Diploma thesis

Genetic variants in the RAD51, XRCC2 and ERCC2 genes as predictors of outcome in soft tissue sarcoma patients

submitted by

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Affidavit

I, hereby, declare that the following diploma thesis has been written only by the undersigned and without any assistance from third parties. Furthermore, I confirm that no sources have been used in preparation other than those indicated in the thesis.

Graz, 19th of April 2012 Signature

Note of thanks

First of all I would like to take the opportunity to thank my parents Ulli and Gerhard, they gave me the greatest gift that a human being ever may get from another, they gave me life! All the confidence they have put in me over the years has helped me to evolve and grow.

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Abstract

Background: Soft tissue sarcomas (STS) represent a rare and heterogeneous group of malignant tumours with mesenchymal cell origin and an incidence rate of 3/100.000/year.

Despite uniform treatment of the same STS-subtypes we observed some patients to show earlier recurrence and tumour progression. A growing number of studies has been published searching for the link between genetic variabilities like single nucleotide polymorphisms (SNPs) and the varying tumour predisposition. SNPs in DNA-repair genes may alter their function which would be devastating for the integrity of the genome. We investigated the following genes from two of the major DNA-repair pathways, homologous recombination (HR) and nucleotide excision repair (NER): Rad51 rs1801320 G>C, XRCC2 rs3218536 G>A and ERCC2 rs13181 A>C.

Patients and methods: For this study 224 patients with histopathologically confirmed soft tissue sarcomas have been recruited at the Department of Orthopaedic Surgery, Medical University of Graz from 1999 till 2010.

The relationship between the genotype frequency of the SNPs in patients and clinical factors was assessed by $\times^2$ and Fisher's exact probability tests. DFS and OS curves were generated by the Kaplan-Meier method and verified by the log-rank test. Cox's proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values.

Results: In the univariate analysis, the minor allele of ERCC2 rs13181 A>C was significantly associated with increased OS (HR 0.509; 95%CI; 0.289-0.897; p=0.019; Figure 7). Patients carrying at least one C allele in ERCC2 rs13181 A>C showed a median OS of 102 months. In contrast, patients with homozygous A/A had a median OS of 63 months. In multivariate analysis, the ERCC2 rs13181 A>C polymorphism remained significantly associated with increased OS (HR 0.449; 95%CI; 0.241-0.836; p=0.012).

Conclusion: Our results indicate an association between ERCC2 rs13181 A>C of the NER-pathway and an increased overall survival for patients with soft tissue sarcoma. It may be due to increased performance of the affected gene caused by structural alteration from a DNA-repair pathway polymorphism.

Zusammenfassung

Hintergrund: Weichteilsarkome stellen eine heterogene Gruppe von malignen Tumoren mit mesenchymalem Ursprung dar. Mit einer Inzidenzrate von ca. 3/100.000/Jahr sind sie mit einer ähnlichen Häufigkeit zu finden wie Gallenblasen- oder Schilddrüsenkarzinome. Heute geht man davon aus, dass Metastasierung und Rezidivierung eng mit spezifischen DNA Polymorphismen zusammenhängen. Einzelnukleotid-Polymorphismen (SNPs) können durch subtile strukturelle Änderungen in DNA-Reperaturenzymen zur Modulation der Krebsprogression führen. Wir untersuchten in diesem Projekt folgende in DNA Reperaturenzymen vorkommenden Genvarianten auf klinische Relevanz: Rad51 rs1801320 G>C, XRCC2 rs3218536 G>A und ERCC2 rs13181 A>C.

PatientInnen und Methoden: Über einen Zeitraum von 11 Jahren (Jänner 1999-Jänner 2010) haben wir Gewebeproben von 224 PatientInnen, welche mit histologisch verifizierten Weichteilsarkomen an der Universitätsklinik für Orthopädie und orthopädische Chirurgie der Medizinischen Universität Graz vorstellig wurden, gesammelt. Die einzelnen Polymorphismen wurden in Bezug auf das Gesamtüberleben (OS) und krankheitsfreiem Intervall (DFS) mit Hilfe der univariaten und multivariaten Cox-Regressions-Analyse hin untersucht.

Ergebnisse: In der univariaten Analyse fanden wir heraus, dass bei ERCC2 rs13181 A>C ein Allel statistisch signifikant mit einem eröhten durchschnittlichen Gesamtüberleben (OS) in Verbindung steht (HR 0.509; 95%CI; 0.289-0.897; p=0.019; Figure 7). Patienten mit zumindest einem C Allel in ERCC2 rs13181 A>C zeigten ein durchschnittliches Gesamtüberleben von 102 Monaten. Im Gegensatz dazu lebten Patienten mit homozygoten A/A durchschnittlich nur 63 Monate. In der multivariaten Cox-Regressions-Analyse des selben Polymorphismus blieb dieser Effekt ebenso erhalten (HR 0.449; 95%CI; 0.241-0.836; p=0.012).

Conclusio: Die Studie konnte eine Relevanz des ERCC2 rs13181 A>C Polymorphismus bestätigen. Die Verlängerung des durchschnittlichen Gesamtüberlebens der Patienten mit Weichteilsarkomen mit dem betroffenen Allel mag in Zusammenhang mit einer gesteigerten Aktivität des besagten DNA Reperatur Gens stehen.

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Abbreviations

AJCC	American Joint Committee on Cancer
CRX	Chest X-ray
DFS	Disease-free survival
EBV	Epstein-Barr virus
EORTC	European Organisation for Research and Treatment of Cancer
FDG	Fluorodeoxyglucose
FNCLCC	Féderation Nationale des Centres de Lutte contre le Cancer
HPF	High power field
HR	Homologous recombination
IHC	Immunohistochemical staining
ILP	Isolated limb perfusion
MFH	Myxofibrosarcoma
MMR	Mis-match repair
MPNST	Malignant peripheral nerve sheath tumor
MSKCC	Memorial Sloan-Kettering Center
NCCN	National Comprehensive Cancer Network
NER	Nucleotide excision repair
NHEJ	Non-homologous end joining
OS	Overall survival
PEComa	Perivascular epithelioid cell tumour
PNET	Primitive neuroectodermal tumour
SNOMED	Systematized Nomenclature of Human and Veterinary Medicine
SNP	Single nucleotide polymorphism
STS	Soft tissue sarcoma
TKI	Tyrosine-kinase inhibitor
TNF	Tumour necrosis factor
TSG	Tumour suppressor gene
UICC	Union for International Cancer Control
WHO	World Health Organisation
XRCC	X-ray repair cross-complementing

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Introduction

Soft tissue sarcomas (STS) represent a rare and heterogeneous group of neoplasms of mesenchymal cell origin.

The World Health Organisation (WHO) has defined more than 50 histological subtypes with individual clinical, prognostic and therapeutic features.

Despite several improvements in early diagnosis, surgical techniques and radio- and chemotherapy, this disease remains a threat to life for a large number of people. The high mortality is related to complications of distant metastasis and tumour dissemination. The choice of treatment of STS is based on independent clinical prognostic factors such as tumour size, depth, histological grade, completeness of resection and presentation status (primary tumour vs. local recurrence).

Unfortunately, to date too little is known about the mechanisms underlying tumour progression. There is increasing evidence that it might be influenced by gene variants involved in DNA repair. Single nucleotide polymorphisms (SNPs) may cause subtle structural alterations in repair enzymes and thereby modulation of cancer progression.

We know more than 130 genes that are involved in the five major DNA-repair pathways. One of them, homologous recombinational repair (HR), plays an important role in the repair pathway of double-stranded breaks (DSBs) which are considered to be the most biological damaging lesion known to us.

RAD51 and X-ray repair cross-complementing (XRCC) group 2 are important genes that participate in HR and are required for correct chromosome segregation and the apoptotic response to DSBs.

SNPs found in these genes may lead to altered DNA repair activity. This may be related to genetic predisposition to several cancer types[i],[ii],[iii] and STS might be one of them. On the other hand, altered DNA-repair activity could lead to better response rates of chemotherapy or could substantially slow down tumour growth. But either way, the role of SNPs in DNA-repair genes for prognosis of STS is unknown.

In this project we investigated SNPs in genes of the HR DNA-repair pathway and outcome in soft tissue sarcoma patients. Despite uniform treatment of the same

STS-subtypes, some patients are observed to show earlier recurrence and different disease progression. Knowledge about the clinical effects of SNPs in DNA repair-pathways is essential because it would lead to a better understanding of STS progression. The existence of susceptible gene variants for disease progression provides a strategy for stratifying patients for risk of metastatic soft-tissue sarcoma, which in turn would allow treatment options to be tailored to the individual. This may result in a decreased incidence of tumour relapse by placing patients on adjuvant therapy that would not have been initiated in current circumstances. Conversely, it may ultimately be possible to avoid treating patients at low risk, thus eliminating the morbidity associated with adjuvant therapies.

1 Soft tissue sarcoma

1.1 Definition

Sarcomas (from greek *σαρχ* meaning “flesh”) are the less common form of malignant soft tissue tumours.

These are divided into two broad categories:

- Sarcomas of the bone
- Sarcomas of the soft tissue (STS)

Because of their mesenchymal cell origin they have the capacity to develop from fat, muscle, cartilage, blood vessels and connective tissue. Although two-third of the human body mass represents soft tissue, sarcomas are a very rare form of cancer.

Approximately 80% of sarcomas originate from soft tissue whereas only 20% originate from bone tissue. Malignant tumours affecting the peripheral nerves are also included because of their similar clinical management and outcome.

As a result, doctors in every field of medicine can be the first ones to be consulted with a suspect lesion. Therefore a correct classification is absolutely necessary.

1.2 Epidemiology

1.2.1 Incidence

Because only one out of 100 soft tissue tumours is malignant, we have to deal with an incidence of 3/100.000 per year in Europe[iv]. For Austria, an age adjusted incidence rate of 2.4/100.000 per year has been reported[v]. This correlates with the appearance of thyroid carcinoma or gallbladder cancer.

1.2.2 Distribution

There are neither significant changes of incidence nor are there geographic differences described in the literature[vi].

However, there is a connection between the type of tumour, symptoms, location and the patient's age. For instance, lipomas are painless, rare in hand, lower leg and foot and very uncommon in children[vii].

Angiolipomas can be associated with pain and are common in young men. Angioleiomyomas are mostly painful and common in lower leg of middle aged women.

Most vascular tumours occur in patients below the age of 20 years[viii].

Whereas only one percent of malignant tumours in adults are soft tissue sarcomas, they are responsible for 15% of the malignant tumours in children.

Benign soft tissue tumours grow in 99% superficially and are in 95% less than 5 cm in diameter[ix]. But soft tissue sarcomas are found superficially in less than a third and are diagnosed on average around 5 cm in diameter[x].

Approximately 45% of sarcomas occur in the lower extremities, 15% in the upper extremities, 10% in the head-neck region, 15% in the retroperitoneum and the remaining 15% in the abdominal and chest wall[xi].

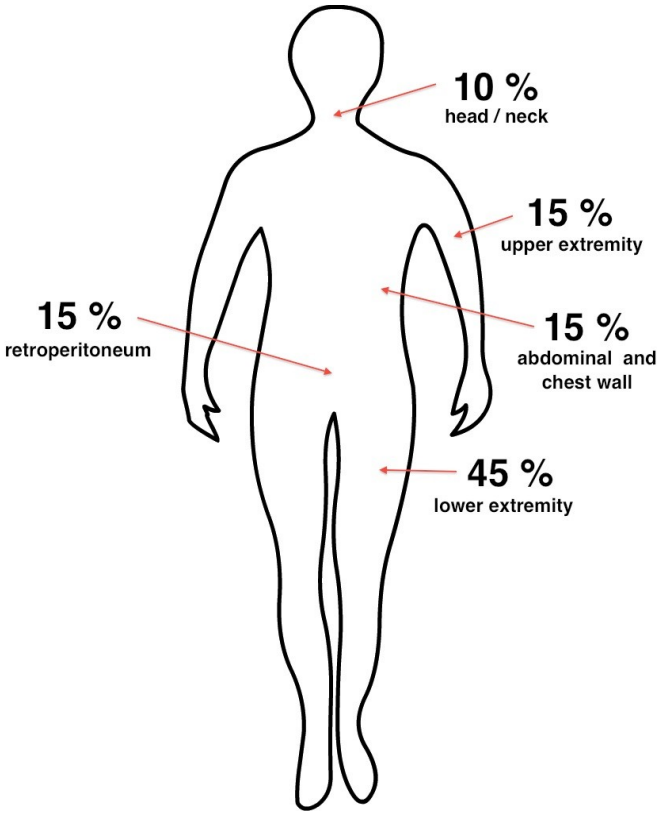

Figure 1: Distribution of STS

1.3 Etiology

There is no specific etiology known for the heterogeneous group of soft tissue sarcomas, but several correlations between exogenous noxae and specific subtypes can be found. Today, soft tissue sarcomas are thought to arise de-novo and not from a preexisting benign lesion as is the case with colon cancer.

Epstein-Barr virus (EBV) is reported to increase the likelihood of soft tissue tumours in immunocompromised patients.

Dupin et al.[xii] reported, that an infectious agent closely related to gamma-herpesvirus is implicated in the pathogenesis of Mediterranean and acquired immunodeficiency syndrome (AIDS)-associated Kaposi's sarcoma.

Similar correlations can be found between the human papilloma virus (HPV) and the occurrence of cervical cancer in women.

Radiation over a period of up to five years can cause so-called fibrohistiocytic tumours, osteosarcomas, fibrosarcomas and also some other malignant tumours as was shown by Brady et al.[xiii] In those cases radiation-induced genetic mutations were found which lead to neoplastic transformations.

Exposure to environmental carcinogens like arsenic, thorium dioxide and vinyl chloride can lead to hepatic angiosarcoma. Soft tissue sarcomas are also slightly more common with people who work with herbicides and chlorophenols.

If a patient suffers from a chronic lymphedema after an operation of breast cancer, there is a higher risk of developing angiosarcoma in the affected arm.

There is no consensus whether trauma can lead to soft tissue tumours or not. The reason for more frequently diagnosing STS with patients who present with trauma could be due to a higher professional awareness of doctors in those circumstances.

In the last ten years scientific progress has led to a better understanding of congenital syndromes associated with soft tissue sarcomas. Because of this high importance they are therefore reviewed below.

1.4 Histology

The embryologic affiliation of most sarcomas is the mesoderm, the middle embryonic germ layer, which lies between the ectoderm and the endoderm. Malignant lymphomas and leukemias also originate from the mesoderm.

Only the neurogenetic sarcoma and the Ewing-sarcoma develop from the ectoderm.

After folding of the several germ layers and the development of the embryo several types of tumours may grow:

- adipocytic tumours
- fibroblastic/myofibroblastic tumours
- so-called fibrohistiocytic tumours
- smoothe muscle tumours
- pericytic (perivascular) tumours
- skeletal muscle tumours

– vascular tumours
– chondro-osseous tumours
– tumours of uncertain differentiation

Dealing with this kind of tumours, it is also important to see the differences in their biological behaviour in a specific tissue. Some may grow locally invasive and are more likely to metastasise, others are not infiltrating at all and are very unlikely to metastasise.

Based on this knowledge we can categorise STS as followed:

- benign
- intermediate
- malignant

Immunohistochemical staining (IHC) can help to identify the presumptive tissue of origin[xiv]. Some characteristic markers are listed below:

– Desmin can be of help in differentiating between rhabdomyosarcoma and leiomyosarcoma
– S100 antigene and neurofilaments indicate tumour arising from neural sheath
– Cytokeratin can help differentiate between synovial sarcoma or epitheloid sarcoma
– Factor VIII is found in tumours of endothelial origin

1.5 Classification

In this project we used a three tier classification that is commonly used by international pathologists[xv,xvi]. It divides STS in sarcomas with specific translocations, amplifications and with complex genetic alterations.

– specific translocations
– amplifications (e.g. dedifferentiated /differentiated liposarcoma)
– complex genetics

Table 1: Classification of STS

Specific translocations	Amplifications	Complex genetics
Synovial sarcoma	Atypical lipomatous tumour	Leiomyosarcoma
Myxoid liposarcoma	Dedifferentiated liposarcoma	Myxofibrosarcoma
Alveolar rhabdomyosarcoma		Undifferentiated pleomorphic sarcoma with prominent inflammation / Inflammatory MFH
Primitive neuroectodermal tumour		Undifferentiated pleomorphic sarcoma / Pleomorphic MFH
Alveolar soft tisue sarcoma		Angiosarcoma
		Malignant peripheral nerve sheath tumour / MPNST
		Mesenchymal chondrosarcoma
		Fibrosarcoma
		Dedifferentiated rhabdomyosarcoma

The most common translocations associated with sarcomas are listed below:

Table 2: Relevant cytogenetic diagnosis[xvii]

Histology	Chromosomal aberration	Frequency [%]
Synovial sarcoma	t(X; 18)(p11;q11)	90
Clear cell sarcoma	t(12; 22)(q13; q12)	>75
Myxoid liposarcoma	t(12; 16)(q13; p11)	77
Alveolar rhabdomyosarcoma	t(2; 13)(q35; q14)	68
Ewing-sarcoma, Primitive neuroectodermal tumour (PNET), Askin tumour	t11; 22)(q24; q12)	86
Desmoplastic small round	t(11; 22)(p13; q12)	60

cell tumour		
Extraskeletal myxoid chondrosarcoma	t(9; 22)(q31; q12)	50

The Systematize Nomenclature of Medicine (SNOMED) is listed below:

Table 3: WHO classification of soft tissue tumours[xviii]

Adipocytic Tumours		
Benign	Intermediate (locally aggressive)	Malignant
Lipoma	Atypical lipomatous tumour / Well differentiated liposarcoma	Dedifferentiated liposarcoma
Lipomatosis		Myxoid liposarcoma
Lipomatosis of the nerve		Round cell liposarcoma
Lipoblastoma / Lipoblastomatosis		Pleomorphic liposarcoma
Angiolipoma		Mixed-type liposarcoma
Myolipoma		Liposarcoma, not otherwise specified
Chondroid lipoma		
Extra-adrenal myelolipoma		
Spindle cell / Pleomorphic lipoma		
Hibernoma		

Fibroblastic / Myofibroblastic Tumours			
Benign	Intermediate (locally aggressive)	Intermediate (rarely metastasising)	Malignant
Nodular fasciitis	Superficial fibromatoses (palmar/plantar)	Solitary fibrous tumour and haemangiopericytoma (incl. lipomatous haemangiopericytoma)	Adult fibrosarcoma
Proliferative	Desmoid-type	Inflammatory	Myxofibrosarcoma

fasciitis	fibromatoses	myofibroblastic sarcoma	
Proliferative myositis	Lipofibromatosis	Low grade myofibroblastic sarcoma	Low grade fibromyxoid sarcoma hyalinizing spindle cell tumour
Myositis ossificans / fibro-osseous pseudotumour of digits		Myxoinflammatory fibroblastic sarcoma	Sclerosing epithelioid fibrosarcoma
Ischaemic fasciitis		Infantile fibrosarcoma	
Elastofibroma			
Fibrous hamartoma of infancy			
Myofibroma / Myofibromatosis			
Fibromatosis colli			
Juvenile hyaline fibromatosis			
Inclusion body fibromatosis			
Fibroma of tendon sheath			
Desmoplastic fibroblastoma			
Mammary-type myofibroblastoma			
Calcifying aponeurotic fibroma			
Angiomyofibroblastoma			
Cellular angiofibroma			
Nuchal-type fibroma			
Gardner fibroma			
Calcifying fibrous tumour			
Giant cell angiofibroma			

So-called Fibrohistiocytic Tumours		
Benign	Intermediate (rarely metastasising)	Malignant
Giant cell tumour of tendon sheath	Plexiform fibrohistiocytic tumour	Pleomorphic 'MFH' / Undifferentiated pleomorphic sarcoma
Diffuse-type giant cell tumour	Giant cell tumour of soft tissue	Giant cell 'MFH' / Undifferentiated pleomorphic sarcoma with giant cells
Deep benign fibrous histiocytoma		Inflammatory 'MFH' / Undifferentiated

pleomorphic sarcoma with prominent inflammation

Skeletal Muscle Tumours	
Benign	Malignant
Rhabdomyoma	Embryonal rhabdomyosarcoma (incl. spindle cell, botryoid, anaplastic)
adult type	Alveolar rhabdomyosarcoma (incl. solid, anaplastic)
fetal type	Pleomorphic rhabdomyosarcoma
genital type	

Vascular Tumours			
Benign	Intermediate (locally aggressive)	Intermediate (rarely metastasising)	Malignant
Haemangioma of - subcutaneous / deep soft tissue - capillary - cavernous - arteriovenous - venous -intramuscular -synovial	Kaposiform haemangioendothelioma	Retiform haemangioendothelioma	Epithelioid haemangioendothelioma
		Papillary intralymphatic angioendothelioma	Angiosarcoma of soft tissue
		Composite haemangioendothelioma	
		Kaposi sarcoma	
Epithelioid haemangioma			
Angiomatosis			
Lymphangioma			

Smooth Muscle Tumours	Pericytic Tumours	Chondro-osseous Tumours
Angioleiomyoma	Glomus tumour (and variants)	Soft tissue chondroma
Deep leiomyoma	malignant Glomus tumour	Mesenchymal chondrosarkoma
Genital leiomyoma	Myopericytoma	Extraskeletal osteosarcoma
Leiomyosarcoma (excl. skin)		

Tumour of uncertain differentiation		
Benign	Intermediate (rarely metastasising)	Malignant
Intramuscular myxoma (incl. cellular variants)	Angiomatoid fibrous histiocytoma	Synovial sarcoma
Juxta-articular myxoma	Ossifying fibromyxoid tumour (incl. atypical / malignant)	Epithelioid sarcoma
Deep ('aggressive') angiomyxoma	Mixed tumour	Alveolar soft part sarcoma
Pleomorphic hyalinizing angiectatic tumour	Myoepithelioma	Clear cell sarcoma of soft tissue
Ectopic harmatomatous thymoma	Parachordoma	Extraskeletal myxoid chondrosarcoma ('chordoid' type)
		PNET / Extraskeletal Ewing tumour pPNET
		Extraskeletal Ewing tmour
		Desmoplastic small round cell tumour
		Extra-renal rhabdoid tumour
		Malignant mesenchymoma
		Neoplasms with perivascular epithelioid cell differentiation (PEComa) clear cell myomelanocytic tumour
		Intimal sarcoma

1.6 Grading and staging

To date there are several grading systems in use, usually only based on histological parameters. Those take only the degree of malignancy and the risk to metastasise in consideration and not one of them is accepted worldwide. The existence of more than 50 histologic subtypes with variable grades makes it difficult to develop a functional classification system.

The French Federation of Cancer Centres Sarcoma Group (FNCLCC) came up with a three-tier system whereas the International Union Against Cancer (UICC) prefers a four-tier system.

Table 4: FNCLCC and UICC grading system

	FNCLCC ('Trojani')	UICC
low-grade	Grade 1	Grade 1
		Grade 2
high-grade	Grade 2	Grade 3
	Grade 3	Grade 4

Coindre et al.[xix] showed impressively how difficult it is to come up with an appropriate grading system with a high reproducibility. They tested their own classification with 15 pathologists which were not involved in developing this system. The crude proportion in agreement was 75% concerning tumour grade but only 61% for the histological type.

To reach a high precision in grading some rules must be observed:

- grading does not differentiate between benign and malignant lesions, therefore a correct histologic diagnosis is elementary
- grading is only used for untreated primary soft tissue sarcomas
- grading needs representative and well processed material
- grading is not suitable for all forms of STS e.g. Malignant peripheral nerve sheath tumour (MPNST), Angiosarcoma, Alveolar soft part sarcoma, Extraskeletal myxoid chondrosarcoma, Clear cell sarcoma and Epithelioid sarcoma[xx]

The grading system mainly used in Europe is called the French system or 'Trojani-Score'. It combines tumour differentiation, tumour necrosis and mitotic index (ratio between number of cells in mitosis and total number of cells).
Each parameter gains points from 0 – 3. Trojani scores the samples and converts it into Grades 1 – 3 with 1 representing a less aggressive tumour.

Parameter	Score
Tumour differentiation	
a) similar to original tissue	1
b) cell type clearly assignable	2
c) cell type not assignable	3
Tumour necrosis	
a) no necrosis	0
b) < 50%	1
c) > 50%	2
Mitotic index	
a) 0-9 / 10 HPF	1
b) 10-19 / 10 HPF	2
c) 20+ / 10 HPF	3

	Score
Grade 1	2,3
Grade 2	4,5
Grade 3	6,7,8

Figure 2: Trojani Score (FNCLCC)

Staging on the other hand is combining clinical and histological parameters and has therefore been developed to increase the prognostic value in terms of overall survival and distant metastases.
A frequently used staging system for STS is the usual tumour, node, metastases (TNM) classification scheme developed by the International Union Against Cancer (UICC) and the American Joint Committee on Cancer (AJCC).

Here, special interest lies in tumour size and depth (T), lymph node involvement (N) and the presence or absence of distant metastases (M). This is complemented with the three-tier grading system developed by Coindre et al. together with the French Federation of Cancer Centers Sarcoma Group (FNCLCC). It is today the most commonly used grading system in Europe.

–grade 1 (well differentiated, low-grade)

–grade 2 (moderately differentiated)

–grade 3 (poorly differentiated, high-grade)

In grading the two most important parameters seem to be the mitotic index and the extent of tumour necrosis. Other important factors are cellularity, pleomorphism and histological type.

Table 5: TNM staging for soft tissue sarcoma[xxi]

Primary tumour (T)	
TX	Primary tumour can not be assessed
T0	No evidence of primary tumour
T1	Tumour 5 cm or less in greatest dimension
T1a	Superficial tumour
T1b	Deep tumour
T2	Tumour more than 5 cm in greatest dimension
T2a	Superficial tumour
T2b	Deep tumour
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1 *	Regional lymph node metastasis
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis
Histologic grade (G)	
Gx	Grade can not be assessed
G1	Grade 1
G2	Grade 2
G3	Grade 3

Anatomic stage / prognostic groups				
Stage I A	T1a	N0	M0	G1, GX
	T1b	N0	M0	G1, GX
Stage I B	T2a	N0	M0	G1, GX
	T2b	N0	M0	G1, GX
Stage II A	T1a	N0	M0	G2, G3
	T1b	N0	M0	G2, G3
Stage II B	T2a	N0	M0	G2
	T2b	N0	M0	G2
Stage III	T2a, T2b	N0	M0	G3
	Any T	N1	M0	Any G
Stage IV	Any T	Any N	M1	Any G

Based on this TNM stage groupings from the AJCC Cancer staging manual 7th edition from 2010 the five-year rates for disease-free survival (DFS) are:

Table 6: Five-year rates for disease-free survival

stage I	86%
stage II	72%
stage III	52%

In our project, surgical margins were classified according to Enneking et al.[xxii] as radical, wide, marginal or intralesional.

Table 7: Surgical Staging System for Musculoskeletal Tumors by Enneking

Ia	Low grade, intracompartmental	G1	T1	M0
Ib	Low grade, extracompartmental	G1	T2	M0
IIa	High grade, intracompartmental	G2	T1	M0
IIb	High grade, extracompartmental	G2	T2	M0
IIIa	Low/High grade, intracompartmental w/ metastases	G1-2	T1	M1
IIIb	Low/High grade, extracompartmental w/ metastases	G1-2	T2	M1

1.7 Diagnosis of sarcomas

For diagnosing STS it is important to begin with an anamnesis that takes special interest in the time of occurrence and the extent of expansion of a suspect lesion. Fast growth and a lack of movability are characteristic for sarcomas and must be seen as red flags. Patients may also present with symptoms of the musculoskeletal system such as: painless neoplasia or lymphoma, signs of inflammation (reddening, hyperthermia), loss of weight, paralysis or night sweats.
Special interest should also been given to possible environmental carcinogens the patient may have been exposed to at work or at home.
A careful clinical examination helps to gain an overview, a special focus should lie on the size and depth of the suspect mass and potential fixation to surrounding structures.
After the clinical examination, diagnostic imaging should be performed. Usually magnetic resonance imaging (MRI) is preferred, because there are several studies that report MRI to be superior to the computer tomography (CT) in evaluating soft tissue sarcomas of the extremity[xxiii] as MRI provides multiplanar images with better spatial orientation. MRI's advantage lies in delineating the extent of the neoplasm and the surrounding structures, especially individual muscle involvement[xxiv].
In case of contraindication for MRI (e.g. the presence of metallic implants in the patient), or if the tumour may develop from bone tissue, the CT is an alternative.
There has also been a multi-centre prospective study, including 133 patients who underwent both CT and MRI within four weeks before surgery for soft tissue sarcoma and there were no statistically significant improved accuracy in combined interpretation of both in determining tumour involvement of muscle, bone, joints or neurovascular structures[xxv].

In some cases, plain radiography of the primary tumour can help rule out soft tissue masses that arise from bone and help detecting intratumoural calcifications, for example in soft tissue osteosarcomas and synovial sarcomas.

In rare cases, a positron emission tomography scan (PET scan) is indicated. There are several studies that report, that PET and integrated PET/CT using

fluorodeoxyglucose (FDG) can distinguish benign STS from sarcomas with highest sensitivity for high grade sarcomas[xxvi]. When it comes to low and intermediate grade sarcomas, the ability of PET/CT is limited and is therefor not indicated for initial work-up of suspect masses.
If cancer is still suspected, the next step is to biopsy the suspect lesion. This should be done after a MRI has been obtained because post-procedural edema can make it difficult to interpret an image. It is also important that adequate tissue is obtained to evaluate and graduate the tumour which is essential for treatment planing later on.

There are several methods for obtaining a biopsy:

- Incisional biopsy

  This has been the gold standard for obtaining diagnostic tissue and is nowadays only performed when larger samples are needed as is required for flow cytometry, cytogenetics or molecular analysis for chromosomal translocations. Longitudinal incision should be performed.
- Core needle biopsy

  Because of the low rate of complications and high accuracy[xxvii] this method is preferred in most cases. To biopsy deep lesions, guidance by CT or ultrasound can help.
- Fine needle aspiration

  This can be useful in confirming disease recurrence, but it is not initially used in diagnostic evaluation because neither subtype nor grade may be provided which are both essential for treatment planning.

All these procedures should be performed by an experienced surgeon who will be planning the definitive tumour resection later on. During the final resection the biopsy duct must be excised completely as well. Therefore it is suggested, that the biopsy is performed at a hospital with experience in treating STS.
The collected tissue should be conserved in alcohol, in formalin, in glutaraldehyd, on dry ice and without addition.

Table 8: Procedure for optimal tissue processing for a soft tissue mass[xxviii]

Tissue preparation	*Studies*
Intraoperative samples	Frozen tissue with cryopreservative
	Cytological, scrape and squash imprints
Formalin-fixed tissue	Routine histopathology
	Immunohistochemical staining
	Reverse transcriptase polymerase chain reaction (RT-PCR)
	Fluorescent in situ hybridization (FISH) for cytogenetics (tissue sections)
Glutaraldehyd-fixed tissue	Electron microscopy
Fresh tissue (tissue culture media)	Cytogenetics
	Molecular studies (translocations)
	Tissue culture
Fresh tissue	Flow cytometry (DNA ploidy, cell surface markers)
Frozen tissue (no cryopreservative)	Molecular studies, gene rearrangement, immunocytochemistry, microarray gene analysis
Alcohol-fixed tissue	Immunocytochemistry (if required improved cytoplasmic preservation)
	Microarray gene analysis
	Cytologic imprinting for FISH studies

When taking routinely blood samples, special interest should be given to alkaline phosphatase (AP) and lactate dehydrogenase (LDH).

Unfortunately delay in diagnosing STS is quite common due to the assumptions of benignity as a result of the mostly painless nature of the tumour.

Most sarcomas metastasise in the lung first. Therefore chest imaging is recommended for every newly diagnosed patient with STS. There is no common consensus weather CT provides benefit over chest X-ray.

On the one hand it is possible that CT has a greater sensitivity in detecting small lung nodules.

Studies have shown on the other hand, that even if CXR misses one-third of all patients with lung metastases, compared to the infrequency of lung metastases the initial staging would be inaccurate overall in only 3.1% of cases[xxix].
Exceptions in terms of metastases are the round cell and the myxoid liposarcoma which should be worked-up initially with a CT of the abdomen and pelvis due to their common presentation of extrapulmonary metastases.
Patients with angiosarcoma have a higher risk of metastases in the central nervous system (CNS) and should therefore be worked-up with an image of it as well.
A full body MRI is still an option and a PET scan can help with the detection of occult distant metastases.

1.8 Therapy of STS

The therapeutic goals in treating STS are long-time survival, avoidance of local recurrence and minimising collateral damage. Surgical resection is elementary for all patients and adding postoperative radiation therapy (RT) for tumours > 5 cm achieves better outcomes. For large, high-grade extremity sarcomas preoperative RT is indicated.
STS are in general chemotherapy-sensitive tumours, even if there are differences in their responsiveness among the histologic subtypes.
Adjuvant chemotherapy, followed after the surgical resection, is standard treatment only for rhabdomyosarcoma.
The benefit of adjuvant chemotherapy for other types of STS remains uncertain and can not be adopted as a universal therapy for extremity primaries until there are better chemotherapy agents.

The largest trial of adjuvant chemotherapy for STS, the EORTC 62931[xxx], is to mention here. Both groups were high-grade patients who underwent surgery whereas one group was additionally treated with adjuvant high-dose *doxorubicin* and *ifosfamid.* The preliminary data has failed to prove any benefit in local control, progression free survival or overall survival. Nevertheless it showed improved survival in both groups compared with previous studies. This is thought to be due

to improved surgical techniques today and the increased use of adjuvant radiotherapy. An up-dated meta-analysis as well as the results of the final analyses are awaited with interest.
Even for those patients in whom the decision has been made to give adjuvant chemotherapy the optimal regimen is still undefined. Nevertheless to date UpToDate[xxxi] recommends to administer five to six cycles of *doxorubicin* and *ifosfamide* supplemented by *mesna*.

Doxorubicin is an anthracyclin antibiotic which works by intercalating DNA. It's most serious side-effect is life-threatening heart damage.
Ifosfamide is a nitrogen mustard alkylating agent. A common side-effect is encephalopathy caused by one of the breakdown products of the molecule. Symptoms can range from fatigue to coma. Besides the brain it can also affect the peripheral nerves hence there should be given lower doses for older patients. There is a higher risk of toxicity of ifosfamide with advancing age. It is also often used in conjunction with mesna to avoid internal bleeding, especially hemorrhagic cystitis.
Mesna is an organosulfur compound often used together with cyclophosphamide and ifosfamide. It's ability to detoxify harmful metabolites from these two anticancer agents makes it a useful addition. Further it increases the urinary excretion of the amino acid cysteine.

The National Comprehensive Cancer Network (NCCN) calls for individualized therapy, taking the patient's performance status, comorbidity factors, age, site of disease and histologic subtype into consideration. Furthermore it has to be taken into account, that potential benefits must be considered in the context of expected treatment-related toxicities (e.g. sterility in younger people, cardiomyopathy, renal damage, second cancers and overall impairment of quality of life).

Neoadjuvant combined therapy is often considered with large (>10 cm) or recurrent, high-grade tumours and if it has to be assumed that the patient will suffer functionally from surgery. Once again, there is no common consensus about how to best integrate radiation therapy, chemotherapy and surgery.

According to NCCN, preoperative RT alone, preoperative chemotherapy followed by postoperative RT or preoperative chemotherapy alone are all acceptable options. It is of course desirable for all, that these patients are treated in the context of a clinical trial so that new insights can be generated and patients can profit from new treatment modalities.

For evaluating the postoperative risk of sarcoma specific death the Memorial-Sloan-Ketterin Cancer Center[xxxii] (MSKCC) in New York has developed a specific nomogram on the basis of a prospectively followed cohort of adult patients with primary soft tissue sarcoma.
This nomogram predicts the probability of 12-year sarcoma specific death using a database of 2,136 treated patients.
Predicting factors are:

- tumour size (<5, 5-10 or >10 cm)
- histologic grade (high or low)
- histologic subtype (fibrosarcoma, leiomyosarcoma, liposarcoma, malignant fibrous histiocytoma, malignant peripheral nerve sheath tumour, synovial tumour or other)
- depth (superficial or deep)
- site (upper extremity, lower extremity, thoracic or trunk, visceral, retro/intraabdominal or head-neck)

This is a useful tool for patients counseling and follow-up scheduling even if its universal applicability remains unproven.

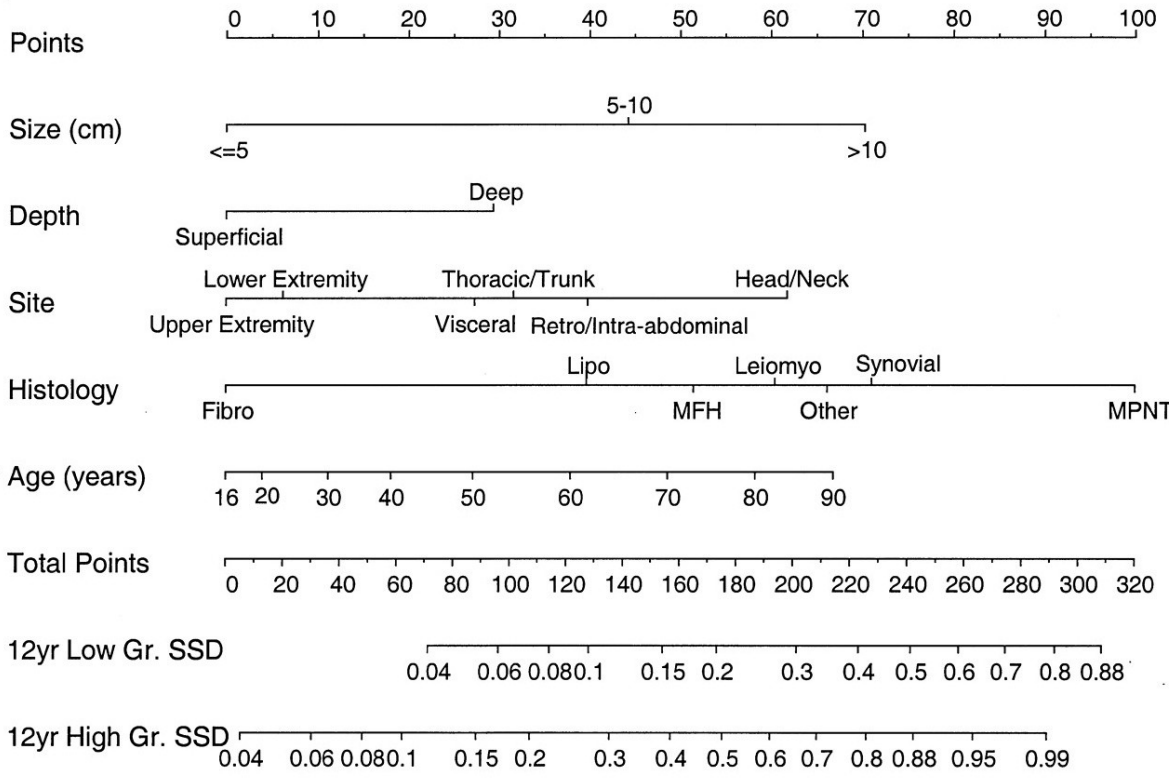

Figure 3: postoperative nomogram for 12-year sarcoma specific death - MSKCC

Instructions for physicians:

Locate the patients's tumour size on the Size axis. Draw a line straight upwards to the Points axis to determine how many points towards sarcoma-specific death the patient receives for tumour size. Repeat this process for the other axes, each time drawing straight upwards to the Points axis. Sum the points achieved for each predictor and locate this sum on the Total Points axis. Draw a line straight down to either the Low Grade or High Grade axis to find the patient's probability of dying from sarcoma within 12 years assuming he or she does not die of another cause first. Of course it is still possible to die of sarcoma after 12 years.

In some inoperable situations amputation can be avoided using an isolated limb perfusion (ILP) with *tumour necrosis factor alpha* (TNF α) and *melphalan*.
During this procedure these two anticancer drugs are delivered directly to the arm or leg while the normal blood flow of the extremity is temporarily stopped. In this way a much higher dose of the drug can be given.
Tumour necrosis factor alpha is a cytokine which promotes systemic inflammation and stimulates the acute phase reaction. By regulating immune cells it is also able to induce apoptotic cell death, inhibit tumorigenesis and viral replication.
Melphalan is a nitrogen mustard alkylating agent which attaches an alkyl group to the guanine base of DNA. Most common side-effects are decreased white blood cell and platelet count as well as nausea and vomiting.
A significant mean tumour decrease can be found after perfusion with this combination[xxxiii]. For this procedure patients must be transferred to specialised centres.

Local complications due to the primary tumour are causing morbidity and sometimes even death. But the most life-threatening aspect of STS is their potential to metastasise hematogenous.
Sarcomas of the extremity, chest wall, head or neck spread mainly to the lung. Extrapulmonary metastases to the retroperitoneum, spine and paraspinous soft tissues occur more often with myxoid and round cell liposarcomas.
Retroperitoneal and visceral sarcomas spread less commonly to the liver.
The rarity of lymph node metastases makes lymph node dissection very uncommon except when there is a suspect imaging in synovial sarcoma, rhabdomyosarcoma, angiosarcoma and epitheloid sarcoma.

Unresectable metastases are a fatal disease and use of systemic chemotherapy eventually combined with radiotherapy brings meaningful palliation and may even prolong survival. The selection of the therapeutic drug depends on the histology and biological behaviour of the tumour as well as the health status and preferences of the patient.
Myxoid and round cell liposarcomas are sensitive to *doxorubicin*-based chemotherapy, whereas synovial sarcoma tends to be especially sensitive to

alkylating agents such as *ifosfamide*. Myxoid and round cell liposarcomas seem also to be sensitive to *trabectedin*.

Trabectedin uses superoxides near the DNA strand which leads to DNA backbone cleavage and cell apoptosis. It kills cells by poisoning the DNA nucleotide excision repair machinery. To avoid vomiting and to protect liver function it is useful to give d*examethason* 30 minutes prior to application.

Leiomyosarcoma of uterine origin, endometrial stromal sarcomas, myxofibrosarcoma, dedifferentiated liposarcoma and malignant peripheral nerve sheath tumours (MPNSTs) show individual variability in their patterns of chemosensitivity. Nevertheless responses have been observed with *anthracylcines*, *ifosfamide* and *gemcitabine*-based regimens.

Gemcitabine is a nucleotide analog which replaces one of the building blocks of the nucleic acid cytidine during DNA replication which leads to apoptosis. As a major side-effect it leads to bone marrow depression.

Angiosarcomas, particularly those arising on the scalp, are sensitive to *taxanes*.

Taxane is a chemotherapeutic drug which is produced naturally by special trees. It is disrupting microtubule during mitosis and are so inhibiting the process of cell division. *Taxanes* lead to so-called "frozen mitosis" and belong to the mitosis inhibitors.

There are new trials that demonstrate tyrosine kinase inhibitors (TKIs) like *sunitinib*, *imatinib* and *sorafenib* to be useful for patients with metastatic alveolar soft parts sarcoma, gastrointestinal stroma tumour (GIST), dermatofibrosarcoma protuberans and desmoid tumour.

But to date there are no clinical trials to indicate the magnitude of any such benefit. Of course, the potential benefits must be taken into consideration with eventual treatment-related toxicities.

1.9 Chromosome abnormalities

In the last years a lot of progress has been made in finding characteristic chromosomal rearrangements associated with soft tissue sarcomas. Molecular alterations play an elementary role in carcinogenesis, especially when they are found in genes involved in the control of cell proliferation or apoptosis, the programmed cell death. Genes that promote tumour growth are called oncogenes whereas genes that suppress it are called tumour suppressor genes (TSG).

Chromosome abnormalities include aneuploidies, chromosome rearrangements, point mutations involving substitution, deletion or insertion of a base pair, DNA duplications and inversions.

Substitution goes along with the replacement of a base pair. If the amino acid that is coded by the new codon is the same, we are talking of silent mutation. If it is not the same, it is called mis-sense mutation. It may happen that substitution creates a STOP codon, causing translation to come to a premature halt. This is called a premature termination or non-sense mutation.

If a deleted or inserted segment is of other than a multiple of three bases, the translation reading frame is also disrupted in a frameshift mutation.

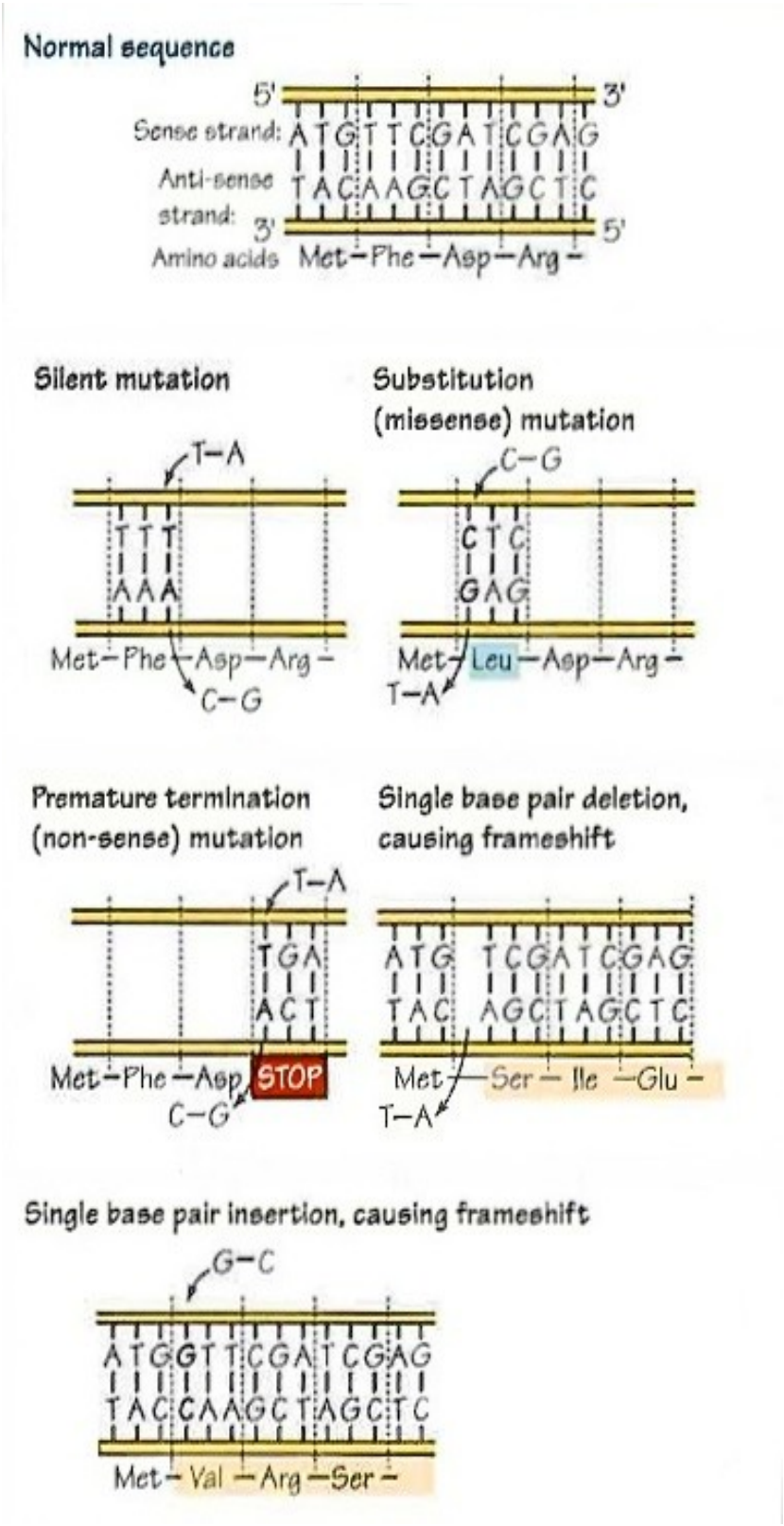

Figure 4: Types of mutations[xxxiv]

The first gene brought in relationship with STS was p53. It is a tumour suppressor gene that regulates the cell cycle, is preventing genome mutations and is so averting cancer. For this reason it is also called ‘the guardian of the genome‘.
In p53, a single point mutation can act dominant negatively affecting the DNA-binding and transcriptional transactivation of the wild-type protein[xxxv]. If this is the case then it can no longer fulfil it's function as a tumour suppressor.
This fact is not only important in the diagnostic approach.
Taubert et al.[xxxvi] have shown, that cells with mutated p53 or without p53 react less sensitively to radiation or chemotherapy. Cytotoxicity of adjuvant therapies seems to depend on p53-dependent apoptosis.
Melanoma, lung cancer, colorectal tumours, bladder and prostate cancer, who have all a high mutational rate of p53, often respond poorly to radiation and chemotherapy.
This goes along with the fact, that tumours like testicular teratocarcinoma with no p53-mutation or like acute lymphoblastic leukemia with wild-type p53 respond well to chemotherapeutic treatment.
Of course, this is not always the case and p53 is not the only factor that influences therapy response.
Molecular alterations likewise play an important role in prognosis and pathogenesis of sarcomas. Today the cytogenetics of cancer has become an integral part of oncology. Most chromosomal aberrations found are translocations and lead to specific histologic subtypes. Other aberrations found are deletions, mutations and amplifications[xxxvii].

1.9.1 DNA repair gene

DNA in human cells is regularly damaged by endogenous and exogenous mutagens. The faithful repair of DNA damage is crucial for genomic integrity and the survival of the cell.

In human, more than 130 genes are involved in the five major DNA repair pathways[xxxviii], homologous recombination (HR), non-homologous end joining (NHEJ), mismatch repair (MMR), DNA strand crosslink repair and nucleotide excision repair (NER).

Soft tissue sarcomas are like many other malignancies associated with genetic defects caused by double-strand breaks (DSBs). DSBs tend to be the biologically most harmful lesions in DNA which are caused mainly by ionizing radiation but also by genotoxic chemicals, reactive oxygen and mechanical stress on the chromosomes during mitosis[xxxix].

Once a DSB has occurred, it can be repaired either by non-homologous end joining (NHEJ) or by homologous recombination (HR).

In the HR the DNA ends are first cut in 5' to 3' direction by nuclease.

The resulting 3´ single-stranded tails then enter into the DNA double helix of an undamaged DNA molecule with which it shares extensive sequence homology, and are extended by a DNA polymerase, which copies information from the partner. Following branch migration, the emerging DNA crossovers (Holliday junctions) are resolved resulting in two intact DNA molecules[xl],[xli].

RAD51 is one of the key proteins for HR and functions by forming nucleoprotein filaments on single-stranded DNA, mediating homologous pairing and strand exchange reactions between single- and double-strand stranded DNA during repair[xlii]. In 2001, Wang et al.[xliii] found a functional SNP of RAD51 rs1801320 G>C by sequencing, which increases the risk of breast cancer within the group of BRCA1/2 mutation carriers.

X-ray repair cross-complementing (XRCC) group 2 is a member of the RAD51 gene family that participates in HR and is required for correct chromosome segregation[xliv]. It interacts directly with RAD51.

One potentially functional SNPs has been identified: XRCC2 rs1799796 is a transition G>A which results in a increased DNA repair activity.

Nucleotide excision repair (NER) is the sole mechanism for removing bulky parts from DNA. It is used to repair damage mainly produced by UV radiation. NER removes a single-stranded segment that includes the lesion and fills the gap using a DNA polymerase which is able to use the undamaged strand as a template.

ERCC2 gene provides instructions for making a protein called XPD. It works as an adenosine triphosphate-dependent 5´-3´ helicase and is a subunit of the basal transcription factor IIH. It separates the double helix during both global genomic repair and transcription-coupled repair[xlv]. One common SNP in the coding region of the XPD gene has been identified. ERCC2 rs13181 A>C causing exon 23 codon 751 Lys to be substituted for Gln is suspected to increase the risk for breast cancer[xlvi] as well as the risk for lung adenocarcinoma in non-smokers[xlvii].

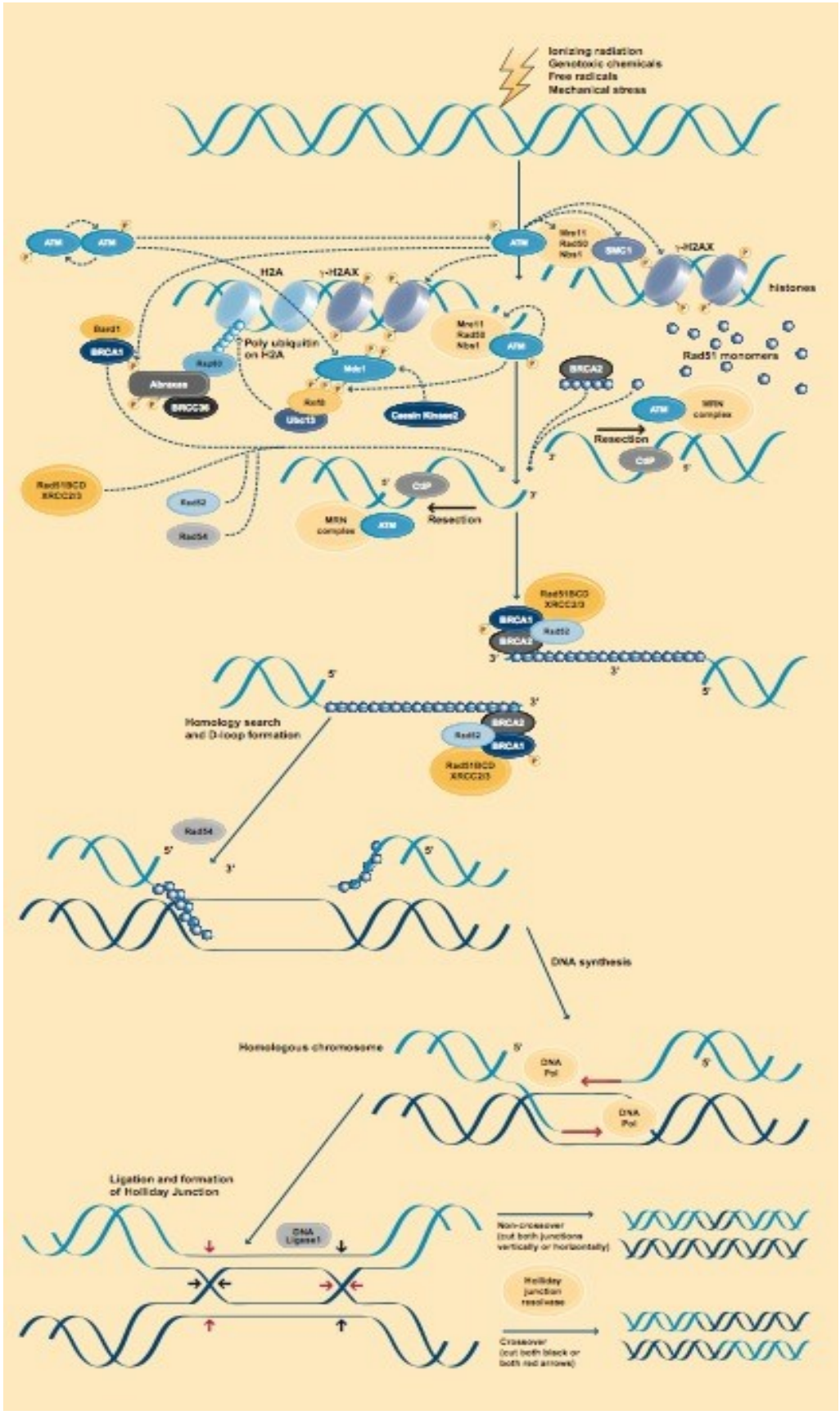

Figure 5: Homologous recombinational repair

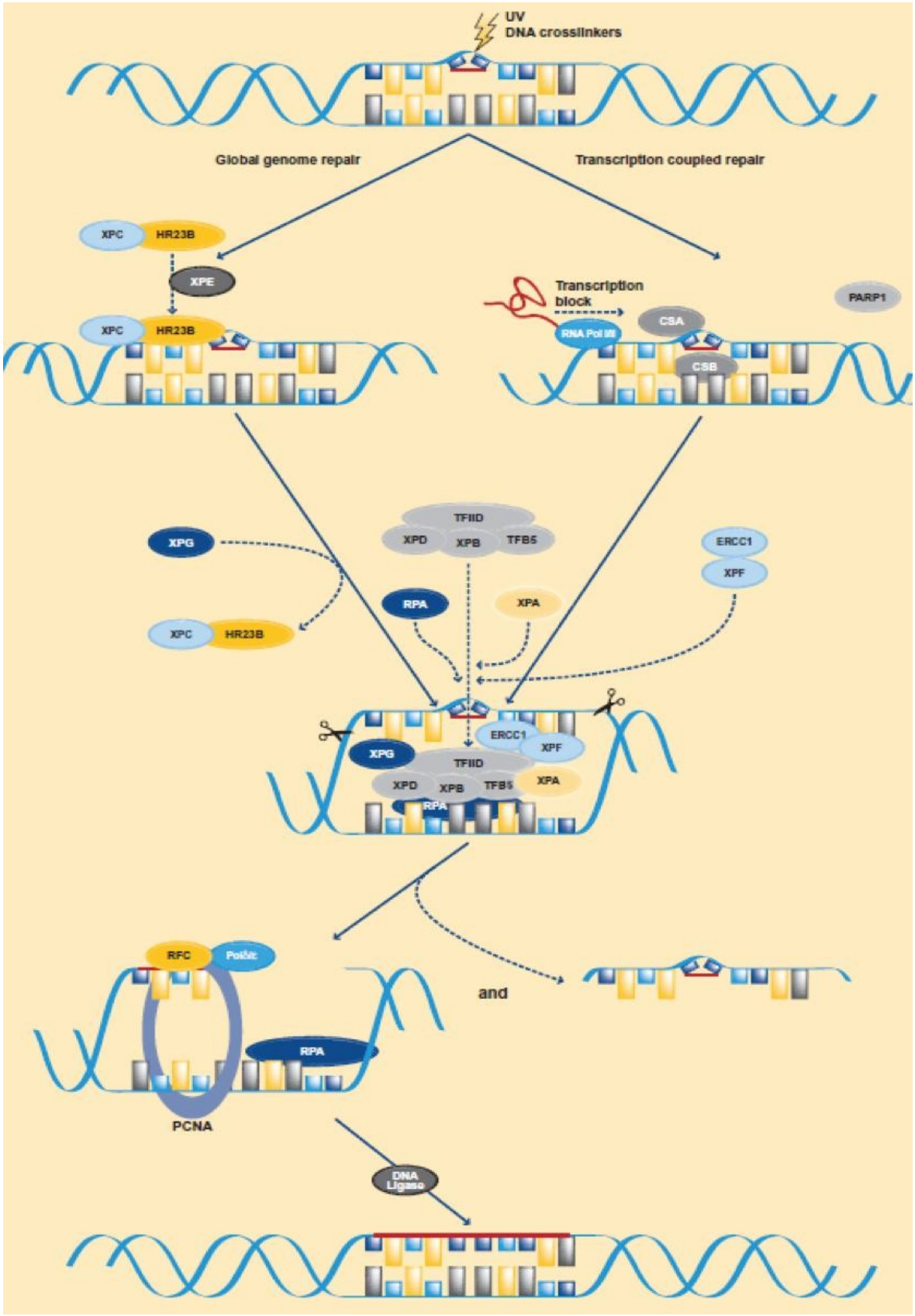

Figure 6: Nucleotide excision repair

1.9.2 Single nucleotide polymorphisms

In the genes encoding RAD51, XRCC2 and ERCC2 several single nucleotide polymorphisms (SNPs) have been described. SNPs are a type of polymorphisms involving variation of a single base pair caused by mutations or errors during cell division. They occur at appreciable frequency in the population (~ 1%), cause small structural alteration and may occur in non-coding regions as well as in coding ones. Polymorphisms in human DNA can effect the development of diseases, the response to pathogens as well as the response to a specific therapy. In DNA-repair genes they may lead to altered DNA-repair activity or up-regulation of analogous proteins and defective repair of DNA breaks. Therefore they are seen as a key to a personalized medicine.

For this project we investigated the following single nucleotide polymorphisms.

Table 9: Selected candidate gene polymorphisms

Gene	Genotype	Base change	NCBI
RAD51	G135C	G>C	rs1801320
XRCC2	Arg188His	G>A	rs3218536
ERCC2	Lys751Gln	A>C	rs13181

2 Material and Methods

2.1 Subjects

Between 1999 and 2010, 224 patients with histopathologically confirmed soft tissue sarcomas have been recruited at the Department of Orthopaedic Surgery, Medical University of Graz for the study. The patients were included in the aftercare measures program, providing follow-ups in regular intervals for adjuvant setting (3 months interval in years 1–3, 6 months interval in years 4–5, and 12 months interval in years 6–15 after diagnosis). Follow-up investigations included clinical check-up and radiological analyses (computer tomography, magnetic resonance imaging, abdominal ultrasound, chest X-ray). Written informed consent was obtained from all patients. Follow-up data of all patients are available.

Age, sex and affected anatomic region were obtained from the patient´s history. Time from first onset of symptoms to definitive curative surgery was recorded. AJCC score was obtained at the time of admission of curative surgery. Surgical margins were classified according to Enneking et al. as radical, wide, marginal or intralesional. Adjuvant treatment modalities and disease free survival (DFS) and overall survival (OS) have been recorded. All histological specimens were evaluated by a group of pathologists specialised in diagnosing soft-tissue sarcoma at our institution. For the present study all specimens have been re-evaluated by one pathologist (Priv. Doz. Bernadette Liegl-Atzwanger).

Data was stored in a MS Access database with strict data protection according to the Austrian Gene Technology Act and to the guidelines of the Ethics Committee of the Medical University of Graz. Patients' characteristics and outcomes were unknown to the investigators performing the genetic analyses.

2.2 Laboratory analyses

2.2.1 Isolation of genomic DNA

Genomic DNA was extracted from paraffin-embedded tissue of the soft tissue sarcoma. Tissue samples were stored at the Biobank of the Medical University of Graz (www.meduni-graz.at/biobank) and DNA isolation was performed by direct use of the QIAamp DNA mini Kit (Qiagen) and according to the manufacturer's instructions. Samples were digested with Qiagen Proteinase K and lysis-buffer at 55°C overnight and loaded onto spin columns. DNA was adsorbed by short centrifugation onto the QIAamp silica membrane, washed and eluted with 100µL water (www.qiagen.com).

2.2.2 PCR - The polymerase chain reaction

The polymerase chain reaction (PCR) is the most widely used method of genetic analysis and has revolutionized the whole field of molecular medicine. Within minutes analyses can be performed on samples that contain only a single nucleus obtained from a mouthwash, hair root or skin dandruff.

Normal DNA replication works in the same way as PCR does, using single-strand DNA and replacing the missing strand.

The reaction uses Taq DNA polymerase which is isolated from *Thermus aquaticus*, a hot-spring bacterium which can withstand temperatures up to 95 °C[xlviii]. It requires a start point of duplex DNA which is provided by single-strand primers, attached one at each end of the sequence which is going to be duplicated.

To start the reaction the DNA sample is heated to 94 °C to melt the hydrogen bonds of the two polynucleotide strands. Afterwards the temperature is reduced to 60 °C and the primers (short oligonucleotides 15 – 30 nucleotides long), which are designed to match and anneal to the flanks of the chosen sequence, are added.

Finally, at 72 °C, the polymerase moves down the DNA strand and synthesizes a complementary strand which recreates a double-stranded molecule.

This is called one 'cycle' and takes only a few minutes. After every cycle the amount of DNA doubles every time and after 30 cycles about 100.000.000 copies of the original sequence are created.

Table 10: Advantages and Disadvantages of PCR[xlix]

Advantages	Disadvantages
applicable to single-genome quantities of DNA	long sequences can not be amplified
procedure is very fast (3-48h)	base sequences of flanking regions must be known
no radioactivity is involved	absolute purity of the sample is essential
product is suitable for further analysis by established molecular techniques	there is no 'proof-reading' or error correction, so that mutations that occasionally arise during the process are also propagated
process can be applied to even badly degraded DNA	

2.3 Statistics

The primary endpoint of the study was disease-free survival (DFS). DFS was calculated from the date of diagnosis of soft tissue sarcoma to the date of the first observation of tumour recurrence. DFS was censored at the last follow-up if the patient remained tumour-free at that time. The secondary study endpoint was overall survival (OS). Overall survival was estimated from the date of diagnosis of STS to the date of death. Allelic distribution of the polymorphisms was tested for deviation from Hardy-Weinberg equilibrium using x^2-test. The true mode of inheritance of all polymorphisms tested is not established yet and we assumed a codominant, additive, dominant or recessive genetic model where appropriate. Odds ratios (OR) and 95% confidence intervals (CI) were determined by logistic regression analysis. The association of polymorphisms with DFS and OS was analyzed using Kaplan-Meier curves and log-rank test. In the multivariate Cox-regression analysis, the model was adjusted for tumour size, tumour site, grading and adjuvant therapy. Case-wise deletion for missing polymorphisms was used in univariate and multivariate analyses. All analyses were performed using the SPSS statistical software package (SPSS Inc., Sunnyvale, USA).

3 Results

Baseline patient characteristics, tumour biological factors and therapy modalities are shown in Table 11. The median age at time of diagnosis was 64,4 years (range 20 to 98 years). The median follow-up time was 48,6 months (range 1 to 155 months).

Genotyping was successful in at least 95% of cases in each polymorphism analysed. In failed cases, genotyping was not successful because of limited quantity and/or quality of extracted genomic DNA. The genotyping quality control by re-analysing of a subsample provided a genotype concordance of >99%. The allelic frequencies for all polymorphisms were within the probability limits of the Hardy-Weinberg equilibrium.

In the univariate analysis, the minor allele of ERCC2 rs13181 A>C was significantly associated with increased OS (HR 0.509; 95%CI 0.289-0.897; p=0.019; Figure 7). Patients carrying at least one C allele in ERCC2 rs13181 A>C showed a median OS of 102 months. In contrast, patients with homozygous A/A had a median OS of 63 months. In multivariate analysis, the ERCC2 rs13181 A>C polymorphism remained significantly associated with increased OS (HR 0.449; 95%CI; 0.241-0.836; p=0.012). In the univariate analysis, RAD51 rs1801320 and XRCC2 rs3218536 were not associated with OS (HR 1,467; 95%CI 0,865-2,489; p=0,155; HR 0,942; 95%CI 0,430-2,063; p=0,880). Furthermore, RAD51 rs1801320 G>C, XRCC2 rs3218536 G>A and ERCC2 rs13181 A>C showed no association with DFS in the univariate analysis (HR 2.026; 95%CI 0.703-5.836; p=0.191; HR 1,140; 95%CI 0,259-5,016; p=0,863; HR 0,930; 95%CI 0,454-1,906; p=0,843).

Table 11: Baseline characteristics

	Frequency	%
	Sex	
female	108	48,20%
male	116	51,80%
	224	100,00%
	Grading	
G1	39	17,40%
G2	46	20,50%
G3	138	61,60%
unknown	1	0,50%
	224	100,00%
	Classification	
specific translocation	52	23,20%
amplifications	32	14,30%
complex genetics	134	59,80%
nos	6	2,70%
	224	100,00%
	Localization	
torso	21	90,60%
other localizations	203	9,40%
	224	100,00%
	Depth	
subcutan	55	24,60%
subfascial	160	71,40%
unknown	9	3,70%
	224	100,00%
	Size	
<5 cm	58	25,90%
>5 cm	147	65,60%
unknown	19	8,50%
	224	100,00%

	adj RTX	
no	75	33,50%
yes	144	64,30%
unknown	5	2,20%
	224	100,00%
	adj CTX	
no	132	58,90%
yes	89	39,70%
unknown	3	1,40%
	224	100,00%

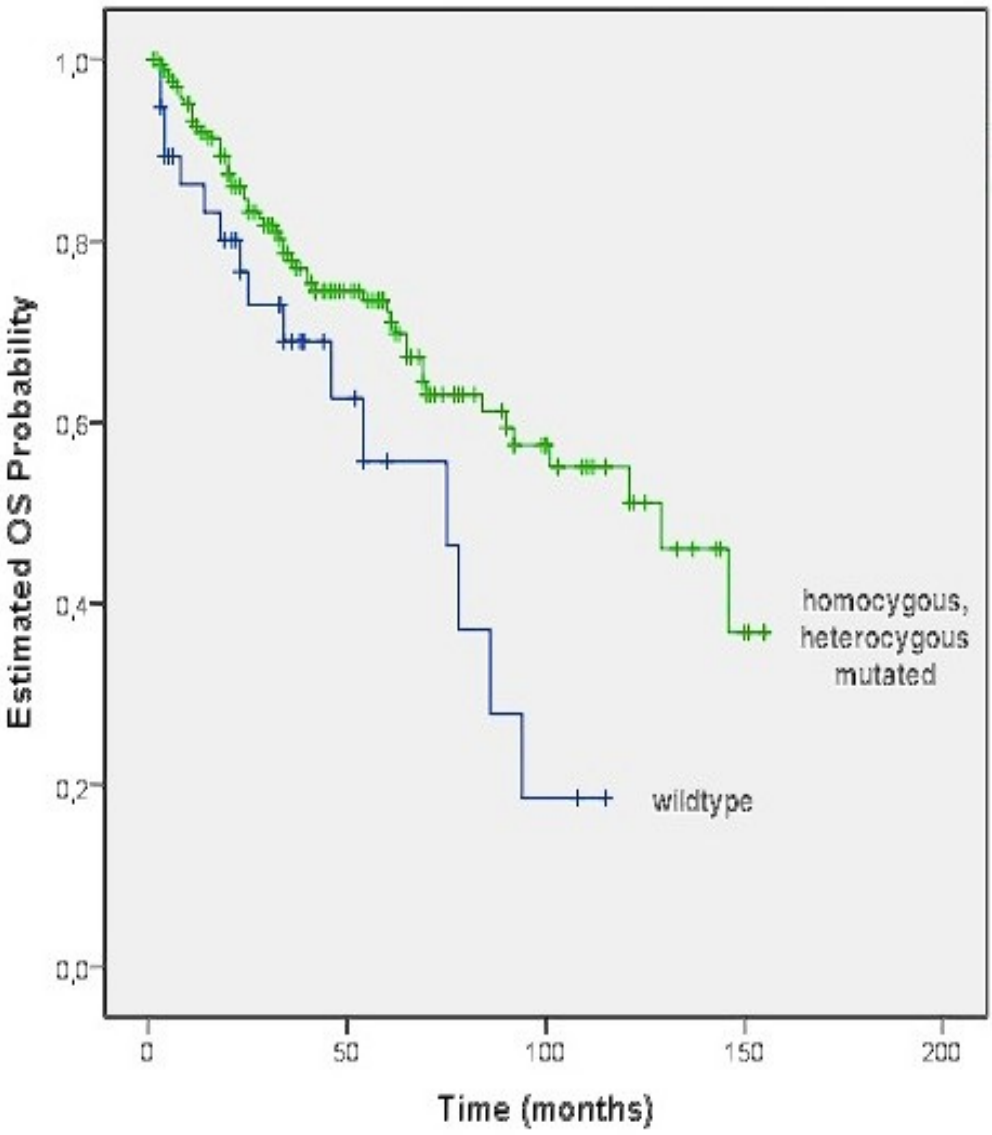

Figure 7: Estimated OS Probability

4 Discussion

The aim of this study was to determine an association between SNPs in genes of the DNA repair pathway and outcome in STS patients because some patients were observed to show earlier recurrence or disease progression despite uniform treatment of the same STS-subtypes.
Therefore we investigated the genes encoding for RAD51, XRCC2 and ERCC2.

We found a statistically significant association between ERCC2 rs13181 A>C and clinical outcome in STS patients. The minor allele of ERCC2 rs13181 A>C was associated with increased OS. XPD, the ERCC2 encoded DNA-repair enzyme, is working as a 5' – 3' helicase and is part of the TFIIH complex. It opens the double-stranded DNA at the site of the lesion.
For RAD51 rs1801320 G>C and XRCC2 rs3218536 G>A no statistically significant correlation with OS or DFS could be found.

Knowledge about the clinical effects of deficient DNA-repair pathways is essential because it leads to a better understanding not only of STS progression but also of the progression of many other forms of cancer.
This provides a strategy for stratifying patients for risk of metastatic soft-tissue sarcoma, which in turn allows treatment options to be tailored to the individual. This may result in a decreased incidence of tumour relapse by placing patients on adjuvant therapy that would not have been initiated in current circumstances. Conversely, it may ultimately be possible to avoid treating patients at low risk, thus eliminating the morbidity associated with adjuvant therapies.
Homologous recombination and nucleotide excision repair represent the two of the mayor DNA repair pathways. HR is used to repair double-strand breaks. Double-stranded breaks can be produced by replication errors as well as by exogenous agents such as ionizing radiation. Fixing DSBs is even more difficult than other types of DNA damage because no undamaged template is available[l].
NER is necessary to correct errors of the DNA caused by UV radiation. It can cut out bulky parts at once and fills up the gap by using a DNA polymerase which uses the complementary single strand as a template.

Gossage et al.[li] reported about the role of DNA repair polymorphisms in individualising cancer therapy. They have come to the conclusion, that the narrow therapeutic index and heterogeneity of patient responses to chemotherapy and radiotherapy implies that the efficacy of these treatments could be enhanced by improving our understanding of the genetic bases for interindividual differences in their effects. They present evidence implicating variations within DNA repair genes as important predictive and prognostic markers in cancer.

Khanna et al[lii] described recent progress in understanding of how cells detect and signal the presence and repair of one particularly important form of DNA damage, the DNA double-strand break (DSB). They discuss accumulating evidence that is connecting deficiencies in cellular responses to DNA DSBs with carcinogenesis.

Another study initiated by Popanda[liii] et al. revealed that lung cancer risk was only moderately affected by a single DNA repair gene variant enhanced up to approximately threefold by specific risk allele combinations.

The functional RAD51 rs1801320 G>C has been reported to be associated with altered gene transcription[liv]. It seems to be important for the increased risk of breast and ovarian cancer for BRCA2 mutation carriers.

In a recent study, Seker et al.[lv] demonstrated that lymphoblastoid cell lines with A/A genotypes show a nearly 2,5-fold higher apoptotic response, representing a direct link between this SNP and its biological significance. The second discussed polymorphism ERCC2 rs13181 A>C may also be important since it is located in the protein-protein interaction domain.

In a study carried out by Le Morvan et al.[lvi] in 2006, normal and tumoral tissues of 93 patients with well-characterised sarcomas were genotyped for the 2 “coding” polymorphisms of XPG and XPD, both DNA-repair enzymes. They found a significant association between these two NER polymorphisms and some subtypes of sarcomas characterised by specific translocations. This leads to the

question whether there might be a link between those SNPs and the occurrence of chromosomal rearrangements. It is further to mention, that DNA repair pathways are also rather important for the chemotherapeutic approach in advanced or metastatic disease. The cytotoxicity of a drug raises with the sensitivity of the cell line for the active substance. Soares et al.[lvii] showed that cells with defects in the homologous recombination (HR) proteins XRCC3, BRCA2, RAD51C and XRCC2 were 8 to 23 times more sensitive to the treatment with trabectedin (Yondelis®).

For the present study we tried to find out if there is a link between SNPs in genes of the two DNA-repair pathway HR and NER and the outcome in soft tissue sarcoma patients. In our analysis the minor allele of ERCC2 rs13181 A>C was significantly associated with increased OS. Our findings suggests that this SNP leads to an increased performance of the affected gene. That is in line with the results from Seker et al. who found higher apoptotic response in lymphoblastoid cells with similar genotypes.

The strength of our study lies in the fact that it was carried out with a relatively large number of patients and long follow-up.
One limitation of our study is the retrospective design, wherefore a selection bias cannot be excluded. Furthermore, as allele frequencies of polymorphisms are known to vary between different ethnicities, our findings may not be transferable to other ethnicities than Caucasian.
In conclusion, our results indicate an association between SNPs in DNA-repair pathways and outcome in soft tissue sarcoma patients.
Before these findings will have an impact in the clinical use and application for public health measures, more large population-based studies and validation of the results will be required.

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