner der Patienten wurde als mikrosatelliteninstabil identifiziert. 25% der Patienten wiesen einen Mangel an der Funktionalität der homologen Rekombination (HRD) auf. Bei 30,8% wurde ein Fusionsgen nachgewiesen, wobei EWSR1-FLI1 und EWSR1- WT1 am häufigsten waren. Insgesamt wurden 38 Kopienzahlveränderungen (CNAs) gefunden, was auf eine erhebliche genomische Instabilität hinweist. Bei 15 Patienten wurden Keimbahnmutationen gefunden, die alle behandlungsrelevant sind, wobei die Mutation im MUTYH-Gen die häufigste ist. Therapierelevante somatische Mutationen wurden bei 47 Patienten gefunden (3,2 Mutationen/Patient). Die am häufigsten betroffenen Gene waren TP53, CDKN2A-C, CDK4, RB1 und ATRX.

Auf der Grundlage der NGS-Ergebnisse erhielten 39,6 % der Patienten eine personalisierte Antitumortherapie. Das mediane Gesamtüberleben (OS) der Patienten mit einer gemäß den Daten der NGS-Analyse ausgerichteten Behandlung betrug 43 gegenüber 33 Monaten bei Patienten ohne zielgerichtete Therapien.

Unsere NGS-Daten aus einer heterogenen Kohorte von 53 Sarkom-Patienten deuten darauf hin, dass personalisierte Therapien, die auf den Ergebnissen einer 720 Gen-Panel-Sequenzierung basieren, zu verbesserten klinischen Ergebnissen bei Sarkom-Patienten führen könnten.

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9. Declaration of own contribution to the dissertation thesis

The work was carried out in the Department of Internal Medicine VIII of the University Hospital Tübingen under the supervision of Prof. Dr. Lars Zender/Prof. Dr. Ulrich M. Lauer and Dr. Martina Hinterleitner. The study was designed by Dr. Martina Hinterleitner. The data collection and processing were conducted by me with the support of Dr. Martina Hinterleitner. The statistical analysis was carried out by me with the support of Prof. Dr. Lars Zender/Prof. Dr. Ulrich M. Lauer, Dr. Martina Hinterleitner and Dr. Clemens Hinterleitner. I wrote the manuscript independently following guidance from Dr. Martina Hinterleitner.

I hereby declare that I have written this dissertation entitled "Molecular-genetic characteristics of sarcomas for identifying prognostic risk groups and potential therapeutic options" using only the aids and sources indicated. I have marked as such all passages that have been taken verbatim or in spirit from published or unpublished writings.

Erklärung zur deutschen Zusammenfassung:
In dieser Arbeit wird aus Gründen der besseren Lesbarkeit lediglich die Sprachform des generischen Maskulinums verwendet. In diesen Fällen werden alle Geschlechteridentitäten ausdrücklich miteingeschlossen.

References

1. **Tobias, J.** *Cancer and its Management (Seventh ed.).* Chichester, West Sussex : John Wiley & Sons, 2015.

2. **National Cancer Institute.** [Online] 12.. May 2015. https://www.cancer.gov/types/metastatic-cancer.

3. **Harper, Douglas.** Online Etymology Dictionary. [Online] https://www.etymonline.com/word/sarcoma.

4. **Peltier, MD PhD Leonard F.** Historical Note on Bone. *Journal of Surgical Oncology.* 1985, Bd. 30, 201-205.

5. **Lahat G MD, Lazar A MD PhD, Lev D MD.** *Sarcoma Epidemiology und Etiology: Potential Environmental and Genetic Factors.* s.l. : Elsevier Saunders, 2008. S. 451-481.

6. **Stewart FW, Treves N.** *Lymphangiosarcoma in post mastectomy lymphedema: a report of six cases of elephantiasis chirurgica.* s.l. : Cancer, 1948. 30:562-72.

7. **Woodward AH, Ivins JC, Soule EH.** *Lymphanigiosarcoma arisng in chronic lyphedematous extremities.* s.l. : Cancer, 1972. 30:562-72.

8. **Brand KG, Buoen LC, Brand I.** *Multiphasic incidence of foreign body-induced sarcomas.* s.l. : Cancer Res, 1976. 36:3681-3.

9. **Mutlu AD, Cavalin LE, Brand I.** *In vivo-restricted and reversible malignancy induced by human herpesvirus-8 KSHV: a cell and animal model of virally induced Kaposi´s sarcom.* s.l. : Cancer Cell, 2007. 11:245-58.

10. **Cattani P, Capuano M, Graffeo R, et al.** *Kaposi´s sarcoma associated with previous human herpesvirus 8 infection in kidney transplant recipients.* s.l. : J Clin Microbiol, 2001. 33:1502-5.

11. **Penn, I.** *Sarcomas in organ allograft recepients.* s.l. : Transplantation, 1995. 60:1485-91.

12. **Deyrup AT, Lee VK, Hill CE, et al.** *Epstein-Barr virus-associated smooth muscle tumors are distincitive mesenchymal tumors reflecting multiple infection events: a clinicopathologic and molecular abalysis of 29 tumors from 19 patients.* s.l. : Am J Surg Pathol, 2006. 30:75-82.

13. **Nur S, Rosenblum WD, Katta UD, et al.** *Epstein-Barr virus-associated multifocal leiomyosarcomas arising in a cardiac transplant recipient: autopsy case report and review of the literature.* s.l. : J Heart Lung Transplant, 2007. 26(9): 944-52.

14. **Rubin BP, Heinrich MC, Corless CL.** *Gastrointestinal stromal tumour.* s.l. : Lancet, 2007. 369:1731-41.

15. **Heinrich MC, Corless CL, Demetri GD, et al.** *Kinase mutations and imatinib respose in patient with metastatic gastrointestinal stromal tumor.* s.l. : J Clin Oncol, 2003. 21:4342-9.

16. **Fraumeni, JF Jr Li FP.** *Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome?* s.l. : Ann Intern Med, 1969. 71:747-52.

17. **Vogelstein B, Lane D, Levine AJ.** *Surfing the p53network.* s.l. : Nature, 2000. 408:307-10.

18. **Lozano, G.** *The oncogenic roles of p53 mutants in mouse models.* s.l. : Curr Opin Genet Dev, 2007. 17:66-70.

19. **Singer S, Socci ND, Ambrosinni G, et al.** *Gene expression profiling ofliposarcoma identifies distinct biological types/subtypes and potential therpeutic targets in well-differentiated and dedifferentiated liposarcoma.* s.l. : Cancer Res, 2007. 67:6626-36.

20. **Shimada S, Ishizava T, Ishizava K, et al.** *The value of MDM2 and CDK4 amplification levels using real-time polymerase chain reaction for the differential diagnosis of liposarcomas and their histologic mimickers.* s.l. : Hum Pathol, 2006. 37:1123-9.

21. **Pollack IF, Mulvihill JJ.** *Nuerofibromatosis 1 and 2.* s.l. : Brain Pathol, 1997. 7:823-36.

22. **Guha, A.** *Ras activation in astrozytomas und neurofibromas.* s.l. : Can J Neurol Sci, 1998. 25:267-81.

23. **Wong FL, Boice JD, Abramson DH, et al.** *Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk.* s.l. : JAMA, 1997. 278:1262-7.

24. **Moll AC, Imhof SM, Bouter LM, et al.** *Second primary tumors in hereditary retinoblastoma: a register based follow-up study 1945-1994.* s.l. : Int J Cancer, 1996. 67:515-9.

25. **Draper GJ, Sanders BM, Kingston JE.** *Second primary neoplasms in patients with retinoblastoma.* s.l. : Br J Cancer, 1988. 53:661-71.

26. **Weinberg, RA.** *The retinoblastoma protein and cell cycle control.* s.l. : Cell, 1995. 81:323-30.

27. **Martin GM, Oshima J.** *Lessons from human progeroid syndromes.* s.l. : Nature, 2000. 408:263-6.

28. **Hickson, ID.** *RecQ helicases: caretakers of the genome.* s.l. : Nat Rev Cancer, 2003. 3:169-78.

29. **Goto M, Miller RW, Ishikawa Y, et al.** *Excess of rare cancers in Werner syndrome (adult progeria).* s.l. : Cancer Epidemiol Biomarkers Prev, 1996. 5:239-46.

30. **Faragher RG, Kill IRm Hunter JA, et al.** *The gene responsible for Werner syndrome may be a celldivision "counting" gene.* s.l. : Proc Natl Acad Sci USA, 1993. 90:12030-4.

31. **Salk D, Au K, Hoehn H, et al.** *Cytogenetics of Werner´s syndrome cultured skin fibroblasts: variegated translocation mosaicism.* s.l. : Cytogenet Cell Genet, 1981. 30:92-107.

32. **German J, Ellis NA.** Bloom syndrome. [Buchverf.] Columbus OH. *The genetic basis of human caner.* s.l. : McGraw-Hill Companies, 1997.

33. **Kansara M, Thomas DM.** *Molecular pathogenesis of osteosarcoma.* s.l. : DNA Cell Biol, 2007. 26:1-18.

34. **Nishijo K, Nakayaa T, Aoyama T, et al.** *Mutation analysis of the RECQL4 gene in sporadic osteosarcomas.* s.l. : Int J Cancer, 2004. 111(3):367-72.

35. **Wang LL, Gannavarapu A, Kozinetz CA, et al.** *Association between osteosarcoma and deleterious mutations in the RECQ$ gene in Rozhmund-Thompson syndrome.* s.l. : J Natl Cancer Inst, 2003. 95:669-74.

36. **Xia SJ, Barr FG.** *Chromosome translocations in sarcomas and the emergence of oncogenic transcription factors.* s.l. : Eur J Cancer, 2005. 41:2513-27.

37. **Scheidler S, Fredericks WJ, Rauscher FJ III, et al.** *The hybrid PAX3-FKHR fusion protein of alveolar rhabdomyosarcoma transforms fibroblasts in culture.* s.l. : Proc Natl Acad Sci U S A, 1996. 93:9805-9.

38. **May WA, Gishizky ML, Lessnick SL, et al.** *Ewing sarcom 11;22 translocation produces a chimeric transcription factor that requires the DNA binding domain encoded by FLI1 for transformation.* s.l. : Proc Natl Acad Sci U S A, 1993. 90:5752-6.

39. **Nagai M, Tanaka S, Tsuda M, et al.** *Analysisof the transforming activity of human synovial sarcoma-associated chimeric protein SYT-SSX1 bound to chromatin remodeling factor hBRM/hSNF2 alpha.* s.l. : Proc Natl Acad Sci U S A, 2001. 98:3843-8.

40. **Schwarzbach MH, Koesters Rm Germann A, et al.** *Comparable transforming capacities and differential gene expression patterns of variant FUS/CHOP fusion transcripts derived ffrom soft tissue liposarcomas.* s.l. : Oncogene, 2004. 23:6798-805.

41. **Aravand A, Welford SM, Teitell MA, et al.** *The COOH-terminal domain of FLI-1 is necessary for full tumourigenesis and transcriptional modulation by EWS/FLI-1.* s.l. : Cancer Res, 2001. 61:5311-7.

42. **Garraway LA, Sellers WR.** *Lineage dependency and lineage-survival oncogenes in human cancer.* s.l. : Nat Rev Cancer, 2006. 6:593-602.

43. **May WA, Gishizky ML, Lessnick sL, et al.** *Ewing sarcoma 11;22 translocation produces a chimeric transcription factor that requires the DNA-binding domain encoded by FLI1 for transformation.* s.l. : Proc Natl Acad Sci U S A, 1993. 98:3843-8.

44. **Davis S, Meltzer PS.** *Ewings´s sarcoma: general insights from a rare model.* s.l. : Cancer cell, 206. 9:331-2.

45. **Sharpless NE, Ferguson DO, o´Hagan RC, et al.** *Impaired nonhomologous end-joining provokes soft tissue sarcomas harboring chromosomal translocations, ampflications and deletions.* s.l. : Mol Cell, 2001. 8:1187-96.

46. **Henson JD, hannay JA, McCarthy SW, et al.** *A robust assay for alternative lengthening of telomeres in tumors shows significance of alternative lengthening of telomeres in sarcomas and satrcytomas.* s.l. : Clin Cancer Res, 2005. 11:217-25.

47. **Scheel C, Schaefer KL, Jauch A, et al.** *Alternative kenghtening aof telomeres is associated with chromosomal instability in osteosarcomas.* s.l. : Oncogene, 2001. 20:3835-44.

48. **Gisselsson D, Pettersson L, Höglund M, et al.** *Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity.* s.l. : Proc Natl Acad Sci U S A, 2000. 97:5357-62.

49. *Occupational poisoning in manufacture of luminous watch dials.* **Martland, HS.** 1929, JAMA. 92:446-73.

50. **Zahm SH, Fraumeni JF Jr.** *The epidemiology of soft tissue sarcoma.* s.l. : Semin Oncol, 199. 24:504-14.

51. **Brady MS, Gaynor JJ, Brennan MF.** *Radiation-associated sarcoma of bone and soft tissue.* s.l. : JAMA, 1929. 92:446-73.

52. **Cha C, Antonescu CR, Quan ML, et al.** *Long-term results with resection of radiation-induced soft tissue sarcomas.* s.l. : Ann Surg, 2004. 238;903-10.

53. **Davidson T, Westbury G, Harmer CL.** *Radiation-induced soft-tissue sarcoma.* s.l. : Br J surg, 1986. 73:308-9.

54. **Huvos AG, Woodard HQ, Cahan WG, et al.** *Postradiation osteogenic sarcoma of bone and soft tissues. A clinicopathologic study of 66 Patients.* s.l. : Cancer, 1985. 55:1244-55.

55. **Pierce SM, Recht A, Lingos TI, et al.** *Long-term radiation complications following conservative surgery (CS) and radiation therapy (RT) in Patients with early stage breast cancer.* s.l. : Int J Radiat Oncol Biol Phys, 1992. 23:915-23.

56. **Wiklund TA, Blomqvist CP, Raty J, et al.** *Postirradiation sarcoma. Analysis if a nation wide cancer registry material.* s.l. : Cancer, 1991. 68:524-31.

57. **Tucker MA, D´Angio GJ, Boice JD Jr, et al.** *Bone sarcomas linked to radiotherapiy and chemotherapy in children.* s.l. : N Engl J Med, 1987. 317:588-93.

58. **Pisters PW, O´Sullivan B, Maki RG, et al.** Soft tissue sarcomas. [Buchverf.] BC Decker. [Hrsg.] Bast RC, Hait WN Kufe DW. *Cancer medicine.* 7th edition. s.l. : Hamilton, 2006.

59. **Cahan WG, Woodward HG, Higinbotham ND, et al.** *Sarcoma arising in irradiated bone: report of eleven cases.* s.l. : Cancer, 1948. 1:3-29.

60. **Arlen M, Higinbotham NL, Huvos HG, et al.** *Radiation-induced sarcoma of bone.* s.l. : Cancer, 1971. 28:1087-99.

61. **Siegel RL, Miller KD, Jemal A.** *Cancer statistics, 2016.* s.l. : CA Cancer J Clin, 2016. 66:7-30.

62. **Surveillance, Epidemiology, and End Results (SEER) Program.** *Incidence - SEER 9 Regs Research Data, Nov 2010 Sub (1973–2008) .* s.l. : Surveillance, Epidemiology, and End Results (SEER) Program, 2011.

63. **Burningham Z, Hashibe M, Spector L, Schiffman JD.** The Epidemiology of Sarcoma. *Clin Sarcoma Res.* 2012, Bd. 14, 2.

64. **Mehren M, Randall RL, Benjamin RS, et al.** *Soft tissue sarcoma, version 2.* s.l. : NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw., 2016. 14:758-786.

65. **ESMO/European, Sarcoma Network Working Group.** *Bone sarcomas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up.* s.l. : Ann Oncol, 2014. 25:iii113-iii123.

66. **Florou V MD, Nascimento AG MD, Gulia A MBBS, and de Lima Lopes Jr G, MD.** Global Health Perspective in Sarcomas and Other Rare Cancers. *American Society of Clinical Oncology Educational Book 38.* s.l. : American Society of Clinical Oncology, 2018.

67. **Kanavos, P.** *The rising burden of cancer in the developing world.* s.l. : Ann Oncol., 2006. 17:viii15-viii23.

68. **Ferlay J, Soerjomataram I, Dikshit R, et al.** *Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012.* s.l. : Int J Cancer, 2015. 136:E359-E386.

69. **Onkopedia. *Onkopedia.* [Online] [Zitat vom: 26. 04 2022.] https://www.onkopedia.com/de/onkopedia/guidelines/weichgewebssarkome-maligne-weichgewebstumoren-des-erwachsenen/guideline/html/index.html.**

70. Abhinav Bansal, Surbhi Goyal, Ankur Goyal, Manisha Jana. WHO classification of soft tissue tumours 2020: An update and simplified. *European Journal of Radiology.* 2021, 143.

71. Kallen ME, Hornick JL. *The 2020 WHO Classification: What's New in Soft Tissue.* s.l. : Am. J. Surg. Patho, 2021. https://doi.org/10.1097/PAS.0000000000001552.

72. Group, The ESMO/European Sarcoma Network Working. Soft tissue and visceral sarcomas: ESMO Clinical. *Annals of Oncology 25.* 2014, iii102–iii112.

73. Domanski HA, Akerman M, Carlen B, et al. Core-needle biopsy performed by the cytopathologist: a technique to complement fine-needle aspiration of soft tissue and bone lesions. *Cancer.* 2005, 105:229-239.

74. Dupuy DE, Rosenberg AE, Punyaratabandhu T, et al. Accuracy of CT-guided needle biopsy of musculoskeletal neoplasms. *Am J Roentgenol.* 1998, 171:759-762.

75. Amanda R. Dancsok, Karama Asleh-Aburaya and Torsten O. Nielsen. *Advances in sarcoma diagnostics and treatment.* 2917.

76. Institute, National Cancer. *National Library of Medicine.* [Online] 2002. https://www.ncbi.nlm.nih.gov/books/NBK65923/table/CDR0000062934__749/.

77. Tanaka, Kazuhiro und Ozaki, Toshifumi. New TNM classification (AJCC eighth edition) of bone and soft tissue sarcomas: JCOG Bone and Soft Tissue Tumor Study Group. *JJCO Japanese Journal of Clinical Oncology.* 2019, 49(2) 103–107.

78. White LM, Wunder JS, Bell RS et al. *Histological assessment of peritumoral edema in soft tissue sarcoma.* s.l. : Int J Radiat Oncol Biol Phys, 2005. DOI:10.1016/j.ijrobp.2004.08.036.

79. Pasquali S, Gronchi A. *Neoadjuvant chemotherapy in soft tissue sarcomas: latest evidence and clinical implications.* s.l. : Ther Adv Med Oncol., 2017. 10.1177/1758834017705588.

80. Taeger G, Grabellus F, Podleska LE et al. *Isolierte Extremitätenperfusion zur lokalen Tumorkontrolle an den Gliedmaßen.* s.l. : Onkologe, 2009. DOI 10.1007/s00761-009-1601-8.

81. Jakob J, Tunn PU, Hayes AJ, Pilz LR, Nowak K, Hohenberger P. *Oncological outcome of primary non-metastatic soft tissue sarcoma treated by neoadjuvant isolated limb perfusion and tumor resection.* s.l. : J Surg Oncol, 2014. DOI:10.1002/jso.23591.

82. Jakob J, Hohenberger P. ***Role of isolated limb perfusion with recombinant human tumor necrosis factor α and melphalan in locally advanced extremity soft tissue sarcoma.*** **s.l. : Cancer, 2016. DOI:10.1002/cncr.29991.**

83. Demetri GD, Elias AD. Results of single-agent and combination chemotherapy for advanced soft tissue sarcomas. [Buchverf.] Benjamin RS Patel S. ***Hematology/Oncology Clinics of North America; Sarcomas, Part II, Vol. 9.*** **Philadelphia : W.B. Saunders Company, 1995.**

84. Sleijfer S, Ouali M, Van Glabbeke M, et al. ***Prognostic and predictive factors for outcome to first-line ifosfamide-containing therapy (IFM) in patients (pts) with advanced soft tissue sarcomas (STS) treated in EORTC-STBSG studies.*** **s.l. : Eur J Cancer, 2010. DOI:10.1016/j.ejca.2009.09.022.**

85. van Oosterom AT, Mouridsen HT, Nielsen OS, et al. ***EORTC Soft Tissue and Bone Sarcoma Group: Results of randomised studies of the EORTC Soft Tissue and Bone Sarcoma Group (STBSG) with two different ifosfamide regimens in first- and second-line chemotherap.*** **s.l. : Eur J Cancer, 2002. PMID:12460784.**

86. Seddon BM, Scurr MR, Jones RL et al. ***Phase II study of gemcitabine and docetaxel as first-line chemotherapy in patients with unresectable leiomyosarcoma.*** **s.l. : Clin Sarcoma Res, 2015. DOI:10.1186/s13569-015-0029-8.**

87. Benjamin R, Legha S, Patel S, Nicaise C. ***Single-agent ifosfamide studies in sarcomas of soft tissue and bone: the M.D. Anderson Experience.*** **s.l. : Cancer Chemother Pharmacol, 1993. PMID:8453693.**

88. Chawla S, Rosen G, Lowenbraun S. ***High dose ifosfamide (HDI) therapy in metastatic soft tissue sarcomas (STS).*** **s.l. : Proc Am Assoc Cancer Res, 1990.**

89. Nielsen OS, Judson I, van Hoesel Q, et al. ***Effect of high-dose ifosfamide in advanced soft tissue sarcomas. A multicentre phase II study of the***

EORTC Soft Tissue and Bone Sarcoma Group. **s.l. : Eur J Cancer, 2000. PMID:10741296.**

90. Dantonello TM, Int-Veen C, Harms D et al. *Cooperative Trial CWS-91 for localized soft tissue sarcoma in children, adolescents, and young Adults.* s.l. : J Clin Oncol, 2009. DOI:10.1200/JCO.2007.15.0466.

91. Le Cesne A, Blay JY, Domont J et al. *Interruption versus continuation of trabectedin in patients with soft-tissue sarcoma (T-DIS): a randomised phase 2 trial.* s.l. : Lancet Oncol, 2015. DOI:10.1016/S1470-2045(15)70031-8.

92. Demetri GD, von Mehren M, Jones RL et al. *Efficacy and safety of trabectedin or dacarbazine for metastatic liposarcoma or leiomyosarcoma after failure of conventional chemotherapy: results of a phase III randomized multicenter clinical trial.* s.l. : J Clin Oncol, 2016. DOI:10.1200/JCO.2015.62.4734.

93. Sanfilippo R, Dileo P, Blay JY et al. *Trabectedin in advanced synovial sarcomas: A multicentre, retrospective study from four European institutions and the Italian Rare Cancer Network.* s.l. : Anticancer Drugs, 2015. DOI:10.1097/CAD.0000000000000228.

94. Sleijfer S, Ray-Coquard I, Papai Z et al. *Pazopanib, a multikinase angiogenesis inhibitor, in patients with relapsed or refractory advanced soft tissue sarcoma: a phase II study from the European organisation for research and treatment of cancer-soft tissue and bone sarcoma group (EORTC study 620.* s.l. : J Clin Oncol, 2009. DOI:10.1200/JCO.2008.21.3223.

95. van der Graaf WT, Blay JY, Chawla SP et al. *Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial.* s.l. : Lancet, 2012. DOI:10.1016/S0140-6736(12)60651-5.

96. Demetri GD, Chawla SP, von Mehren M et al. *Efficacy and safety of trabectedin in patients with advanced or metastatic liposarcoma or leiomyosarcoma after failure of prior anthracyclines and ifosfamide: results*

of a randomized phase II study of two different schedules. **s.l. : J Clin Oncol, 2009. DOI:10.1200/JCO.2008.21.0088.**

97. Hartmann JT, Oechsle K, Huober J et al.: An open label, non-comparative phase II study of gemcitabine as salvage treatment for patients with pretreated adult type soft tissue sarcoma. s.l. : Invest New Drugs, 2006. DOI:10.1007/s10637-005-3537-1.

98. Patel SR, Gandhi V, Jenkins J et al. *Phase II clinical investigation of gemcitabine in advanced soft tissue sarcomas and window evaluation of dose rate on gemcitabine triphosphate accumulation.* s.l. : J Clin Oncol, 2001. PMID:11481354.

99. Maki RG, D'Adamo DR, Keohan ML et al. *Randomized phase II study of gemcitabine and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of sarcoma alliance for research through collaboration study 002 [corrected].* s.l. : J Clin Oncol, 2007. DOI:10.1200/JCO.2006.10.4117.

100. Garcia Del Muro X, Lopez-Pousa A, Maurel A et al. *Randomized phase II study comparing gemcitabine plus dacarbazine versus dacarbazine alone in patients with advanced soft tissue sarcoma: A Spanish Group for Research on Sarcomas (GEIS) study.* s.l. : J Clin Oncol, 2011. DOI:10.1200/JCO.2010.33.6107.

101. National Human Genome Research Institute. [Online] 06.. Septmeber 2022. [Zitat vom: 11.. November 2022.] https://www.genome.gov/genetics-glossary/DNA-Sequencing.

102. Behjati S, Tarpey PS. *What is next generation sequencing?* s.l. : Archives of Disease in Childhood: Education and Practice Edition, 2013. doi:10.1136/archdischild-2013-304340.

103. Chmielecki J, Meyerson M. DNA sequencing of cancer: what have we learned? *Annual Review of Medicine.* 2014, Bd. 65.

104. Abate AR, Hung T, Sperling RA, Mary P, Rotem A, Agresti JJ, et al. DNA sequence analysis with droplet-based microfluidics. *Lab on a Chip.* 2013, Bd. 13.

105. Pekin D, Skhiri Y, Baret JC, Le Corre D, Mazutis L, Salem CB, et al. Quantitative and sensitive detection of rare mutations using droplet-based microfluidics. *Lab on a Chip.* 2011, Bd. 11, 13.

106. Watson JD, Crick FH. *The structure of DNA.* 1953.

107. Marks, L. *The path to DNA sequencing: The life and work of Frederick Sanger.*

108. Wu, R. Nucleotide sequence analysis of DNA. *Nature New Biology.* 68, 1972, Bd. 236, 198-200.

109. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 12, 1977, Bd. 74, 5463–77.

110. Maxam AM, Gilbert W. A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA.* 2, 1977, Bd. 74, 560-64.

111. Anger F, Air GM, Barrell BG, Brown NL, Coulson AR, Fiddes CA, Hutchison CA, Slocombe PM, Smith M. Nucleotide sequence of bacteriophage phi X174 DNA. *Nature.* 1977, Bd. 265, 687-95.

112. Beck S, Pohl FM. DNA sequencing with direct blotting electrophoresis. *EMBO J.* 12, 1984, Bd. 3, 2905.09.

113. Prober JM, Trainor GL, Dam RJ, Hobbs FW, Robertson CW, Zagursky RJ, Cocuzza AJ, Jensen MA, Baumeister K. A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides. *Science.* 1987, Bd. 238, (4825): 336–41.

114. Staden, R. A strategy of DNA sequencing employing computer programs. *Nucleic Acids Research.* 1979, Bd. 6, (7): 2601–10.

115. Ronaghi M, Karamohamed S, Pettersson B, Uhlén M, Nyrén P. Real-time DNA sequencing using detection of pyrophosphate release. *Analytical Biochemistry.* 1996, Bd. 242, (1): 84–89.

116. Kawashima, Eric H., Farinelli, Laurent und Mayer, Pascal. *Patent: Method of nucleic acid amplification.* 12. May 2005.

117. Brenner S, Johnson M, Bridgham J, Golda G, Lloyd DH, Johnson D, Luo S, McCurdy S, Foy M, Ewan M, Roth R, George D, Eletr S, Albrecht G, Vermaas E, Williams SR, Moon K, Burcham T, Pallas M, DuBridge RB, Kirchner J, Fearon K, Mao J, Corcoran K. *Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays.* s.l. : Nature Biotechnology, 2000. (6): 630–34.

118. de Magalhães JP, Finch CE, Janssens G. Next-generation sequencing in aging research: emerging applications, problems, pitfalls and possible solutions. *Ageing Research Reviews.* 2010, Bd. 9, (3): 315–23.

119. Straiton J, Free T, Sawyer A, Martin J. From Sanger sequencing to genome databases and beyond. *BioTechniques.* 2019, Bd. 66, (2): 60–63.

120. Campbell PJ, Getz G, Korbel JO, et al. Pan-cancer analysis of whole genomes. *Nature.* 578:82–93, 2020.

121. Bailey MH, Tokheim C, Porta-Pardo E, et al. Comprehensive characterization. *Cell .* 2018, 173:371–385.e18.

122. Gerstung M, Jolly C, Leshchiner I, et al. The evolutionary history of 2,658. *Nature.* 2020, 578:122–128.

123. Colomer R, Mondejar R, Romero-Laorden N, et al. When should we order a next generation sequencing test in a patient with cancer? *EClinicalMedicine.* 2020, 25:100487.

124. Berger MF, Mardis ER. The emerging clinical relevance of genomics in cancer. *Nat Rev Clin Oncol.* 2018, 15:353–365.

125. Fancello L, Gandini S, Pelicci PG, Mazzarella L. Tumor mutational burden quantification from targeted gene panels: Major advancements and challenges. *J Immunother Cancer.* 2019, 7:183.

126. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in nonsmall cell lung cancer. *Science.* 2015, 348:124–128.

127. Carbone DP, Reck M, Paz-Ares L, et al. First-line nivolumab in Stage IV or recurrent non-small-cell lung cancer. *N Engl J Med .* 2017, 376:2415–2426.

128. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to. *N Engl J Med.* 2014, 371:2189–2199.

129. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm. *Lancet.* 2016, 387:1909–1920.

130. Yamamoto H, Imai K. *An updated review of microsatellite instability in the era.* 2019.

131. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science 2017.* 357:409–413.

132. Sztupinszki Z, Diossy M, Krzystanek M, et al. Migrating the SNP array-based homologous recombination deficiency measures to next generation sequencing data of breast cancer. *Breast Cancer.* 2018, 4:16.

133. Audeh MW, Carmichael J, Penson RT, et al. Oral poly(ADP-ribose) polymerase inhibitor Olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet.* 2010, 376:245–251.

134. Tutt A, Robson M, Garber JE, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and

advanced breast cancer: a proof-of-concept trial. *Lancet.* 2010, 376:235–244.

135. Du XH, Wei H, Zhang P, et al. *Heterogeneity of soft tissue sarcomas and its implications in targeted therapy.* 2020.

136. Jour G, Scarborough JD, Jones RL, et al. Molecular profiling of soft tissue sarcomas using next-generation sequencing: a pilot study toward precision therapeutics. *Hum Pathol.* 2014, 45:1563–1571.

137. Gounder MM, Ali SM, Robinson V, et al. Impact of next-generation sequencing (NGS) on diagnostic and therapeutic options in soft-tissue and bone sarcoma. *J Clin Oncol .* 2017, 35:11001–111001.

138. Carmagnani Pestana R, Groisberg R, Roszik J, Subbiah V. Precision oncology in sarcomas: divide and conquer. *JCO Precis Oncol.* 2019, 3:1–16.

139. Vyse S, Thway K, Huang PH, Jones RL. Next-generation sequencing for the management of sarcomas with no known driver mutations. *Curr Opin Oncol.* 2021, 1;33(4):315-322.

140. Tawbi HA, Burgess M, Bolejack V, et al. Pembrolizumab in advanced soft tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort single-arm, open-label, phase 2 trial. *Lancet Oncol.* 2017, 18:1493–1501.

141. Ben-Ami E, Barysauskas CM, Solomon S, et al. *Immunotherapy with single agent Nivolumab for advanced leiomyosarcoma of the uterus: Results of a phase 2 study.* 2017.

142. He M, Abro B, Kaushal M, et al. Tumor mutation burden and checkpoint immunotherapy markers in primary and metastatic synovial sarcoma. *Hum Pathol.* 2020, 100:15–23.

143. Abeshouse A, Adebamowo C, Adebamowo SN, et al. Comprehensive and integrated genomic characterization of adult soft tissue sarcomas. *Cell.* 2017, 171:950–965.e28.

144. Petitprez F, de Reynie` s A, Keung EZ, et al. B cells are associated with survival. *Nature.* 2020, 577:556–560.

145. Doyle LA, Nowak JA, Nathenson MJ, et al. Characteristics of mismatch repair deficiency in sarcomas. *Mod Pathol.* 2019, 32:977–987.

146. Campanella NC, Penna V, Ribeiro G, et al. *Absence of microsatellite instability in soft tissue sarcomas.* 2015.

147. Chudasama P, Mughal SS, Sanders MA, et al. Integrative genomic and transcriptomic analysis of leiomyosarcoma. *Nat Commun.* 2018, 9:1–15.

148. Kovac M, Blattmann C, Ribi S, et al. Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. *Nat Commun.* 2015, 6:8940.

149. Lord CJ, Ashworth A. BRCAness revisited. BRCAness revisited. *Nat Rev Cancer.* 2016, 16:110–120.

150. Ingham M, Allred JB, Gano K, et al. NCI protocol 10250: A phase II study of temozolomide and olaparib for the treatment of advanced uterine leiomyosarcoma. *J Clin Oncol.* 2020, 38:TS11570–TS111570.

151. Grignani G, D'Ambrosio L, Pignochino Y, et al. Trabectedin and olaparib in patients with advanced and nonresectable bone and soft-tissue sarcomas (TOMAS): an open-label, phase 1b study from the Italian Sarcoma Group. *Lancet Oncol.* 2018, 19:1360–1371.

152. Weymann D, Pataky R, Regier DA. Economic evaluations of nextgeneration precision oncology: a critical review. *JCO Precis Oncol.* 2018, 2:1–23.

153. Florou V, Rosenberg AE, Wieder E, et al. Angiosarcoma patients treated with immune checkpoint inhibitors: a case series of seven patients from a single institution. *J Immunother Cancer.* 2019, 7:213.

154. Farhana A, Lappin SL. *Biochemistry.* s.l. : StatPearls Publishing, January 2023. NBK557536.

155. Planas-Paz L, Pliego-Mendieta A, Hagedorn C, Aguilera-Garcia D, Haberecker M, Arnold F, Herzog M, Bankel L, Guggenberger R, Steiner S, Chen Y, Kahraman A, Zoche M, Rubin MA et al. *Unravelling homologous recombination repair deficiency and therapeutic opportunities in soft tissue and bone sarcoma.* s.l. : EMBO Mol Med, 2023. doi.org/10.15252/emmm.202216863.

156. Cranmer LD, Loggers ET, Pollack SM. *Pazopanib in the management of advanced soft tissue sarcomas.* s.l. : Ther Clin Risk Manag, 2016. doi: 10.2147/TCRM.S84792. PMID: 27354810; PMCID: PMC4907704.

157. Gómez J, Tsagozis P. *Multidisciplinary treatment of soft tissue sarcomas: An update.* s.l. : World J Clin Oncol, 2020. doi: 10.5306/wjco.v11.i4.180. PMID: 32355640; PMCID: PMC7186235.

158. Meyer M, Seetharam M. *First-Line Therapy for Metastatic Soft Tissue Sarcoma.* s.l. : Curr Treat Options Oncol, 2019. doi: 10.1007/s11864-019-0606-9. PMID: 30675651.

159. Painter CA, E, Tomson BN, Dunphy M, Stoddard RE, Thomas BS, Damon AL, Shah S, Kim D, Gómez Tejeda Zañudo J, Hornick JL, Chen Y, Merriam P, Raut CP, Deme GD. *The Angiosarcoma Project: enabling genomic and clinical discoveries in a rare cancer through patient-partnered research.* s.l. : Nat Med, 2020. https://doi.org/10.1038/s41591-019-0749-z.

160. Gounder MM, Agaram NP, Trabucco SE, Robinson V, Ferraro RA, Millis SZ, Krishnan A, Lee J, Attia S, Abida W, Drilon A, Chi P, Angelo SP, Dickson MA, Keohan ML, Kelly CM, Agulnik M, Chawla SP, Choy E, Chugh R, Meyer CF, Myer PA, Moore JL, Okimoto RA et al. *Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma.* s.l. : Nat Commun, 2022. 10.1038/s41467-022-30496-0. PMID: 35705558.

161. Painter CA, Jain E, Tomson BN, et al. The Angiosarcoma Project: enabling genomic and clinical discoveries in a rare cancer through patient-partnered research. *Nat Med.* 2020, 26:181–187.

162. Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, Altekruse SF, Kosary CL, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA, Edwards BK. *SEER Cancer Statistics Review, 1975–2008.* s.l. : National Cancer Institute, 2010.

163. Paul Riviere, Aaron M. Goodman, Ryosuke Okamura, Donald A. Barkauskas, Theresa J. Whitchurch, Suzanna Lee. *High Tumor Mutational Burden Correlates with Longer Survival in Immunotherapy-Naïve Patients with Diverse Cancers.* s.l. : Mol Cancer Ther, 2020. doi.org/10.1158/1535-7163.MCT-20-0161.

164. Aurélien Marabelle MD, Marwan Fakih MD, Juanita Lopez MB BChir, Manisha Shah, MD, Ronnie Shapira-Frommer MD, Kazuhiko Nakagawa MD et al. *Association of tumor mutational burden with outcomes in patients with advanced solid tumors treated with Pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study.* s.l. : Lancet, 2020. https://doi.org/10.1016/S1470-2045(20)30445-9.

165. Ben-Ami E, Barysauskas CM, Solomon S, et al. *Immunotherapy with single agent nivolumab for advanced leiomyosarcoma of the uterus: Results of a phase 2 study.* s.l. : Cancer, 2017. doi: 10.1002/cncr.30738.

166. Pokuri VK, Merzianu M, Gandhi S, Baqai J, Loree TR, Bhat S. *Interdigitating dendritic cell sarcoma.* s.l. : J Natl Compr Canc Netw., 2015. doi: 10.6004/jnccn.2015.0020. PMID: 25691604..

167. Yang C, Zhang Z, Tang X, Zhang X, Chen Y, Hu T, Zhang H, Guan M, Zhang X, Wu Z. *Pan-cancer analysis reveals homologous recombination deficiency score as a predictive marker for immunotherapy responders.* s.l. : Hum Cell, 2022. 34628623.

168. Niu B, Ye K, Zhang Q, et al. *MSIsensor: microsatellite instability detection using.* 2014.

169. Overman MJ, Lonardi S, Wong Kym, et al. *Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite*

instability-high metastatic colorectal cancer. **s.l. : J Clin Oncol, 2018. doi: 10.1200/JCO.2017.76.9901.**

170. Saito, T., Oda, Y., Kawaguchi, Ki. et al. *Possible Association Between Higher β-Catenin mRNA Expression and Mutated β-Catenin in Sporadic Desmoid Tumors: Real-Time Semiquantitative Assay by TaqMan Polymerase Chain Reaction.* s.l. : Lab Invest, 2002. doi.org/10.1038/labinvest.3780399.

171. Ericson K, Engellau J, Persson A, Lindblom A, Domanski H, Akerman M, Nilbert M. *Immunohistochemical Loss of the DNA Mismatch Repair Proteins MSH2 and MSH6 in Malignant Fibrous Histiocytomas.* s.l. : Sarcoma, 2004. doi: 10.1080/13577140400010856.

172. Wooster R, Cleton-Jansen AM, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BA, von Deimling A, Wiestler OD, et al. *Instability of short tandem repeats (microsatellites) in human cancers.* s.l. : Nat Genet, 1994. doi: 10.1038/ng0294-152.

173. Abkevich V, Timms KM, Hennessy BT, Potter J, Carey MS, Meyer LA, Smith-McCune K, Broaddus R, Lu KH, Chen J, Tran TV, Williams D, Iliev D, Jammulapati S, FitzGerald LM, Krivak T, DeLoia JA, Gutin A, Mills GB, Lanchbury JS. *Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer.* s.l. : Br J Cancer, 2012. doi: 10.1038/bjc.2012.451; PMID: 23047548.

174. Birkbak NJ, Wang ZC, Kim JY, Eklund AC, Li Q, Tian R, Bowman-Colin C, Li Y, Greene-Colozzi A, Iglehart JD, Tung N, Ryan PD, Garber JE, Silver DP, Szallasi Z, Richardson AL. *Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents.* s.l. : Cancer Discov, 2012. doi: 10.1158/2159-8290; PMID: 22576213.

175. Popova T, Manié E, Rieunier G, Caux-Moncoutier V, Tirapo C, Dubois T, Delattre O, Sigal-Zafrani B, Bollet M, Longy M, Houdayer C, Sastre-Garau X, Vincent-Salomon A, Stoppa-Lyonnet D, Stern MH. *Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with*

BRCA1/2 inactivation. **s.l. : Cancer Res, 2012. oi: 10.1158/0008-5472; PMID: 22933060..**

176. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ, Tutt AN, Zdzienicka MZ, Smith GC, Ashworth A. ***Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition.*** **s.l. : Cancer Res, 2006. doi: 10.1158/0008-5472.CAN-06-0140. PMID: 16912188..**

177. Chenlu Zhang, Xi Guo, Fei Xu, Yang You, Zhiming Wang, Rongyuan Zhuang, Wenshuai Liu, Li-Jie Ma, Hanxing Tong, Yong Zhang, Weiqi Lu, Jun Liu, Yuhong Zhou. ***Evaluation of homologous recombination deficiency (HRD) score and immuno-tumor microenvironment (iTME) as biomarkers of soft tissue sarcoma (STS) survival and response to immune checkpoint inhibitor therapy.*** **s.l. : Journal of Clinical Oncology, 2021. DOI: 10.1200/JCO.2021.39.15_suppl.11568.**

178. Nguyen, L., W. M. Martens, J., Van Hoeck, A. et al. ***Pan-cancer landscape of homologous recombination deficiency.*** **s.l. : Nat Commun, 2020. https://doi.org/10.1038/s41467-020-19406-4.**

179. Li H, Tu J, Zhao Z, Chen L, Qu Y, Li H, Yao H, Wang X, Lee DF, Shen J, Wen L, Huang G, Xie X. ***Molecular signatures of BRCAness analysis identifies PARP inhibitor Niraparib as a novel targeted therapeutic strategy for soft tissue Sarcomas.*** **s.l. : Theranostics, 2020. doi: 10.7150/thno.45763. PMID: 32863940; PMCID: PMC7449912.**

180. Seligson ND, Kautto EA, Passen EN, Stets C, Toland AE, Millis SZ, Meyer CF, Hays JL, Chen JL. ***BRCA1/2 Functional Loss Defines a Targetable Subset in Leiomyosarcoma.*** **s.l. : Oncologist, 2019. doi: 10.1634/theoncologist.2018-0448. Epub 2018 Dec 12. PMID: 30541756; PMCID: PMC6656468.**

181. Melinda L. Telli, Kirsten M. Timms, Julia Reid, Bryan Hennessy, Gordon B. Mills, Kristin C. Jensen et al. ***Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant***

Chemotherapy in Patients with Triple-Negative Breast Cancer. **s.l. : Clin Cancer Res, 2016. doi.org/10.1158/1078-0432.CCR-15-2477.**

182. Choi J, Manzano A, Dong W, Bellone S, Bonazzoli E, Zammataro L, Yao X, Deshpande A, Zaidi S, Guglielmi A, Gnutti B, Nagarkatti N, Tymon-Rosario JR, Harold J, Mauricio D, Zeybek B, Menderes G, Altwerger G, Jeong K, Zhao S, Buza N, Hui P, Ravaggi A et al. *Integrated mutational landscape analysis of uterine leiomyosarcomas.* s.l. : Proc Natl Acad Sci U S A, 2021. doi: 10.1073/pnas.2025182118. PMID: 33876771; PMCID: PMC8053980.

183. Heeke AL, Pishvaian MJ, Lynce F, Xiu J, Brody JR, Chen WJ, Baker TM, Marshall JL, Isaacs C. *Prevalence of Homologous Recombination-Related Gene Mutations Across Multiple Cancer Types.* s.l. : JCO Precis Oncol, 2018. doi: 10.1200/PO.17.00286. Epub 2018 Jul 23. PMID: 30234181; PMCID: PMC6139373.

184. Kim H, Ahn S, Kim H, Hong JY, Lee J, Park SH, Park JO, Park YS, Lim HY, Kang WK, Kim KM, Kim ST. *The prevalence of homologous recombination deficiency (HRD) in various solid tumors and the role of HRD as a single biomarker to immune checkpoint inhibitors.* s.l. : J Cancer Res Clin Oncol, 2022. doi: 10.1007/s00432-021-03781-6. Epub 2021 Sep 12. PMID: 34510272; PMCID: PMC9349061.

185. Teramura Y, Tanaka M, Yamazaki Y, et al. *Identification of Novel Fusion Genes in Bone and Soft Tissue Sarcoma and Their Implication in the Generation of a Mouse Model.* s.l. : Cancers, 2020. doi:10.3390/cancers12092345.

186. Mertens F, Antonescu CR, Mitelman F. *Gene fusions in soft tissue tumors: Recurrent and overlapping pathogenetic themes.* s.l. : Genes Chromosomes Cancer, 2016. doi:10.1002/gcc.22335.

187. Ben-David U, Amon A. *Context Is Everything: Aneuploidy in Cancer.* s.l. : Nat Rev Genet, 2020. doi: 10.1038/s41576-019-0171-x.

188. Taylor BS, Barretina J, Maki RG, et al. *Advances in sarcoma genomics and new therapeutic targets.* s.l. : Cancer, 2011. DOI: 10.1038/nrc3087. PMID: 21753790; PMCID: PMC3361898.

189. Harbers Luuk, Agostini Federico, Nicos Marcin, Poddighe Dimitri, Bienko Magda, Crosetto Nicola. *Somatic Copy Number Alterations in Human Cancers: An Analysis of Publicly Available Data From The Cancer Genome Atlas.* s.l. : Frontiers in Oncology, 2021. DOI=10.3389/fonc.2021.700568.

190. *Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas.* Network, Cancer Genome Atlas Research. 171(4):950-965.e28, s.l. : Cell, 2017. doi: 10.1016/j.cell.2017.10.014. PMID: 29100075; PMCID: PMC5693358..

191. Dancsok AR, Asleh-Aburaya K, Nielsen TO. *Advances in sarcoma diagnostics and treatment.* s.l. : Oncotarget, 2017. doi: 10.18632/oncotarget.12548. PMID: 27732970; PMCID: PMC5351692.

192. Chan SH, Lim WK, Ishak NDB, Li ST, Goh WL, Tan GS, Lim KH, Teo M, Young CNC, Malik S, Tan MH, Teh JYH, Chin FKC, Kesavan S, Selvarajan S, Tan P, Teh BT, Soo KC, Farid M, Quek R, Ngeow J. *Germline Mutations in Cancer Predisposition Genes are Frequent in Sporadic Sarcomas.* s.l. : Sci Rep, 2017. doi: 10.1038/s41598-017-10333-x. PMID: 28878254; PMCID: PMC5587568.

193. Ballinger ML, Goode DL, Ray-Coquard I, James PA, Mitchell G, Niedermayr E, Puri A, Schiffman JD, Dite GS, Cipponi A, Maki RG, Brohl AS, Myklebost O, Stratford EW, Lorenz S, Ahn SM, Ahn JH, Kim JE, Shanley S, Beshay V, Randall RL, Judson I, Seddon B, et al. *International Sarcoma Kindred Study. Monogenic and polygenic determinants of sarcoma risk: an international genetic study.* s.l. : Lancet Oncol, 2016. doi: 10.1016/S1470-2045(16)30147-4. Epub 2016 Aug 4. PMID: 27498913.

194. Martínez-Jiménez, F, Muiños, F, Sentís, I et al. *A compendium of mutational cancer driver genes.* s.l. : Nat Rev Cancer, 2020. https://doi.org/10.1038/s41568-020-0290-x.

195. *Comprehensive and integrated genomic characterization of adult soft tissue sarcomas.* Network, Cancer Genome Atlas Research. 171: 950–965, s.l. : Cell, 2017. doi: 10.1016/j.cell.2017.10.014. PMID: 29100075; PMCID: PMC5693358..

196. Groisberg R, Hong DS, Holla V, Janku F, Piha-Paul S, Ravi V, Benjamin R, Kumar Patel S, Somaiah N, Conley A, Ali SM, Schrock AB, Ross JS, Stephens PJ, Miller VA, Sen S, Herzog C, Meric-Bernstam F, Subbiah V. *Clinical genomic profiling to identify actionable alterations for investigational therapies in patients with diverse sarcomas.* s.l. : Oncotarget, 2017. doi: 10.18632/oncotarget.16845. PMID: 28424409; PMCID: PMC5503611.

197. Shern JF, Chen L, Chmielecki J, Wei JS, Patidar R, Rosenberg M, Ambrogio L, Auclair D, Wang J, Song YK, Tolman C, Hurd L, Liao H, Zhang S, Bogen D, Brohl AS, Sindiri S, Catchpoole D, Badgett T, Getz G, Mora J, Anderson JR, Skapek SX, Barr FG et al. *Comprehensive genomic analysis of rhabdomyosarcoma reveals a landscape of alterations affecting a common genetic axis in fusion-positive and fusion-negative tumors.* s.l. : Cancer Discov, 2014. doi: 10.1158/2159-8290.CD-13-0639. Epub 2014 Jan 23. PMID: 24436047; PMCID: PMC4462130.

198. Lim YH, Zaki TD, Levinsohn JL, Galan A, Choate KA, Hanlon AM. *Somatic Mutation Profile of Atypical Fibroxanthoma and Cutaneous Undifferentiated Pleomorphic Sarcoma.* s.l. : Dermatol Surg, 2021. doi: 10.1097/DSS.0000000000002342. PMID: 32079866.

199. Liu KX, Lamba N, Hwang WL, Niemierko A, DuBois SG, Haas-Kogan DA. *Risk stratification by somatic mutation burden in Ewing sarcoma.* s.l. : Cancer, 2019. doi: 10.1002/cncr.31919. Epub 2019 Jan 2. PMID: 30602061.

200. Rickel K, Fang F, Tao J. *Molecular genetics of osteosarcoma.* s.l. : Bone, 2017. doi: 10.1016/j.bone.2016.10.017. Epub 2016 Oct 17. PMID: 27760307; PMCID: PMC5393957.

201. Italiano A, Di Mauro I, Rapp J, Pierron G, Auger N, Alberti L, Chibon F, Escande F, Voegeli A-C, Ghnassia J-P et al. *Clinical effect of molecular methods in sarcoma diagnosis (GENSARC): a prospective, multicentre, observational study.* s.l. : Lancet Oncol, 2016. doi: 10.1016/S1470-2045(15)00583-5. Epub 2016 Mar 10. PMID: 26970672.

202. Lucchesi C, Khalifa E, Laizet Y, Soubeyran I, Mathoulin-Pelissier S, Chomienne C, Italiano A. *Targetable Alterations in Adult Patients With Soft-Tissue Sarcomas: Insights for Personalized Therapy.* s.l. : JAMA Oncol, 2018. doi: 10.1001/jamaoncol.2018.0723. PMID: 29801054; PMCID: PMC6233783.

203. Waxweiler RJ, Stringer W, Wagoner JK, et al. *Neoplastic risk among workers exposed to vinyl chloride.* s.l. : Ann N Y Acad Sci, 1976. 271:40-8.

204. Barshir R, Fishilevich F, Iny-Stein T, Zelig O, Mazor Y, Guan-Golan Y, Safran M, Lancet D. www.genecards.org. *GeneCards – the human gene database.* [Online] [Zitat vom: 20. 07 2022.] www.genecards.org.

205. Grünewald TG, Alonso M, Avnet S, Banito A, Burdach S, Cidre-Aranaz F, Di Pompo G, Distel M, Dorado-Garcia H, Garcia-Castro J, González-González L, Grigoriadis AE, Kasan M, Koelsche C, Krumbholz M, Lecanda F, Lemma S, Longo DL, Madrigal-Esquivel C et al. *Sarcoma treatment in the era of molecular medicine.* s.l. : EMBO Mol Med, 2020. doi: 10.15252/emmm.201911131. Epub 2020 Oct 13. PMID: 33047515; PMCID: PMC7645378.

206. Marabelle A, Fakih M, Lopez J, et al. *Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label,phase 2 KEYNOTE-158 study.* 2020.