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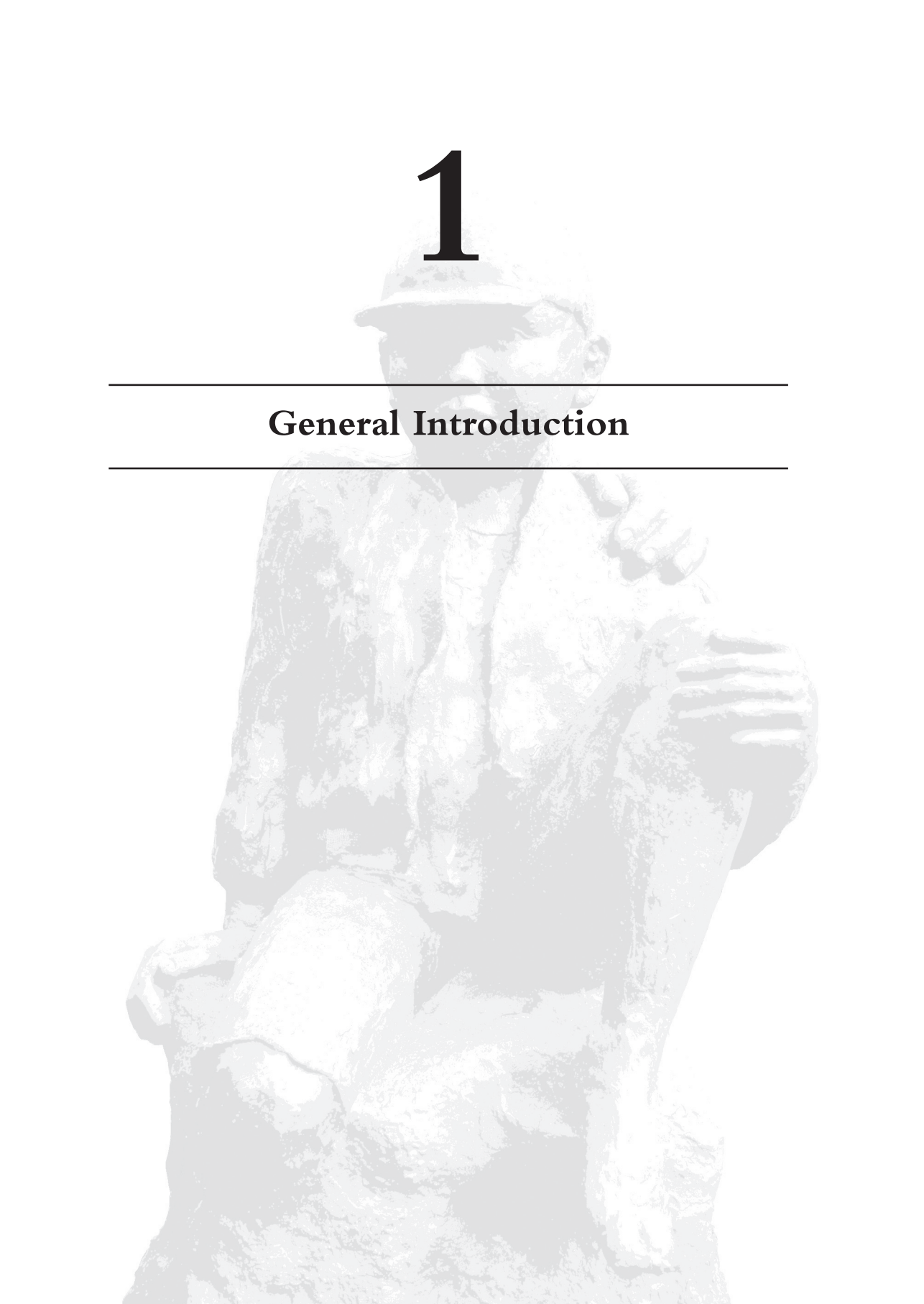
General Introduction



INTRODUCTION

Osteosarcoma is a primary, high-grade malignant spindle cell tumour, in which neoplastic osteoid or bone is produced by the proliferating malignant cells (1, 2). In this introduction epidemiology, genetics and pathology of osteosarcoma will be discussed against the background of this thesis, which focusses on clinical and pathologic aspects.

Epidemiology of Osteosarcoma.

Incidence and gender distribution of Osteosarcoma.

Cancer in general is a major health problem in the world, it is the 2nd leading cause of death for all ages (3). Not only primary cancer, but also the increased incidence of secondary malignancies (4-7) or side effects after treatment contribute to the health problem (8). Osteosarcoma is a rare type of tumour. The proportion of osteosarcoma among all cancers varies with age. In children up to 15 years, osteosarcoma comprises 2.3%(1.6%-2.6%) of all tumours (9-13), in adolescents 15-25 years, 2.6% (11, 14), but in patients older than 25 years, osteosarcoma represents less than 1% of all malignancies (3, 12, 13, 15-21). This variation in occurrence of osteosarcoma is reflected in table 1 and figure 1. Table 1 shows the incidence of osteosarcoma in patients younger than 25 years of age (14, 15, 18, 22-24). The highest incidence is found in children 10-19 year where osteosarcoma accounts for 8.6 new cases per 10^6 population per year (10, 14, 16). Conversely, osteosarcoma in children less than 5 years old is extremely rare. Among 6023 osteosarcoma patients, 105 (1.7%) children were less than 5 years old (25-28). In these young patients, osteosarcoma presents more often in the humerus (up to 32% of the cases), with the telangiectatic subtype more frequently diagnosed than in older patients, suggesting a possible difference in biology compared to osteosarcoma in the later age groups.

TABLE 1.

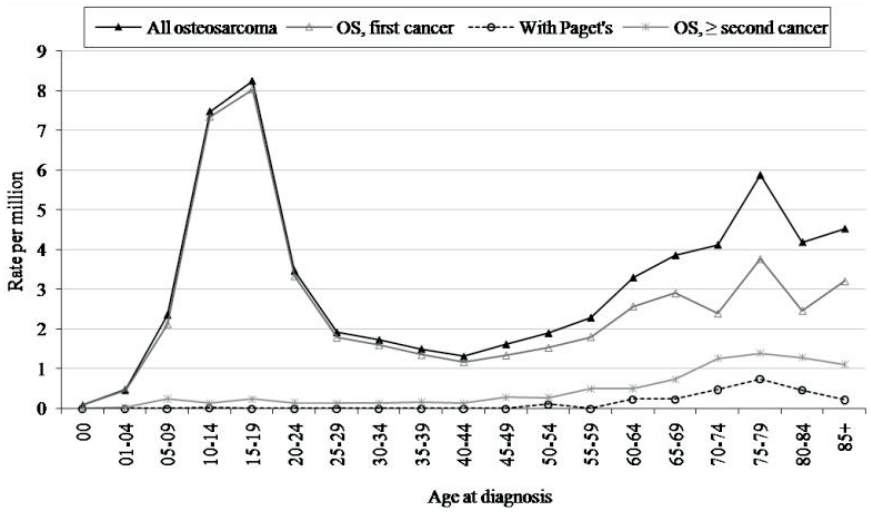
Age adjusted incidence rates of osteosarcoma, given as n/10^6/year^{-1}, as given by different authors in non-SEER series for patients to 25 years of age.*Patients 12-14 yr.

Author (ref)	0-4 y	5-9 y	10-14 y	15-19 y	20-24 y
Eyre (24)	0.4	2.6	5.7	-	-
van den Berg (23)	0.8	3.6	10.9	13.6	-
Stiller (22)	0.2	2.4	6.8	8.4	-
Birch (14, 18)	-	-	7.5*	7.7	3.3
McWhirter (15)	0.0	2.0	5.0	-	-

Figure 1 covers the distribution of osteosarcoma throughout all ages and is based on data from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program (29). The incidence as number of patients per 10^6 population per year shows a triphasic pattern. After a steep raise in the age group of 5-14 years old the first peak of 8.4-8.6 cases per 10^6 persons per year is present in the age group of 15-20 years. This peak is followed by a plateau with a low incidence rate of on average $1.7/10^6$ per year in the age group 25 to 59 years. After 60 years the incidence gradually increases to a second peak, with an annual incidence of $4.9/10^6$ per year in patients of 77-79 years of age. In this age group secondary osteosarcoma and osteosarcoma in the context of Paget's disease contribute for 24% and 9% respectively and differ in localisation (see paragraph 1.2) (29).

FIGURE 1.

Incidence of osteosarcoma, showing a triphasic pattern with a peak during adolescence, a plateau during adulthood and a second peak in older patients (29).



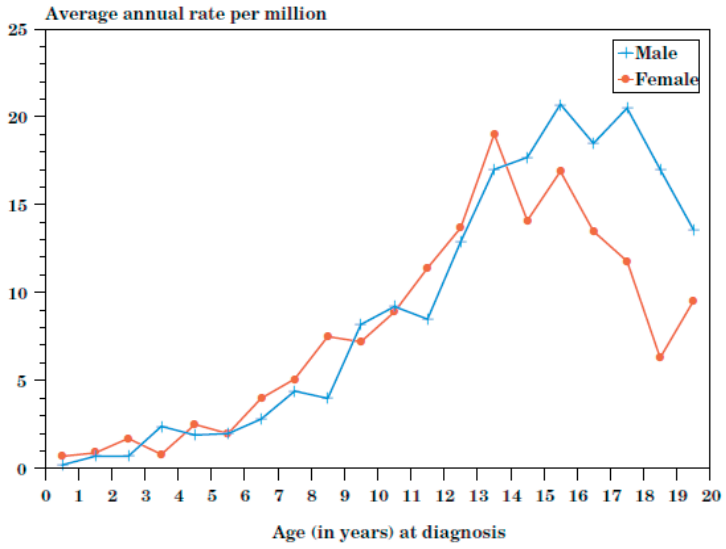
Consistent with other reports, osteosarcoma in older patients differs from that in the younger patients in localization (more non-extremity site), size (larger) and more metastatic disease at diagnosis or secondary osteosarcomas (30-35).

Gender and Osteosarcoma. The SEER data showed clear differences in male-female ratios in different age groups (17, 29). In patients younger than 15 years, the incidence of osteosarcoma in females is higher than in male (figure 2), but after 15 years, this ratio reverses to male

predominance (ratio male:female = 1.34:1). In patients 25-59 years still more males are affected by osteosarcoma (ratio male:female = 1.2:1), but after 60 years of age, osteosarcoma is less common in males (ratio male:female = 0.9:1), except in osteosarcoma in Paget's disease, that affects more males (ratio male:female = 1.58:1).

FIGURE 2.

Incidence of osteosarcoma in males and females, showing that in younger patients the incidence in females in higher than in males, which is reversed in the age group above the 15 years (17).

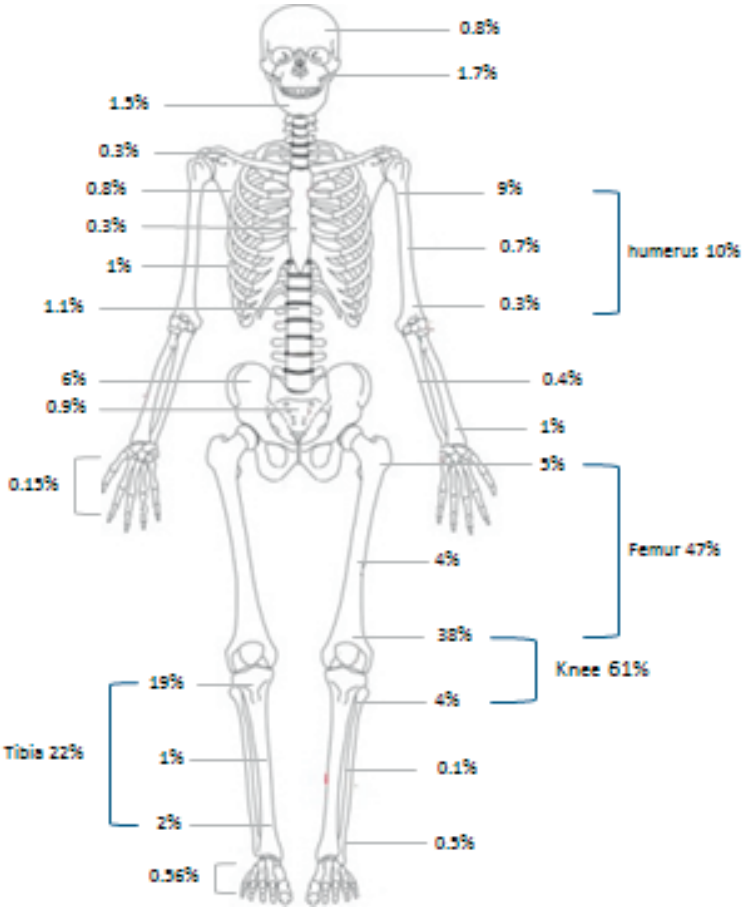


Localization of osteosarcoma

Osteosarcoma in patients is mainly localized in the long tubular bones, as is demonstrated in figure 3. The data of this figure are retrieved from more than 6.000 cases reported in 4 large studies (36-39) and in 2 atlases of bone tumours (40, 41). As can be seen, nearly 75% of the osteosarcomas are located in the long bones of the lower extremity, more than 60% in the metaphyseal region around the knee, and 10% in the long bones of the upper extremity. In the axial skeleton (vertebral column, sacrum, scapula and clavicle) 3% of all osteosarcomas are located, the chest accounts for 1.3%, the pelvis for 6% and the facial bones and skull for 4%. The location, found in the epidemiologic study of Mirabello shows different sites in the age groups, older than 25 years, whereas in the younger age groups the sites are similar to these in the large studies of figure 3.

FIGURE 3.

Distribution of osteosarcoma in the skeleton. Data from 6.454 cases of osteosarcoma (see text for references).



The variable distribution of osteosarcoma in the skeleton in the different ages (table 2) and the diverse histology of the different subtypes (29) indicates that osteosarcoma is not an uniform disease, and behaves different in younger people than in older patients.

TABLE 2.

Difference in osteosarcoma localisation in 3 age groups. This table clearly demonstrates more axial and extra-osseous location in older patients, compared to the younger patients (29).

site	0-24 yr	25-59 yr	≥ 60 yr
Lower Long bones	75%	43%	27%
Upper long bones	11%	10%	8%
Pelvis	4%	11%	19%
Facial bones/skull	3%	10%	5%
Chest	2%	4%	4%
Vertebral column	1%	4%	5%
Extra-osseal	< 1%	7%	19%

Survival in Osteosarcoma

The survival rates shown in figure 4 represent a general trend over the past 4 decades in children and adolescents. These American data do not differ from European studies, as is demonstrated in table 3.

FIGURE 4.

Five-year survival rates for children and adolescents with osteosarcoma, diagnosed during the period 1973-2002, and with follow-up until 2006. Data from the SEER-9 registries and Centers for Disease Control and Prevention (42).

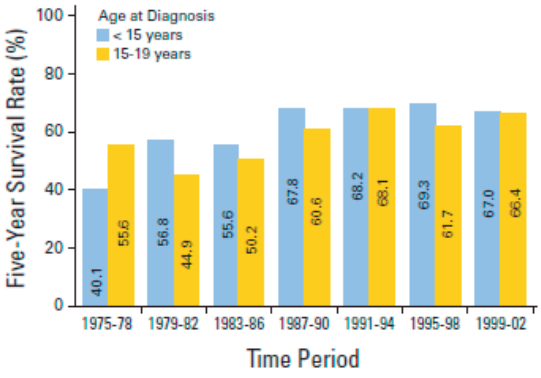


Figure 4 shows an substantial increase in 5-year overall survival from the mid 1970-ties onwards to the mid 1980-ties, due to the introduction of chemotherapy. Since the mid-eighties no further improvement in survival was achieved (9, 22, 29, 42-47). The effect of the development of chemotherapy on survival is discussed in **chapter 2** of this thesis. The mean 5-year overall survival compiled from these data bases is 62% (53%-77%). Overall survival after 5 year follow-up does not decrease much, because less than 5% of all osteosarcoma patients have a late relapse (48-50).

TABLE 3.
Population based studies reporting (5-year or more) overall survival (OAS) in children, adolescents or patients less than 40 years of age with osteosarcoma. These studies were selected on the basis of large international data bases, like SEER, EUROCARE and ACCIS.

Author (ref)	≥ 5 yr OS
Smith 2010 (42)	68%
Mirabello 2009 (29)	60%
Gatta 2009 (11)	77%
Arndt 2007 (47)	58%
Magnani 2006 (46)	61%
Stiller 2006 (22)	55%
Stiller 2006 (51)	53%
Gatta 2005 (9)	59%
Gatta 2003 (44)	66%
Stiller 2001 (43)	60%

Risk factors in Osteosarcoma from population based data.
Risk factors for osteosarcoma will be discussed as reported in population based data bases and in clinical treatment trials. Because this introduction is mainly focussed on the epidemiology of osteosarcoma, the patient- and tumour related factors will be discussed in more detail and for completeness, the treatment related factors will shortly be listed.

Patient related factors

Age

The age-dependent incidence pattern has been discussed earlier in the paragraph about epidemiology of osteosarcoma (paragraph 1.1). With respect to survival, children, younger than 5 years old have a survival of 52%-60% (25-27), but patients older than 60 years of age have reduced survival (22%-58%), due to secondary or Pagetoid osteosarcomas (30-35). It

seems that the presentation of osteosarcoma in the very young and elderly patients is different from the age group between puberty and 40 years of age on the bases of underlying biologic differences. This observation suggests a different biologic behaviour of osteosarcoma in the several age groups.

Length

The pattern of incidence of osteosarcoma (figure 1) suggests a relationship with pubertal growth and development of osteosarcoma. Since Fraumeni reported a relationship between large stature and osteosarcoma more than 40 years ago (52), other authors published a similar relationship between height and osteosarcoma in young patients (53-56). In a pooled analysis taller than average (51^{th}-89^{th} percentile) and very tall (≥ 90^{th} percentile) patients, mainly younger than 25 years, had an increased risk on osteosarcoma (57). The risks, expressed as odds ratios, were 1.35[(1.18-1.54) 95% CI] and 2.60[(2.19-3.07) 95% CI] respectively. A meta-analysis found that patients with osteosarcoma were 0.26 SD[(0.088-0.432) 95% CI] taller than the reference population and that 62% [(57%-67%) 95% CI] of the patients had a height above the median for the reference group (58). These data may suggest that particularly pubertal growth plays a role in the genesis of osteosarcoma.

Pre-malignant conditions as risk factors: Paget; fibrous dysplasia, chronic osteomyelitis and others

Osteosarcomas arising in Paget's disease and in fibrous dysplasia are more frequently occurring, but have to be distinguished from secondary osteosarcoma after radiotherapy or as second neoplasm after chemotherapy, because of a different causal relationship. Osteosarcoma arising in benign precursors, like chronic osteomyelitis, bone infarction and giant cell tumours of bone have rarely been reported (59, 60), and therefore will briefly be listed.

Osteosarcoma in Paget's disease of bone

Paget's disease of is a skeletal disorder, characterized by focal increased bone turnover, occurring in 1%-3.6% of the Caucasian population above the 55 years of age (61-64). The basic defect in Paget's diseases is an increased osteoclastic bone reabsorbtion, with reactive, disorganised bone formation. The cause is unknown, but based on familial history of Paget's disease, which occurs in 5%-40% (65-68), an autosomal dominant inheritance is assumed (63, 69-71). Other Paget's disease related syndromes have been recognized (70, 72), connected to each other by a activating mutation of the RANK-NF-κB pathway, which is the molecular basis of this bone disease (63, 70, 72, 73).

Osteosarcoma as complication of sporadic cases occurs in 0.4%-5.5% of the cases (56, 74-80). Most (80%) of these cases develop in the poly-ostotic form of Paget's disease (75, 76). Sparse case-reports of familial Paget's disease or related disorders documented the development of osteosarcomas (81-84), suggesting a shared susceptibility region on chromosome 18q21-22 between osteosarcoma and Paget's disease (83, 85-87).

Osteosarcoma complicating Paget's disease is localized in the deformed bones. Mainly the large limb bones (femur, humerus, tibia) or the flat bones (pelvis, skull, scapula) are affected in osteosarcoma, secondary to Paget's disease (59, 76, 78, 80). Survival in Paget related osteosarcoma is very low, with a median survival 8-11 months, and a 5-year overall survival of around 10% (78, 80).

Osteosarcoma in Fibrous Dysplasia

Fibrous dysplasia of bone is a focal bone disease where abnormal differentiation of osteoblasts, due to a mutation in the α-subunit of the G-protein on chromosome 20q13 (GNAS), causing a fibrous displacement of bone tissue (61, 88-90). Sixty percent presents as a mono-ostotic form, and 40% as a poly-ostotic disease. In less than 5%, the poly-ostotic form is associated with precocious puberty, other endocrine dysfunctions (hyperthyroidism, hypercortisolism, hyperprolactinaemia and renal phosphate wasting) and café-au-lait skin pigmentation, an entity which is called the McCune Allbright syndrome (89, 91). Symptoms of this bone disease are bone pain, deformations of the bones and in some cases pathological fractures, and occur in 80% before 15 years of age. Osteosarcoma occurs rarely, in 0.4%-1.6% of the cases of fibrous dysplasia in larger series (92, 93), either in the mono-ostotic or poly-ostotic form (56, 94-98). However, this proportion might be overestimated because almost the half of the patients were irradiated for fibrous dysplasia (93). Several case reports of osteosarcoma in the McCune-Allbright (99-102) or in fibrous dysplasia in association with muscular myxoma's, the Mazabraud syndrome have been published (103-106).

Osteosarcoma in other benign conditions

Osteosarcomas have sporadically been reported to occur in chronic osteomyelitis (107, 108), bone infarcts (109-111), Giant cell tumours (112-115), solitary (116-118) or multiple osteochondroma (119, 120), osteogenesis imperfecta (121, 122), aneurysmal bone cysts (108, 123, 124) or solitary bone cysts (108, 125). There is no explanation for a relationship between these benign conditions and the development of osteosarcoma (108).

Prognostic factors in Osteosarcoma

Prognostic factors in osteosarcoma are related to the *tumour features* (volume, location, histological subtype, pathological fracture and the presence of metastases at diagnosis) or *related to its treatment* (chemotherapy regimen, type and timing surgery, completeness of surgery of the primary tumour and, chemotherapy induced histologic response of the tumour). In this introduction large studies have been chosen to avoid missing significant prognostic factors (126)(table 4). In this table the results of the most powerful prognostic factors for overall survival are shown, as result from multivariate analysis in these studies (38, 127-132). An extensive list of molecular markers is not included in this table, because the emphasis of this introduction is clinico-pathological markers.

TABLE 4.
Clinico-pathologic factors related to overall survival in osteosarcoma found to be relevant after multivariate analysis in 7 large studies.

Author (ref)	No. patients	Prognostic factor	HR	95% CI	p-value	comment
Whelan (132)	1067	early time line surgery	1.80	1.17-2.76	0.007	stage IIb
		female gender	0.79	0.64-0.99	0.036	
		distal site tumour	0.66	0.51-0.87	0.003	
		good histol resp	0.48	0.38-0.61	<0.001	
McTiernan (131)	533	Nausea/vomiting gr 1-2	0.37	0.16-0.85	0.020	stage IIb
		good histol response	0.48	0.38-0.61	<0.001	
		Thrombocytopenia gr 1-2	0.49	0.27-0.87	0.016	
		0ral mucositis gr 3-4	0.51	0.29-0.91	0.023	
		distal site in bone	0.66	0.51-0.87	0.003	
		female gender	0.79	0.64-0.99	0.036	
Pakos (130)	1135	metastatic disease	6.59	4.77-9.09	< 0.001	all stages
		poor histol resp	1.67	1.29-2.16	< 0.001	patients > 1990
		surgery: amputation	1.56	1.20-2.03	0.001	
		site bone: tibia	0.66	0.51-0.88	0.004	CT (≥2 drugs)
Bacci (128)	789	protocol CT IOR 1/2/3	2.3/1.5/1.6	1.0-3.4	0.008	stage IIb
		AF elevated	2.1	1.6-2.7	<0.0001	stage IIb
		poor histol resp	2.0	1.6-2.6	< 0.0001	
		tumour volume ≥ 200 ml	1.4	1.1-1.8	0.01	
		surg margin inadequate	1.3	1.0-1.7	0.044	
		age ≤ 14 yr	1.3	1.0-1.7	0.044	
Petrilli (129)	225	poor histol resp	3.15	1.61-6.17	0.001	all stages
		metastatic disease	3.02	1.72-5.29	< 0.001	
		size T > 12 cm	1.93	1.20-3.12	0.007	
Smeland (127)	113	gender male	3.7	1.59-8.66	0.002	stage IIb
		volume T > 190 ml	2.4	1.18-5.05	0.017	
		mean MTX_{t24} > 4500 μM	0.4	0.21-0.88	0.017	
Bielack (38)	1702	residual T > surgery	4.01	2.66-6.04	< 0.0001	for all sites
		poor histol resp	2.44	1.98-3.01	< 0.0001	
		metastatic disease	1.88	1.33-2.65	0.0003	
		axial site	1.87	1.25-1.80	0.002	
		tumour size > 1/3	1.30	1.08-1.56	0.005	only sign for EFS

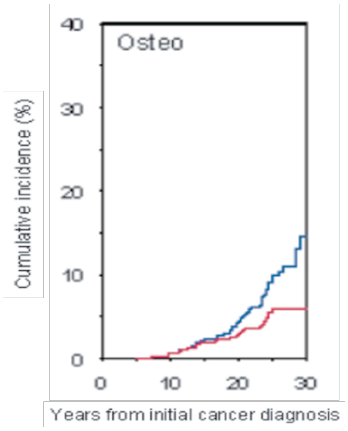
The most important *patient related* prognostic factors for poor overall survival include metastatic disease at diagnosis, large tumour volume and proximal or axial tumour site, whereas chemotherapy induced toxicity was prognostic favourable .
Treatment related prognostic factors are incomplete surgery resulting in residual disease, effectiveness of the chemotherapy regimen and a poor histologic response to pre-operative chemotherapy. Axial site is related to the difficulty in getting a complete resection (38), and early time-line surgery is often necessary in case of early progressive disease (132), which biases the outcome of the disease obviously. Young age as a relevant poor prognostic factor was relevant in the study of Bacci et al (128), but not in other studies (38, 132). Males had an unfavourable outcome relative to females in 2 studies (127, 132), but not in the 4 others (38, 128-130). Although histologic response to preoperative chemotherapy is one of the most important prognostic factors for survival of osteosarcoma, a recent trial from the European Osteosarcoma Intergroup (EOI) did not find a better event-free or overall survival in patients who were treated with a more dose-intensive chemotherapy arm despite the fact that this group showed a significant higher proportion of histologic good responders (133). These results called the use of histological response as measure for outcome into question. This issue will be extensively discussed in **chapter 2**.

Secondary malignancies, second malignancies following Osteosarcoma, and Osteosarcoma as 2nd malignant disease

Contemporary average survival in cancer in general is about 60% (3). In children the overall survival is higher, around 70%-80% (42). Due to the high survival, a growing population of survivors is at risk for the development of second malignancies. The cumulative risk for a second malignant disease is between 3.1% after 25 years to 7.9% after 30 years (6, 134-136). After treatment for cancer in childhood, a lifelong risk on a second malignancy was between 3.3 to 9.2-fold higher than in the general population (5-7, 134, 135). The cumulative incidence of subsequent malignant neoplasms following osteosarcoma is on average 1.3% after 10 years, 5.2% after 20 years and 6% after 30 years (figure 5) (6, 137-144), representing a 3.5-fold higher risk of a malignancy than the general population. Radiotherapy, female gender, genetic factors, such as the Li-Fraumeni syndrome or Rothmund-Thomson syndrome, young age at primary diagnosis and possibly chemotherapy, particularly alkylating agents are risk factors for the development of subsequent neoplasms.

FIGURE 5.

Cumulative incidence of 2nd primary malignancies after osteosarcoma. Blue line is all subsequent neoplasms, red line is subsequent malignant neoplasms (SMN). Estimated Cumulative incidence for a SMN is after 30 years is 6%(3.9%-8.1%) 95% CI. From ref (6).



Bone tumours in general account for 6.5% (3.3%-9.9%) of all second malignancies (5-7, 134, 136), representing a 20-30 fold excess risk compared to the normal population (6, 7, 134). Although the exact incidence of osteosarcoma as secondary malignancy is difficult to establish, because of the different results in the several data bases, the relative risk on a 2nd osteosarcoma after treatment for a previous malignancy is calculated to be 22-133 fold (6, 134, 139, 142, 143, 145-149).

Irradiation as risk factor for osteosarcoma has been recognized since 1929 in radium dial painters osteogenic sarcoma was observed as consequence of their work (150). Osteosarcoma after radiotherapy represents about 3.2% (1.0%-5.5%) of all osteosarcomas (40, 41, 147, 148, 151-161). Radiotherapy-induced osteosarcomas differ from conventional osteosarcomas in that the male-female ratio is equal (male:female = 0.98:1), and present more often in axial sites or skull, and less in extremity sites. In general, the same rate of metastases as in conventional osteosarcoma, around 13% have been reported in 5 studies (147, 154, 155, 157, 161). Two other studies documented an higher proportion of metastases rate at diagnosis, more than 20% (148, 160).

The average latency time between radiotherapy treatment and the occurrence of the subsequent osteosarcoma was 11.5 years but shorter latency times, for example 3-5 years, do not rule out radiotherapy induced osteosarcomas (151, 154-157). In most of these cases genetic causes, such as hereditary Retinoblastoma or the Li-Fraumeni syndrome contribute to the occurrence of these osteosarcomas (153). It also has been suggested that the latency was

shorter after concomitant use of chemotherapy than when radiotherapy was used as single treatment in some studies (145, 152, 156, 159, 160) or in younger patients (151).
Despite previous contradicting reports (148, 151, 162), aggressive treatment with neo-adjuvant chemotherapy and surgery leads to an overall survival of radiotherapy induced osteosarcoma between 40%-50% (147, 154, 157, 163), which is close to the outcome in primary conventional osteosarcoma. Adequate surgical margins are highly important for curative treatment in this subtype of osteosarcomas (147, 154, 160). Due to the localization of secondary osteosarcomas in the axial sites, adequate margins often are difficult to be achieved (147). Multi-agent chemotherapy has successfully been used in patients with secondary/ radiotherapy induced osteosarcoma despite the modifications that need to be given because of prior treatment with cytotoxic agents (147, 154, 160, 161).

Germline mutations and Osteosarcoma

It has been estimated that 1%-10% of all childhood cancers arise in the context of cancer predisposition syndromes (164, 165). Reports of familial clustering of osteosarcoma have been restricted to sparse case-reports and small series (table 5) (166-177).
Osteosarcoma is one of the cancers that has been associated with syndromes like the Li-Fraumeni syndrome, (hereditary) Retinoblastoma, and with RECQL-helicase mutation syndromes like the Rothmund-Thomson-, RAPADILINO-, Baller-Gerold-syndrome and others, as listed in table 6.

TABLE 5.
Case-reports of familial occurrence of osteosarcoma in the literature. Hillmann reviewed 41 other cases in the literature that were not listed in this table (166).

		Type	
Relation	**Age (year)**	**Osteosarcoma**	**Author (ref)**
sister-brother	11 and 12	(telangiectatic) OS	Ottaviani (167)
2 brothers	18 and 21	osteo-/chondroblastic	Chin (168)
son-father	13 and 44	HG conventional/osteoblastic	Longhi (169)
2 brothers	15 and 21	HG conventional/chondroblastic	
brother-sister	14 and 11	osteoblastic (both)	Hillmann (166)
brother-sister	11 and 14	osteoblastic/sclerosing	Danckwerth (170)
2 cousins	11 and 8	Telangiectatic	Nishida (171)
2 sisters	17 and 15	not specified	Miller (172)
sister-brother	11 and 9	not specified	Schimke (173)
daugther-father;	6 and 25	not specified	Swaney (174)
2 brothers	10 and 4	not specified	
daugther-father	13 and 40 (2x)	not specified	Epstein (175)
2 cousins	22 and 18	not specified	Robbins (176)
2 sisters, 2 brothers	11, 15, 20 and 22	HG conventional (3x), fibroblastic	Harmon (177)

Li-Fraumeni syndrome

The Li-Fraumeni syndrome (LFS) is a clinical and genetic heterogeneous cancer predisposition syndrome with multiple early onset sarcomas and other tumours within an individual (proband) and in first and/or second degree relatives in the same lineage (178, 179, 182); OMIM, MIM ID #151623. The most frequent tumours in LFS are osteosarcomas and soft tissue sarcomas, premenopausal breast cancer, brain tumours, adrenocortical carcinoma and leukemias (178, 182, 183).

TABLE 6.
Hereditable syndromes that have been related to the occurrence of osteosarcoma.

Disease/syndrome	Gene	Chromosome	Gene Function	Inheritance	Spectum of Cancers	References
Li-Fraumeni syndrome	p53	17p13.1	TSG	AD	(osteo-)sarcoma, breast cancer, adrenocortical carcinoma	(178-185)
Retinoblastoma	Rb	13q14.1-q14.2	TSG	AD	Retinoblastoma, osteosarcoma, soft tissue tumours	(186-192)
Rothmund-Thomson	RECQL-4	8q24.3	DNA-helicase	AR	osteosarcomas (32%), squamous cell carcinoma, basal cell carcinoma, myelodysplasia	(193-197)
RAPADILINO	RECQL-4	8q24.3	DNA-helicase	AR	mainly osteosarcomas	(198-200)
Baller-Gerold	RECQL-4	8q24.3	DNA-helicase	AR	one osteosarcoma	(201, 202)
Bloom	RECQL-3	15q26.1	DNA-helicase	AR	leukemia/lymphoma, carcinoma's (skin, breast, colon), osteosarcoma, Wilms' tumour	(203, 204)
Werner	RECQL-2	8p12-p11.2	DNA-helicase	AR	thyroid carcinoma, skin-, melanoma, soft tissue sarcoma; osteosarcoma (7%); meningeoma; hematologic	(204-207)
unknown	RECQL-1	12p12.1	DNA-helicase	?	unkown	(193)
Paget Sporadic	SQSTM1	5q35-qter	ubiquitin binding protein	AD	Osteosarcoma, undifferentiated sarcoma, fibrosarcoma	(78, 80, 208)
Paget Familial	TNFRSF11A	18q21-22	TNF-receptor super fam-11	AD	osteosarcoma, Kaposi sarcoma, chondrosarcoma	OMIM 601530, (82, 83, 85, 209)
Familial Expansile Osteolysis	TNFRSF11A	18q21-22	Rank signalling	AD	osteosarcoma	OMIM 174810,(84)
McCune-Albright (MAS)/ Mazabraud syndrome	GNAS	20q13.32	G-protein α- subunit	somatic mutation	osteo-and chondrosarcoma, breast- and thyroid cancer	(99-102, 104-106, 210)

TABLE 7.
Criteria for classical Li-Fraumeni syndrome (LFS), Li-Fraumeni-like syndrome (LFS-L), Chompret and revised Chompret criteria (185).

Classical LFS	Proband with sarcoma at age < 45 yr **AND**
	A first degree relative with any cancer at age <45 yr **AND**
	Another 1st or 2nd degree relative with either cancer at age < 45y **OR**
	a sarcoma at any age
Li-Fraumeni like	Proband with any childhood cancer or sarcoma, brain- or or adrenal cortical tumour at < 45 yr **AND**
syndrome (Birch)	First or 2nd degree relative with a spectrum tumour* at any age **AND**
	First or 2nd degree relative in the same lineage with any cancer < 60 yr
LFS-Chompret	Proband affected by spectrum tumour < 36 yr, **AND**
Criteria	≥ 1 first or 2nd degree relative with a spectrum tumour** < 46 yr or multiple primary tumours **OR**
	Proband with multiple primaries, 2 of which are spectrum tumours and the first at < 36 yr **OR**
	Proband with adreno-cortical tumour at any age
LFS-Chompret	Proband with spectrum (incl lung broncho-alveolar) tumour < 46 yr and ≥ 1 first or 2nd degree relative
criteria revised	with an spectrum tumour** < 56 yr or multiple primary tumours **OR**
	Proband with multiple primary tumours, 2 of which are spectrum tumours and the first at < 46 yr **OR**
	Proband with adreno-cortical tumour or choroid plexus tumour, irrespective family history
	* spectrum tumours are: bone or soft tissue sarcoma, pre-menopausal breast cancer, brain tumour, adrenal cortical carcinoma (leukemia/lymphoma); narrow spectrum cancer with hematologic cancers
	** other than breast cancer if the proband is affected by breast cancer

The classic criteria of for the LFS (178), the Li-Fraumeni-Like syndrome (LFL) (211), the Chompret criteria (212) and revised Chompret criteria (213) are listed in table 7, based on Ruijs (185). Birch reduced the age for families that were suspected for LFS (182). Chompret studied the incidence of unaffected mutation carriers or patients with multiple primary

cancers (212) and Tinat extended the Chompret criteria with respect to age at onset of the LFS-spectrum tumours in order to cover families with identified TP53 mutations (213). Malkin et al. demonstrated that germline mutations of the p53 gene were responsible for the excess of cancers in these families (179). Subsequent studies showed that 50%-85% of the families fulfilling the classical criteria for LFS harbour a germ-line TP53 mutation (180, 184). TP53 negative cases in classical LFS families are explained by de novo mutations, which occur in 7%-20% of the cases (214), posttranslational p53 alterations, abnormalities in regulation or modifier genes, or other genes that are of influence on the phenotype (for review see Malkin 2011(215)). The prevalence of osteosarcoma in families that met the classical LFS-criteria varied between 6%-16% (table 8) (178, 179, 183, 216, 217), not different from when other criteria are use, like the LFL-syndrome (182, 184, 218) or Chompret-criteria were used (181, 185).

TABLE 8.
Incidence of osteosarcoma (OS) in Classical Li-Fraumeni syndrome, Li-Fraumeni like syndrome (LFS/LFL; Birch criteria) and in the Li-Fraumeni syndrome according to the Chompret criteria (LFS-Chompret).

Author (ref)	Li-Fraumeni criteria	prevelance OS in LFS syndrome
Li (178)	Classical LFS	12.0%
Li (216)	Classical LFS	15.7%
Malkin (179)	Classical LFS	6%
Hisada (217)	Classical LFS	12.5%
Nichols (183)	classical LFS	12.1%
Varley (180)	LFS/LFL (Birch)	12.3%
Birch (182)	LFS/LFL	6.8%
Olivier (184)	LFS/LFL	14.9%
Chompret (181)	Chrompet	17.6%
Ruijs (185)	LFS/LFL/Chrompet	8.5%
	LFL/TP53 mut pos	0%
	LFL/TP53 mut neg	3.6%

However, Ruijs found the highest incidence of bone tumours in families with the typical Li-Fraumeni syndrome criteria (8,5%), which was higher than using the LFL-criteria of Birch (0-3.6%) (185). Mutant TP-53 was found in 26.3% of the sporadic osteosarcoma cases (table

9) (219-231), whereas only 4.9% of the investigated patients had a germ-line mutation (232-234). These germline mutations were often reported in patients without typical LFS history. This suggests that although TP53 alterations contribute significantly to the sarcomagenesis of osteosarcoma, familial cases are present in less than 5% of the cases.

TABLE 9.

Proportion of osteosarcoma patients with TP53 abnormalities, detected by southern blot (SB), (PCR)single strand conformation polymorphism (SSCP), immunehistochemistry (IHC), Microsatellite analysis (MSA) or DNA sequencing (DNA-seq). In 2 reports, no correlation with clinical features were reported (NR).

Author (ref)	Number OS	TP53 alteration (%)	Technique	Clinical correlation
Wunder (231)	196	19.4%	SB, SSCP	no
Entz-Werle (230)	54	53%	MSA, PCR	no
Kawaguchi (229)	23	21.7%	SSCP, DNA-seq	no; older age
Gokgoz (228)	272	22.1%	SSCP	no
Tsuchiya (227)	30	50%	SB, SSCP	EFS
Goto (226)	32	40.6%/28.1%	MSA, IHC	PR
Yokoyama (225)	17	23.5%	SSCP	no
Lonardo (224)	83	26.5%	IHC	no
Pellin (223)	19	21.1%	IHC and SSCP	no; older age
Miller (222)	42	30.1%	SB, SSCP	NR
Ueda (221)	18	27.7-61.1%	IHC	no
Toguchida (232)	76	23.7%	SB, SSCP	no
Miller (219)	60	18.3%	SB	NR

From table 9 can also be concluded that no relationship could be established between abnormalities of the TP53 and clinical features, e.g. progression of disease, survival or response on chemotherapy of osteosarcoma. Some authors found that TP53 rearrangements were more frequently encountered in older patients. This suggests that the TP53 is an early event in tumorigenesis, for example inducing chromosomal instability in osteosarcoma (235), rather than an indicator for tumour progression.

The lack of concordance between TP53 mutation and outcome might be related to germ-line variations in TP53 (215, 236) or polymorphisms in modifier genes, such as the MDM2 SNP309 variation (237) although most of the reports in table 9 ruled out normal variants.

Retinoblastoma and Osteosarcoma

Retinoblastoma (Rb) is a tumour of the retina and occurs in 3% of the childhood cancers (238). It occurs in 60% of the cases as a non-heritable and unilateral form, in 10%-15% as an unilateral but heritable disease and in 25%-30% as an heritable, bilateral disease (192, 239).

TABLE 10.

Number of patients with a secondary malignancy (SMN) and osteosarcoma (OS) as proportion of the 2nd maligancy in heritable Retinoblastoma (her Rb) or in sporadic Retinoblastoma (non-her Rb).

				SMN (number)		OS as SMN	
Author (ref)	**No Rb-pat**	**Hereditary RB**	**No SMN's**	**hereditary Rb**	**non-her Rb**	**hereditary Rb**	**non-her Rb**
MacCarthy (192)	1927	809	121	108	13	32	2
Marees (191)	668	298	74	62	12	15	0
Acquaviva (190)	1111	408	38	31	7	10	0
Mohney (189)	180	82	20	17	3	4	0
Wong (240)	1604	961	199	190	9	70	0
Fontanesi (186)	172	65	6	6	0	4	0
	5662	2623 (46.3%)	458 (8.1%)	414 (15.8%)	44 (1.4%)	135 (32.6%)	2 (4.5%)

Based on the fact that the Rb-gene is a tumour suppressor gene, mutations in this region implicate a higher incidence of cancer. Table 10 shows the 10-fold higher incidence of second malignancies in hereditary retinoblastoma than in sporadic retinoblastoma, which is explained by the genetic susceptibility of these patients for subsequent cancers. Osteosarcomas comprise nearly 1/3 of all second malignancies in long term survivors of retinoblastoma, and all but two patients with osteosarcoma fall into the group of hereditary Retinoblastoma. This suggests a relation between the genetic defect in hereditary retinoblastoma and osteosarcoma.
The risk of getting an osteosarcoma after hereditary retinoblastoma is around 500-fold (191, 241). The high risk on secondary osteosarcoma among survivors of retinoblastoma is for a part explained by the use of radiotherapy, although patients who were not treated with radiotherapy also have an high risk on getting an osteosarcoma during their life (242, 243). The different latency periods of osteosarcomas that develop inside and outside the radiation field suggest that multiple genes are involved in the radiation induced tumours in these patients, but one of these gene is the mutated Rb-gene (242). Retinoblastoma has been a model for carcinogenesis since the 2-hit hypothesis has been published by Knudson (244). The penetrance of this autosomal dominant disorder is complete in the bilateral disease, but not in the unilateral disease (245). Non-sense or frameshift mutations and splice mutations account for the most of the gene abnormalities in Retinoblastomas (246-248). Somatic

abnormalities in the Rb-gene were found in 14%-72% patients with osteosarcoma, using either LOH-analysis (234, 249-253), Southern or Northern Blotting (222, 249, 250, 254-256), Immunostaining or gel electrophoresis (table 11) (223, 250, 257). Some investigators found a correlation between Rb-LOH and clinical parameters as survival (234, 252) or metastases formation (257), whereas others could not confirm that (253).

TABLE 11.

Frequency of abnormalities in the Retinoblastoma (Rb) gene in patients with osteosarcoma. See table 9 for abbreviations. LOH is loss of heterozygosity. MI is microsatellite instability.

Author (ref)	patients	technique	mutation rate	comment
Heinsohn (253)	41	LOH	39%	LOH not progn sign
Patiño-Carcia (234)	76	LOH	37%	LOH Rb associated with reduced (E)FS
Benassi (257)	39	IHC; EF	53%	pRb- associated with metastases ($p<0.05$)
Pellin (223)	19	IHC; EF	26%	no progn correlation with clinical parameters
Belchis (251)	18	LOH	72%	LOH or MI in 14/18; MI in 8/18 (44%); LOH in 72%
Feugeas (252)	34	LOH	71%	EFS $Rb^{-/-}$ 43%, EFS $Rb^{+/+}$ 100% ($p=0.008$) and $Rb^{-/+}$.
Miller (222)	37	SB	19%	60% had alterations in Rb and/or p53
Wadayama (250)	63	LOH	63%	LOH not necessarily correlated with
		SB	29%	inactivation Rb gene at protein level
		IHC	54%	
Scholz (256)	14	SB	14%	1 DNA abn samples also had no prot exp
		NB	4/8 -, 2/8 -/+, 2/8?	5 prot def samples had no Rb abn
Araki (254)	23	SB; NB	35%	1 $SB^{-/+}$ had also $NB^{-/+}$; 4 cases $NB^{-/-}$ were $SB^{+/+}$
Wunder (255)	12	SB; NB	50%	All $Rb^{-/-}$ or $Rb^{-/+}$ abnormal Rb-RNA expression
Toguchida (249)	30	LOH	64%	40% of the OS patients has Rb abn.
		SB	43%	LOH and SB do not correlate

RECQL-Helicases mutation syndromes

RECQL-helicases are a highly conserved family of genes and proteins that have an important role in adapting to cellular stress, and thereby maintaining genomic stability, preventing epigenetic drift and early senescence (204, 258-260). Examples of abnormal repair in humans

are present in rare genetic disorders of the RECQL genes as listed in table 6. Of the 5 known RECQL-genes in humans, 3 are known with a recessive inherited gene mutations, leading to the Werner syndrome (RECQL-2 mutations) (261), Bloom syndrome (RECQL-3 mutations) (203) and the RECQL-4 mutation spectrum syndromes: Rothmund-Thomson (RTS), RAPADILINO and Baller-Gerold syndrome (reviewed in Lindor 2000 (193)). Mutations in the RECQL-1 and RECQL-5 genes are not known to cause syndromes or diseases, particular no cancer. Particularly in the RTS, the incidence of osteosarcoma is extremely high (194-197). Osteosarcoma in patients with RTS behaves almost similar as in non-syndrome patients. Age at presentation was lower in some series (195, 262, 263), but location, response to pre-operative chemotherapy and outcome was like sporadic osteosarcoma. However the proportion metachronous or secondary tumours in RTS patients was 17% compared to the 2.6%-5.4% in sporadic osteosarcomas, reflecting the genetic basis in syndromatic patients (263).
Not all germline RECQL-mutations have the similar genetic consequences for the development of osteosarcoma. In the Werner syndrome (RECQL-2 mutation), the incidence of osteosarcoma is 7.6% of 157 cancers (205), the peak age was 35-55 years and the osteosarcomas were located in unusual sites in the skeleton, for example the patella, the radius or the foot (205-207, 264). The incidence of osteosarcoma in the Bloom syndrome (RECQL-3 mutation) was low, not more than 2%, but still higher than in the general population (203). Overall, these syndromes with germline mutations in RECQL helicases 2,-3 and -4 predispose to an increased risk of osteosarcoma (265).

Paget's disease, Familial Osteosarcoma and the McCune-Allbright/Mazabraud syndrome
For osteosarcoma in Paget's disease, see paragraph 1.4.2., for familial osteosarcoma see the introduction of this paragraph. Osteosarcoma in McCune-Allbright/Mazabraud syndrome, see paragraph 1.4.2.2.

Pathology
The term osteosarcoma historically developed from osteogenic sarcoma (266) which encompassed all tumours derived from bone. From the period after 1946 osteosarcoma is defined as a primary, intramedullary high-grade bone tumour producing malignant osteoid (267, 268).
Since the first 2 editions of the WHO classification used a similar framework, based on histologic criteria (270) progress in biological and genetic understanding of these malignancies was made. In 2002 the third revision of the classification of bone and soft tissue tumours was published, which integrated morphological data with tumour specific cytogenetic and molecular data (269). Table 12 shows the different subtypes of osteosarcoma, based on the WHO-classification 2002. The unusual histological forms of high-grade conventional osteosarcoma will be discussed here in more detail, because in **chapter 6** of this thesis some of these rare subtypes are more present among the patients.

TABLE 12.
Subtypes of osteosarcoma according to the site in the bone (269)

SITE IN BONE	GRADE of MALIGNANCY	TYPE	SUBTYPE
Intramedullar	*High*	Conventional OS	Osteoblastic
			Chondroblastic
			Fibroblastic
		Unconventional OS	Osteoblastic-sclerosing
			Osteoblastoma resembling
			Chondromyxoid fibroma-like
			Chondroblastoma-like
			Clear-cell
			Malignant fibrous histiocytoma-like
			Giant cell rich
			Epitheloid
		Teleangiectatic OS	
		Small Cell OS	
		Secondary Osteosarcoma	M.Paget
			Post-Irradiation
			In various bone diseases
	Low	Low Grade Central OS	
Surface	*High*	High-Grade Surface OS	
	Intermediate	Periosteal (Juxta Cortical Chondroblastic OS)	
	Low	Parosteal (Juxta-cortical OS)	
Intra-cortical	*High*		
Extra-Skeletal	*High*		

Conventional High-Grade Osteosarcoma

The proportion of high-grade conventional osteosarcoma is between 70%-90% of all osteosarcomas in larger studies (2, 269, 271, 272). Histologically, the malignant cells of an osteosarcoma consist of anaplastic, pleomorphic spindle cells, although other forms can be present like epitheloid, plasmacytoid, ovoid, round or fusiform cells or the tumour may contain multinucleated giant cells. The malignant osteoid, formed by the pleomorphic tumour cells, is highly variable in thickness and ranges from tiny amounts to a frank ossifying tumour, as is visible on a plain radiograph. Besides osteoid, high-grade conventional osteosarcoma can also produce cartilage and/or fibrous tissue. Depending on the amount of matrix, conventional osteosarcoma is divided into osteoblastic (50%), chondroblastic (25%) and fibroblastic (25%) (see also table 12). This subdivision has no prognostic value, because the outcome in these three subgroups did not differ, despite a significant better response rate among the fibroblastic group (271).

Unconventional types of high-grade conventional Osteosarcoma

Sclerosing subtype Osteosarcoma

The sclerosing subtype of osteosarcoma has been classified by most authors under multifocal osteosarcoma (273-281). This multifocal variant was diagnosed in young patients, age 5-16 years, and is characterized by multiple foci of high-grade osteosarcoma, sclerotic on the radiographs and all localized in the metaphyseal part of the long tubular bones. The clinical behaviour of this variant is very aggressive, most patients died within 1 year after diagnosis with widespread disease. However, not all sclerosing variants are clinically highly malignant (282-289). These variants of sclerosing osteosarcoma are of low to intermediate grade, occur generally in older patients, are located predominantly in multiple sites of the axial skeleton and skull, either with or without involvement of long bones or occur as recurrent disease. This subtype is mentioned here only for completeness to define this subtype of osteosarcoma appropriately.

Osteoblastoma-like subtype of Osteosarcoma

The osteoblastic resembling subtype of osteosarcoma has been estimated to occur in less than 1.5% of all osteosarcomas (290). The localization in 33 cases differs from HG conventional osteosarcoma. Thirty nine percent of the cases are found in the axial skeleton and skull, and 61% in the appendicular skeleton (290-296). The most common involved bone was the tibia, and this subtype presents often in unusual sites like the foot (290, 291, 296) or rare locations in the bone, like the condyles of the femur (294, 295). As other high-grade osteosarcoma, osteoblastoma-like osteosarcoma has a similar tendency to metastasize as the conventional subtype of osteosarcoma (290).

Chondromyxoid fibroma like Osteosarcoma
This type has been described in 2 case reports (297, 298) and by Mirra (299). Although this subtype has been described as a low grade osteosarcoma (297, 299), local and systemic recurrences were described in both patients, with an unusual metastases in the left atrium (297).

Chondroblastoma-like Osteosarcoma
Schajowicz published one case of chondroblastoma-like osteosarcoma in the tibia of a 12-year old boy with a tumour located in the diaphysis of the femur (300). Clinically, the tumour was highly malignant as was demonstrated by a fast local growing and recurrence tendency and the development of pulmonary metastases, one months after resection.

Clear Cell type Osteosarcoma
Four cases of the clear cell type osteosarcoma have been described, in 3 children and one adult patient (301, 302). All lesions were located around the knee, in the meta-epiphyseal part of the distal femur in 3 cases or proximal tibia in one case. Two of the 4 patients died from metastases, and the follow up of the other 2 was less than 1 year.

Malignant Fibrous Histiocytoma type of Osteosarcoma
This subtype of osteosarcoma needs to be distinguished from Malignant Fibrous Histiocytoma of bone (MFH) (303-306). Nine patients were reported, aged 8-75 years, with lytic lesions in the meta-epiphyseal part of the distal femur (n=6) or the proximal tibia (n=2) (304, 305). MFH-like osteosarcoma has no p53 overexpression and a low Ki-67 labelling index compared with conventional osteosarcomas or MFH of bone (306). The reports are conflicting with respect to clinical aggressiveness. Whereas Ballance reports the development of pulmonary metastases in 4 of 6 patients within one year (304), Naka finds an 5-year overall survival of 67% in 7 cases (306).

Giant cell rich Osteosarcoma
Giant cell rich osteosarcoma has been reported to occur in 0.6%-3% of all primary osteosarcoma and is defined as an undifferentiated sarcoma with scanty tumour osteoid and an abundance of osteoclast-like giant cells (41, 307, 308). Fifteen cases were described in detail (307-311). All but one showed ill-defined lytic lesions, with a wide zone of transition, located in the meta-diaphyseal part of the femur(n=10) or tibia (n=4) and one was located in the navicular bone of the foot (311). The mean age was 21(6-41) years, older than in conventional high-grade osteosarcoma, but younger than in giant cell tumours of the bone. The differential diagnosis is high-grade conventional osteosarcoma with giant cells (more abundant osteoid), telangiectatic osteosarcoma (septae with sarcomatous cells), giant cell tumours of bone (epiphyseal location in the bone) and aneurysmal bone cyst (no malignant cells) (307, 308, 311). Prognosis is difficult to give, because the few well documented cases, but seems to be similar as in conventional high-grade osteosarcoma (307, 312)

Epitheloid Osteosarcoma
In the epithelial subtype of osteosarcoma, epitheloid-differentiated osteoblasts are arranged in nesting or gland-like structures, admixed with osteoid producing malignant spindle cells, forming a biphenotypic tumour (313, 314). The histological picture resembles (metastatic) carcinoma (315, 316). Variable immunohistochemical expression of cytokeratins, vimentin or epithelial membrane antigen have been reported in these cases (313, 316-320). Overall a male predominance is observed, an average age of 29 years, ranging from 4.5-66 years, and most often, the osteosarcomas are located in the femur (313, 315-323). Apart from a poor outcome in the Rosette-formed epithelial subtype, prognosis is similar as in high-grade conventional osteosarcoma (324).

Teleangiectatic Osteosarcoma
This variant of osteosarcoma is defined as a one forming tumour, characterized by large spaces, filled with blood with or without septa (325). This type has been reported to occur in 5% (0.9%-11%) of all cases of osteosarcoma (2, 40, 325-330). Radiologically, it is an aggressive, purely lytic lesion, with destruction of the cortex, periosteal reaction, soft tissue invasion and a relatively high proportion of pathological fractures (40, 325, 328, 330, 331). Mineralization, typical for osteosarcoma, is scant on plain films but can best be shown by CT. MRI is effective in distinguishing a telangiectatic osteosarcoma from other types or benign blood filled lesions by marrow replacement on T1-, and high signal on T2-weighted images (40, 332). With contemporary neoadjuvant chemotherapy, outcome in patients with this variant are similar (328, 330, 333) or even better (329) than conventional osteosarcoma.

Small cell Osteosarcoma
The small cell variant of osteosarcoma is composed of small cells with a variable degree of osteoid production (334). The mean incidence rate from 4 different studies is 2.2% (1.1%-4%) (335-337). Males and female were equally affected in 147 cases, dissimilar like conventional osteosarcoma (40, 335-337). Age distribution shows the highest incidence in the adolescent and young adult group, and the localization in the skeleton were similar like in conventional osteosarcoma, with a relatively a high proportion (18%) located in the humerus. This variant of osteosarcoma has to be distinguished from Ewing sarcoma, another small cell tumour of bone, which is sensitive for radiotherapy, in contrast to the small cell osteosarcoma. This can be done using the characteristic translocation t(11;22) in Ewing sarcoma, which is not present in small cell osteosarcoma. Although small cell osteosarcoma seems to be sensitive for platinum analogs (335), survival is worse than in HG conventional osteosarcoma, although this is based on older reports, with less effective medical treatment (335, 337, 338).

Low-grade central Osteosarcoma
A low grade central osteosarcoma is a well differentiated subtype, arising from the medullary cavity of the long tubular bones (339). This subtype has a better prognosis than its high-grade counterpart but also has another location than other non-high-grade subtypes, parosteal and

peri-osteal osteosarcoma (339-341) and accounts for approximately 1-2% of all osteosarcomas (40, 339-341). The age at presentation is generally around the 3rd decade, and patients have a prolonged history of symptoms of on average 1 year of nonspecific pain with or without swelling in the diaphyseal site of the femur or tibia. Occasionally, a low grade central osteosarcoma is diagnosed in the small bones of the hand or foot (340-342) or in the flat bones of the ribs (340, 342, 343) or skull (340, 344). Histological, the low grade osteosarcomas are hypo-to moderate cellular spindle cell tumours with slight atypia and occasional mitotic figures, irregular bone formation in a parosteal, desmoid or fibrous dysplasia like pattern (340). If this tumour is inadequately excised, progression into higher grade of malignancy occurs in 15% of the patients with recurrence (340, 341, 345, 346) with the potential for developing distant metastases and leading to death. Dedifferentiation not only occurs at recurrence, but has been reported at diagnosis in rare instances (347-349).

Surface Osteosarcomas

Surface osteosarcomas arise by definition from the surface from the bone, and can be of high-grade (high-grade surface osteosarcoma, also known as juxtacortical osteosarcoma), intermediate grade (periosteal osteosarcoma; juxtacortical chondroblastic osteosarcoma) or of low grade (parosteal osteosarcoma or juxtacortical osteosarcoma) (350-352).

TABLE 13.
Characteristics of 3 types of surface osteosarcomas. FU follow-up; LR local relapse; SR systemic relapse; OAS overall survival; DOD death of disease; OS osteosarcoma.

	Parosteal	Periosteal	High-Grade Surface
incidence	4%	1.5%	< 1%
male:female	1:1.4	1.4:1	4:1
peak incidence (decade)	3rd	2nd	2nd-3rd
symptoms	swelling ≥ pain	swelling ± pain	pain + swelling
duration of symptoms	1-5 yr	0.1-2 yr	< 1 yr
involved bones	femur, tibia	tibia, femur, ilium	femur, tibia, radius
involved site bone	distal meta-diaphysis	shaft, prox/dist	shaft/dia-metaphyseal
specific X-feature	broad based sclerotic, lobulated mass	small, cortical, lytic, extending soft tissue	broad based T, cortex destruction, periost ++, medullary involvement
metastases at Diagnosis	no	no	no
metastases during FU	30% LR, 6% SR	LR in 22%; 17% SR	12-26% LR, 5y-OAS 46-82%
preferred treatment	wide excision	wide resection	as HG conventional OS
prognosis	91% 5y OAS	17% DOD; 10y OAS 84%	as HG conventional OS
typical histology	hypocellular stroma with absent to moderate atypia (≤ 20%) between well formed bony trabeculae	appearance of moderately differentiated chondroblastic OS	high-grade anaplastic tumor cells, as in HG conventional OS

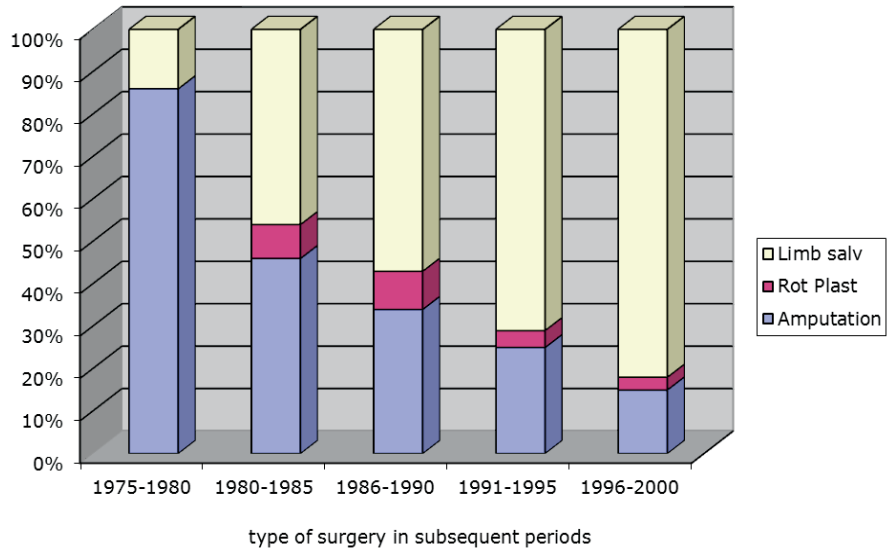
Table 13 shows the clinic-pathological differences between the subtypes of surface osteosarcoma. As is shown in this table parosteal osteosarcoma is the most frequent type (353-356), whereas the periosteal osteosarcoma and high-grade surface osteosarcoma accounts for 1.5% (357, 358) and less than 1% respectively (359, 360).
Despite the lower grade of parosteal and periosteal osteosarcoma, focal dedifferentiated areas into higher grade have been described(353, 355, 361-363), which occur more often in recurrences in these tumours(355, 356, 364, 365).

Treatment of Osteosarcoma

Modern treatment of osteosarcoma consists of pre-operative and postoperative (neoadjuvant) chemotherapy providing systemic tumour control in conjunction with adequate resection of the tumour. Because an extensive meta-analysis of chemotherapy is presented in **chapter 2**, the surgical treatment will only be discussed shortly here.
For local tumor control, limb salvage has replaced ablative surgery as surgical option in the majority of patients (figure 6), it became clear that chemotherapy contributes to local treatment as well (163, 366-370).

FIGURE 6.

Different types of surgery in subsequent 5-year periods.



Limb salvage is a challenge due to the diversity of sites in which the tumours arise, the extension of the tumour to adjacent soft tissue, the proximity of neurovascular structures and the age (356, 371-373). This raises the question about safety in terms of local recurrence and survival after such a procedure.
Table 14 shows an average rate of local recurrence of 6% among more than three thousand three hundred patients, nearly 2x higher after limb salvage (7.8%) than after ablative surgery (4.0%) or rotation plasty (3.8%).

TABLE 14.
Local recurrence rate (LR) among osteosarcoma patients who underwent an amputation, limb salvage surgery or and rotation plasty.

	No.	LR rate (%) after			
Author (ref)	**patients**	**amputation**	**limb salvage**	**rotation plasty**	**total**
Bacci (163)	1126	2.8	6.3	5.6	5.3
Rodriguez-Galindo 2004	397	6.7	6.0	-	6.5
Weeden (374)	559	2.4	10.2	0	7.5
Brosjo (375)	223	3.1	10.5	-	6.3
Bielack (368)	440	2.9	8.8	incl Amp	5.2
Rougraff (376)	227	5.5	10.9	-	7.4
Glasser (366)	279	1.9	9.2	-	6.5
Eckardt (377)	116	7.8	3.8	-	5.2
total	3367	4.0	7.8	3.4	6.1

Survival after local recurrence is poor, on average 21% (5-41%), especially in the presence of concurrent systemic metastases (163, 370, 378-380). However, table 15 shows that the outcome after limb salvage does not fare worse compared with amputation or rotation plasty, indicating limb salvage is a safe procedure.

TABLE 15.
Five year survival in 5 studies of patients who underwent an amputation, a limb salvage procedure or an rotation plasty.

		5-year overall survival			
Author (ref)	**No pats**	**amputation**	**limb salvage**	**rotation plasty**	**p-value**
Bacci (381)	1.148	53%	61%	58%	< 0.001
Bielack (38)	1.702	66%	70%	-	0.089
Weeden (374)	559	42%	61%	59%	< 0.01
Rougraff (376)	227	51%	48%	-	0.84
Glasser (366)	279	73%	80%	-	-
total /mean	3.915	62%	64%	59%	

Factors that significantly relate to the risk of local recurrence were the application of chemotherapy (367, 368, 380), histologic response on pre-operative chemotherapy (163, 367, 368, 379), tumour volume (379) and surgical margin (163, 367, 368, 379, 382). An adequate (radical or wide) surgical margin has the lowest risk for local recurrence, whereas inadequate (marginal or intralesional) margins have high local recurrence rates in most studies, up to 24%(163, 383). Poor histological response has a high additional risk for local recurrence. Particularly when also inadequate margins are present, local recurrence rates can raise to 16%-31% (163, 368, 383). In these studies nearly all patients had chemotherapy, which was essential in limb salvage.
It can be concluded that limb salvage surgery is feasible in contemporary osteosarcoma treatment, but only after pre-operative chemotherapy has been given, and adequate surgical margins can be achieved.

AIM OF THIS THESIS

Despite the enormous number of papers about osteosarcoma that has appeared last decades, there are still many unanswered questions about this bone tumour. One question is whether or not osteosarcoma is one disease or has to be considered as a complex of different disease entities. If osteosarcoma consists of different disease entities, the consequence of that conclusion would be not only a different treatment approach, but it raises then also the question how these different forms are related to each other. For example, osteosarcoma is considered as a high-grade malignant disease, and modern treatment protocols are based on surgical excision of the tumour in combination with neo-adjuvant chemotherapy. However,

16% of the patients will survive, despite the fact that they were treated with local treatment only (384, 385). Around 20% of the patients with recurrent disease can be cured with surgery only (386), while relapsed disease is considered as one of the most disastrous presentations of osteosarcoma. These clinical experiences may suggest that there exist some subgroups of osteosarcoma, that have a less malignant behavior than others. If indeed a less malignant subgroup could be defined, the next question should be whether or not chemotherapy could be reduced or even avoided in this group, in order to prevent the serious side effects of chemotherapy, stressing the importance of this question.
So far, we are not able to distinguish osteosarcomas with unfavorable or favorable outcome by clinical parameters. It might be asked if that would be possible, based on different molecular signatures. Questions about the molecular behaviour of osteosarcoma are not only important from scientific point of view, but may reveal insight in the development of new treatment options. Studies about the pathophysiology of osteosarcoma are hindered by complex genetic changes in this tumour (387-393). Although concerns were raised about the use of *in vitro* models in osteosarcoma research (394, 395), recently it was demonstrated that research on osteosarcoma cell lines is representative for clinical osteosarcoma (396). However, it remains important to understand the complete picture of osteosarcoma that clinical data, filtered by statistical systems need to be transferred into the laboratory and the other way around.
The present study was undertaken to meet some of these questions in order to understand the evaluation of treatment for high-grade conventional osteosarcoma at usual and unusual sites in an attempt to get evidence from clinical and pathological point of view if therapy can become more tailored. Furthermore, we wanted to study a possible relationship between benign osseous lesions and high-grade malignant osteosarcoma using a high throughput method.
To meet these questions, in **chapter 2** the background of chemotherapeutic treatment was investigated with emphasis on chemotherapy. Evidence was found that the drugs that are used in modern treatment protocols indeed are valuable, but are limited to four effective drugs. In this study trials in stage IIb osteosarcoma were investigated, in order to get as much homogeneity as possible. Treatment in metastatic or relapsed osteosarcoma is poorly defined, experience based but no randomized trials were found in these subgroup of patients. In this meta-analysis a new statistical tool, a multivariate random effects analysis with survival data at several time points (Fiocco et al) was applied, heading the heterogeneity of the used studies for this paper. The value of salvage of patients with a poor response the pre-operative chemotherapy is also critically reviewed in this chapter.
Chapter 3 goes deeper to the genetic basis of osteosarcoma. In this study, we tried to find a RNA-expression profile which distinguishes histologic response on pre-operative chemotherapy and/or outcome of patients. A small size of patient samples was available for this study due to the limited amount of tumour tissue at diagnosis, necrosis of tumour in patients with good histological response and uniformity of treatment of patients. In this study RNA expression of tumour tissue was compared with the expression profile of benign osteoblastomas, mesenchymal stem cells that were altered into osteosarcomas and mesenchymal stem cells that were altered into osteosarcomas. Being at the start of the this high

output techniques, we were faced with problems of the analysis and interpretation of these approach. However, some important issues were concluded from this pilot and the technique is nowadays more routine.

Chapter 4 deals with the background of the statistical analysis is given in a paper of Goeman et al, who is working on the department of biostatistics in the Leiden University.

Chapter 5 describes the transition from clinical to laboratory investigations. In this chapter, the expression of the *HER*-2 oncogene in osteosarcoma is critically reviewed. The aim of this study was to investigate whether or not the *HER*-2 oncogene can be demonstrated by several techniques, as Real-time PCR (RT-PCR), Immunohistochemistry (ICH) and Fluorescent in situ Hybridisation (FISH). The importance of this oncogene lies in the fact that treatment whit the monoclonal antibody *Herceptin*, which is regularly used in Breast carcinoma, is supported when the results of such an investigation confirm the presence of this receptor on the membrane surface of the osteosarcoma cells.

In **Chapter 6** a study is presented which comprises osteosarcoma in rare localizations: the hand and the feet. It is not well-known if osteosarcomas in these unusual sites need a similar treatment approach than osteosarcoma elsewhere in the body. Is surgery in addition to chemotherapy acquired for these sites? The assumption may be that local excision only will be sufficient to treat osteosarcomas in the hand and foot, because it can be recognized quite early. However, the outcome and factors that influence the outcome of osteosarcomas in these locations are not exactly known, and no definite conclusions about appropriate treatment can be given before such is better described.

Chapter 7 is concerned with factors that determine the outcome of patients with pulmonary metastasized osteosarcoma. Our questions in this single institute experience were mainly if repeated surgery is valuable in these patients and if factors could be defined for resectability of the tumours in these patients. Unfortunately the series was too small to make a definite conclusion about the role of repeated chemotherapy, but a suggestion was done about the value, based on the relationship between vitality of the tumours and the outcome.

In **Chapter 8** a summary of the chapters is given and some concluding remarks about future research are made, which is in Dutch in **chapter 9**.

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