
PhD thesis by

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Cancer Registry of Norway

&

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1. Scientific environment

This project comprises a collaboration of all hospitals involved in sarcoma management in Norway during the period 2011–2015. However, most of the work was carried out at the Cancer Registry of Norway and the Department of Oncology at the Norwegian Radium Hospital, which is currently part of Oslo University Hospital.

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Professor Øyvind S. Bruland, MD, PhD was the main supervisor, and senior consultant Tom Børge Johannesen, MD, PhD was the co-supervisor.
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Oslo, February 2016
3. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ALP</td>
<td>Serum alkaline phosphate</td>
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<tr>
<td>CDR</td>
<td>Cause of Death Registry in Norway</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>Classical OS</td>
<td>Patients &lt; 40 years at diagnosis with extremity localised high-grade skeletal osteosarcoma without evidence of metastasis at primary diagnosis</td>
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<tr>
<td>COSS</td>
<td>The Cooperative Osteosarcoma Study Group</td>
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<td>CRN</td>
<td>The Cancer Registry of Norway</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
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<td>DLGOS</td>
<td>Dedifferentiated low-grade osteosarcoma</td>
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<td>EFS</td>
<td>Event-free survival</td>
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<td>ESOS</td>
<td>Extraskeletal osteosarcoma</td>
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<td>EURAMOS</td>
<td>The European and American Osteosarcoma Study Group</td>
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<tr>
<td>F-18 FDG</td>
<td>Fluorine-18 fluorodeoxyglucose</td>
</tr>
<tr>
<td>GIST</td>
<td>Gastrointestinal stromal tumour</td>
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<tr>
<td>HUH</td>
<td>Haukeland University Hospital</td>
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<td>ICD</td>
<td>International Classification of Disease</td>
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<td>ISG</td>
<td>The Italian Sarcoma Group</td>
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<td>LDH</td>
<td>Serum lactate dehydrogenase</td>
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<td>LFS</td>
<td>Li-Fraumeni syndrome</td>
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<td>LGCOS</td>
<td>Low-grade central osteosarcoma</td>
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<td>LGOS</td>
<td>Low-grade osteosarcoma</td>
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<tr>
<td>MAP</td>
<td>High-dose methotrexate with leucovorin rescue, doxorubicin and cisplatin</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>Term</td>
<td>Definition</td>
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<td>------------</td>
<td>-----------------------------------------------------------------------------</td>
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<tr>
<td>Non-classical OS</td>
<td>All high-grade skeletal osteosarcoma patients except classical OS</td>
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<td>NRH</td>
<td>Norwegian Radium Hospital</td>
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<tr>
<td>OPF</td>
<td>Osteosarcoma predisposing factor</td>
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<tr>
<td>OS</td>
<td>Osteosarcoma in the skeleton</td>
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<tr>
<td>PAS</td>
<td>Patient Administrative Data System</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>POS</td>
<td>Parosteal osteosarcoma</td>
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<tr>
<td>RB</td>
<td>Retinoblastoma</td>
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<tr>
<td>RESOS</td>
<td>Radiation-induced extraskeletal osteosarcoma</td>
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<tr>
<td>ROS</td>
<td>Radiation-induced skeletal osteosarcoma</td>
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<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SCS</td>
<td>Spindle cell non-osteosarcoma arising from bone tissue</td>
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<tr>
<td>SEER</td>
<td>The National Cancer Institute’s Surveillance, Epidemiology and End Results</td>
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<tr>
<td>SSG</td>
<td>The Scandinavian Sarcoma Group</td>
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<tr>
<td>SSS</td>
<td>Sarcoma-specific survival</td>
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<td>WHO</td>
<td>World Health Organization</td>
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4. Synopsis

4.1 Background
Despite skeletal osteosarcoma (OS) being the most common primary malignant bone tumour, it is a rare disorder that displays considerable heterogeneity and appears as various clinical entities showing a great span in tumour biology and prognosis. Since the mid-1970s, developments in multimodal treatment, including multi-agent chemotherapy, have been essential to improve the survival of high-grade OS patients. Our knowledge concerning the outcome of OS patients is mainly based on cancer registries, institutional series, clinical trials or experiences from cooperative sarcoma societies, in some cases with paediatric and adolescent age cohorts only. These studies have predominantly focused on high-grade OS patients with tumours localised in the extremities who are under 40 and who have no detectable metastasis at primary diagnosis (Hattinger et al., 2015); this subgroup has been defined as having ‘classical OS’ (Bruland et al., 1997; Smeland et al., 2011). The prognosis is dismal for other subgroups of OS, such as the ‘non-classical OS’ subgroup (Chapter 9.2). Patients with OS in the axial skeleton (i.e. not limb-localised OS) may die due to lack of local control, even without detectable metastases. The chemoresistant disease in patients presenting with overt metastases is also an unsolved clinical challenge. The poor tolerance of adequate chemotherapy in the elderly represents another hurdle.

To our knowledge, very few nationwide studies on the clinical presentation and prognosis of OS have been published (Chapter 7.7). None of these previous publications have specifically addressed a scope beyond the classical patient and nor have they addressed topics relating to the clinical epidemiology of low-grade OS (LGOS) and dedifferentiated OS (DLGOS). Further, as far as we know, no nationwide study of extraskeletal osteosarcoma (ESOS) has been published during the modern chemotherapy era.

4.2 Purpose
We have attempted to secure a complete nationwide cohort of all Norwegian cases of histologically confirmed OS and ESOS within a time frame of 35 years based on registry sources supplemented with clinical records from hospitals involved in sarcoma management.

4.3 Key research questions and hypotheses
- There is a significant difference in survival between classical and non-classical OS.
How big is the difference? Which factors may explain this discrepancy?

- To what extent have changes in therapy over time influenced the treatment results?
- How does our nationwide approach affect the anatomical distribution within subgroups and histological subentities of OS compared to previous studies?
- We also attempted to identify incidence rates, patient characteristics and prognostic factors for both low- and high-grade OS and ESOS.

4.4 Main findings

- The average annual incidence rate for all OSs and ESOSs was 3.5 per million, in line with international figures—that is, 473 OS cases and 37 ESOS cases between 1975 and 2009 in Norway.
- The overall 10-year survival rate increased from about 30% during the late 1970s to around 50% 20 years later for all OS patients, with no subsequent improvement during the last two decades.
- We could not confirm any stage migration over time related to an increased proportion of patients with primary metastatic disease due to better imaging.
- Only 48% of all high-grade OS cases were classical OS. A considerable discrepancy in survival between classical and non-classical OS was observed: 61% versus 26% 10-year sarcoma-specific survival (SSS) for the entire time period. Twice as many of the former cases received both adequate surgery and chemotherapy compared to the latter. This could only partly explain the differences in survival.
- An inherent chemoresistance appears evident in primary metastatic disease.
- We identified a higher rate of local relapse among patients with high-grade axial tumours than among patients with high-grade extremity OS, but no corresponding differences regarding the first metastatic event were documented. Twelve patients died due to their local relapse and without metastasis.
- Fifty-four patients had LGOS or DLGOS (12 cases). LGOS has an excellent prognosis when surgically resected with a free margin. However, LGOS has the potential to dedifferentiate and metastasise, resulting in a poor outcome.
- The median age among ESOS patients was 68 years. They had a dismal outcome with only 16% five-year SSS for all ESOSs, probably due to both primary chemotherapy resistance and the different biologic characteristics of these tumours compared to OS.
4.5 ‘New’ knowledge?

High-grade OS:

- The number of patients in the non-classical group, representing just over half of all patients in our cohort, is higher than previously reported.
- A considerable discrepancy in adequate surgery and chemotherapy between classical and non-classical OS was documented.
- Adequate versus inadequate treatment could only partly explain the dismal difference in survival between these two subgroups of OS.
- There were no significant differences in survival dependent on age or tumour site among adequately treated patients.

Low-grade OS:

- More than half of all LGOSs were classified as low-grade central OS (LGCOS), which is clearly higher than previously reported. The anatomical distribution of LGCOS, with the mandible/maxilla as the most frequent site, also differed substantially from the available literature.

ESOS:

- Nearly one quarter of all cases were radiation-induced ESOS (RESOS), a significantly higher proportion than previously published.
- The prognosis for ESOS was dismal, compared to the available literature.
5. List of publications

I

II

III

IV
6. Aims of the present study

The overall aim was to obtain a complete nationwide cohort of all OS and ESOS patients in Norway during 1975–2009. We also attempted to ensure the quality of the histopathological diagnosis, the epidemiological variables and the clinical information during treatment and follow-up. All four articles in the present thesis are based on this cohort but have different approaches regarding various subgroups of OS/ESOS and clinical findings.

The selected time period coincides with modern multimodal assessment and treatment. The starting year of 1975 was chosen to increase the number of patients and capture the time well before the prospective clinical OS studies began in Scandinavia in the early 1980s. The ending year of 2009 was chosen because it was the last available year for complete registration of primary diagnosis according to the Cancer Registry of Norway (CRN) when the current project began.

Paper I
To calculate time trends on incidence and survival outcomes for all patients with OS during the last three to four decades. To investigate the anatomical distribution of all subgroups and histological subentities for this OS cohort.

Paper II
To analyse the prognostic factors and treatment outcome for all high-grade OS patients during the modern chemotherapy era, with a focus on patients with primary metastatic disease, non-extremity tumour localisation or age > 40 at the time of diagnosis (non-classical OS). 

Paper III
To describe the epidemiological and clinical characteristics related to treatment outcomes in all LGOS and DLGOS patients.

Paper IV
To investigate the histopathological and clinical characteristics of all ESOS patients in Norway between 1975 and 2009.
7. Introduction

7.1 General considerations

OS is the most frequent primary malignant bone tumour worldwide (Mirabello et al., 2009a; Hogendoorn et al., 2010; Fletcher et al., 2013). The term sarcoma\(^1\) is derived from the Greek *sarx*, meaning flesh, and the term OS was first introduced at the turn of the 19\(^{th}\) century (Boyer, 1814). The incidence of OS is bimodally distributed by age with a dominant peak in adolescence and a smaller, less well-studied one among the elderly (Mirabello et al., 2009a). The annual incidence rate among children and adolescents appears to be relatively consistent throughout the world (Mirabello et al., 2009a), and most rates range from 3–5 per million in men and 2–4 per million in women (Mirabello et al., 2009a). The incidence rate of OS varies more with older age (Mirabello et al., 2009a).\(^2\) The incidence of OS may depend on ethnicity (Ottaviani et al., 2009a; Mirabello et al., 2009b).

In Norway, bone cancers currently constitute about 4% of all childhood cancers (0–14 years) for males and 3% for females during 2010–2014 (CRN, 2014). Further, the cumulative risk of developing bone cancer by the age of 75 is 0.1% in Norway (CRN, 2014).

OS comprises a wide spectrum of malignant bone tumours where the neoplastic cells show varying degrees of dedifferentiation and aggressiveness (Rosen et al., 1982). This family of neoplasms is characterised by the production of osteoid or immature bone due to the malignant proliferation of spindle stromal cells (Huvos, 1991; Unni et al., 2010; Fletcher et al., 2013). A distinction is generally drawn between histologically different subgroups of OS (Fletcher et al., 2013). The conventional type is the most common high-grade OS and develops inside the bone. It has been subdivided by the predominant phenotype of the stroma, which may either be bone-, cartilage- or fibrous-like (hence osteoblastic/osteosclerotic, chondroblastic and fibroblastic OS). Other high-grade types are telangiectatic, small cell and high-grade surface OS (Fletcher et al., 2013). LGCOS (Unni et al., 1977) and parosteal OS (POS) (Geschickter et al., 1951) are most commonly low-grade malignancies, while

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\(^1\) The term sarcoma was used to describe all bone and fleshy tumours until the middle of the 19\(^{th}\) century, when the development of cellular pathology made it possible to separate sarcomas from carcinomas on the basis of their tissues of origin (Peltier, 1985).

\(^2\) The majority were in the range of 2.5–5 per million in men > 60 years and 1.5–4 in women > 60 years (Mirabello et al., 2009a).
periosteal OS is an intermediate- or high-grade OS believed to arise from the bone surface (Fletcher et al., 2013).

Most commonly, OS affects the metaphyseal part of the distal femur, the proximal tibia and the proximal humerus (Huvos, 1991; Unni et al., 2010; Fletcher et al., 2013). The axial skeleton is more rarely affected (Luetke et al., 2014). The tendency of OS to occur in the extremity bones decreases with age (Jawad et al., 2011; Savage et al., 2011; Whelan et al., 2012a).

ESOS (Wilson, 1941) consists of a malignant group of neoplasms that resemble undifferentiated pleomorphic sarcoma except for the presence of an osteoid and, sometimes, a chondroid matrix (Guillou et al., 2010; Fletcher et al., 2013). This group of neoplasms accounts for 1–2% of all soft tissue sarcomas (Chung et al., 1987; Lee et al., 1995) and approximately 4–6% of all OSs (Sordillo et al., 1983; Thampi et al., 2014). ESOS typically arises during mid and late adulthood (Fletcher et al., 2013; Thampi et al., 2014).

7.2 Aetiology
The aetiology of OS is not well understood (Ottaviani et al., 2009b; Savage et al., 2011). An association between rapid bone growth and OS has been postulated, given the tumours’ typical metaphyseal location close to a growth plate and its peak incidence during adolescence and early adulthood (Savage et al., 2011). OS in elderly patients more often develops as tumours in previously abnormal bone(s) (Eftekhar, 2009; Savage et al., 2011). These cases are commonly reported as ‘secondary’ OS (Fletcher et al., 2013). The best known example of an OS predisposing factor (OPF) is probably the exposure to self-luminous paint containing the radioactive substance radium, which was used to make watches glow in the dark in the early 20th century (Martland, 1931). Many women who were exposed to this experienced anaemia, bone fractures and necrosis of the jaw several years later, and a dose-dependent development of mainly OS was observed among these workers (Polednak et al., 1978). Therapeutic radiation is also a known risk factor for OS (Wiklund et al., 1991; Bjerkehagen et al., 2008; Savage et al., 2011). Radiation-induced OS (ROS) was, for example, noted to occur more frequently than expected in survivors of Hodgkin’s disease following therapeutic radiation (Dores et al., 2002). Nevertheless, very low doses of radiation used for medical evaluation, such as computed tomography (CT) or positron emission tomography
PET scans, are seemingly not associated with the increased risk of OS (Linet et al., 2009; Savage et al., 2011).

Paget disease is another condition associated with the increased risk of OS. It typically occurs in elderly individuals (Hansen et al., 2006; Ottaviani et al., 2009b) and is believed to represent about half of all cases of OS in patients aged 60 years or older (Savage et al., 2011; Fletcher et al., 2013). Paget disease is characterised by highly exaggerated bone remodelling caused by abnormalities in osteoclast regulation, localised to one or several bones (Colina et al., 2008). The disease is twice as common in men as in women (Ottaviani et al., 2009b), and there is a marked geographic variation, with a particularly high prevalence noted in the United Kingdom, Australia, New Zealand and North America (Colina et al., 2008). In addition, patients with fibrous dysplasia (Harris et al., 1962) appear to have an increased risk of developing OS (Huvos et al., 1972; Raymond et al., 2009b) despite many of the reported cases also having received previous radiotherapy (Ruggieri et al., 1994). Fibrous dysplasia may occur at one or more sites, and the clinical spectrum varies from asymptomatic, monostotic lesions to extensive skeletal deformation (Raymond et al., 2009b).

OS also occurs in certain genetic syndromes. This is a heterogeneous group of disorders the pathogenesis of which is often based on the inactivation of tumour suppressor genes (Ottaviani et al., 2009b; Savage et al., 2011). Li-Fraumeni syndrome (LFS) is a cancer syndrome characterised by frequent familial sarcomas, breast cancer and other cancers in close relatives (Li et al., 1969). It has an autosomal dominant inheritance pattern and is caused by a mutation in the tumour suppressor gene TP53 (Malkin et al., 1990) in most cases (Savage et al., 2011). Retinoblastoma (RB) is another example of genetic alterations by mutation in the RB1 tumour suppressor gene (Lohmann, 1999). It is a malignant retinal tumour that normally occurs prior to the age of five. Loss of normal RB function has a substantial impact on the risk of subsequent cancers in RB patients, including OS (Savage et al., 2011), which is further increased by previous radiotherapy (Wong et al., 1997).

7.3 Diagnosis
Typical symptoms at first clinical presentation are a history of localised bone pain followed by a palpable mass (or swelling) and limitations of joint movement (Widhe et al., 2000). The first symptoms may often be linked to a recent trauma, which is the most likely reason patients’ attention is drawn to the body part affected by OS (Widhe et al., 2000). The disease
might also present with a pathological fracture, either spontaneously or after a minor trauma (Sun et al., 2015).

Definitive diagnosis requires histological examination of suspected tumour material, ideally by open biopsy (Bielack et al., 2009a; Hogendoorn et al., 2010). Patients with findings suggestive of OS should be sent to a reference hospital before biopsy, as an inappropriate diagnostic procedure can irrevocably compromise any chance for limb-salvage or even a cure (Bielack et al., 2009a; Hogendoorn et al., 2010). Both X-rays in two planes and magnetic resonance imaging (MRI) should be performed before biopsy to evaluate the tumour’s intramedullary and soft tissue extension and its relationship to vessels and nerves (Bielack et al., 2009a). The region assessed by MRI should include the entire involved bone, including the neighbouring joint in order not to miss skip lesions (Enneking et al., 1975), that is, intramedullary tumour foci without direct connection to the primary tumour. To exclude metastatic disease at the time of diagnosis, staging should include chest X-rays, CT scanning of at least the thorax and a radionuclide bone scan (Bielack et al., 2009a). In addition, F-18 FDG\(^3\) PET/CT has been shown to be a useful method in evaluating bone tumors during initial staging and for monitoring therapeutic response (Costelloe et al., 2014).

7.4 Treatment - historical aspects

The treatment of OS has been described in the medical literature since the second part of the 19th century (Gross, 1879). However, the therapeutic strategy for OS remained controversial until the early 1970s (Ham et al., 1998), with primary surgical ablation recommended by some authors (Coventry et al., 1957; Weinfeld et al., 1962; Dahlin et al., 1967) and primary radiation therapy with or without delayed surgery by others (Cade, 1955; Allen et al., 1973). The latter strategy was advocated by Cade due to the unsatisfactory survival rates in patients treated with surgery alone (Cade, 1955). In his procedure, high-dose radiotherapy (6,000 rad over six weeks) of the primary tumour area was performed, followed by a ‘holding action’ with delayed amputation only if metastases did not appear after several months (Harvei et al., 1981; Jaffe et al., 2013). This regimen was widely practised in many countries since the 1950s (Ham et al., 1998) and was practised in Norway until 1975 (Harvei et al., 1981).

In the pre-chemotherapy era, up to 20% of all classical patients were cured by surgery and/or

\(^3\) Fluorine-18 (F-18) fluorodeoxyglucose (FDG)
radiotherapy (Weinfeld et al., 1962; Dahlin et al., 1967; Friedman et al., 1972; Harvey et al., 1981). This poor outcome revealed that micrometastases were present in the majority of OS patients at primary diagnosis (Marcove et al., 1970; Jaffe et al., 1976; Bacci et al., 2001b; Bruland et al., 2005; Bruland et al., 2009b). The great majority of these patients succumbed to pulmonary metastases within two years of diagnosis despite primary amputation (Ham et al., 1998).

Prior to the 1960s, OS was considered chemoresistant (Jaffe, 2014). However, from the 1970s several investigators reported that chemotherapy, including the concept of using high-dose methotrexate with leucovorin ‘rescue’ (Goldin, 1971; Jaffe et al., 1974), was effective against relapsed or metastatic OS (Cortes et al., 1974; Baum et al., 1979; Gasparini et al., 1985). This constitutes the rationale for the development of potent and efficient combinations of chemotherapy given to all patients with high-grade histology since the late 1970s or early 1980s to combat micrometastases. At the beginning of this ‘modern’ chemotherapy era, Rosen and co-workers introduced the concept of giving chemotherapy before carrying out definitive surgery of the primary tumour (Rosen et al., 1975). Such neoadjuvant chemotherapy arose in conjunction with the development of limb-sparing surgery for control of the primary tumour (Meyers et al., 1997). Nevertheless, any tumour mass that was large enough to be detected by conventional techniques was considered too large for chemotherapy alone to eradicate (Meyers et al., 1997). The only effective tool for local control of clinically detectable disease is surgery. Therefore, the combined treatment of surgery and chemotherapy is mandatory to achieve optimal long-term survival of OS.

7.5 Current therapeutic strategies

7.5.1 High-grade osteosarcoma

Current management of high-grade OS comprises neoadjuvant chemotherapy followed by surgical removal of all detectable disease and postoperative (adjuvant) chemotherapy, preferably by a multidisciplinary team at a sarcoma centre (Federman et al., 2009; Isakoff et al., 2015). Therapy in such settings is usually given within the framework of prospective and often collaborative clinical studies or established treatment protocols (Bielack et al., 2009a).

Surgery

Localised disease: The goal of surgery is to safely remove the tumour and yet preserve as much function as possible. A complete removal of the tumour must always be attempted
(Enneking et al., 1980), including the biopsy tract surrounded by an unviolated cuff of normal tissue, as narrower margins are associated with an increased risk of local recurrence. Nevertheless, no general definition exists regarding the adequate width of the normal cuff, as this varies depending on the layers of reactive tissue surrounding the tumour and the responsiveness to preoperative chemotherapy (Andreou et al., 2011; Luetke et al., 2014; Anderson, 2016).

Advances in imaging and surgical techniques and the positive effects of neoadjuvant chemotherapy have led to a major shift from amputation towards limb-salvage surgery in the majority of patients during the last two to three decades (Bielack et al., 2002; Yasko, 2009; Bruland et al., 2009a; Anderson, 2016). Various limb-sparing procedures are available today, such as the use of biological solutions (auto and allografts) and/or metallic endoprosthesis (Wafa et al., 2006; Marulanda et al., 2008; Mangat et al., 2011). Limb-salvage surgery can also be achieved in patients with pathological fractures (Abudu et al., 1996; Papagelopoulos et al., 2007). Nevertheless, there is still potential for improvements in tissue engineering to preserve even more normally functioning bones, joints and muscles (Anderson, 2016).

Currently, amputation remains a good option for a small number of patients, that is, those with poor histological response to neoadjuvant treatment and where the limb-salvage procedures cannot achieve with negative margins as in obvious infiltration of tumour tissue into neurovascular structures (Marulanda et al., 2008; Yasko, 2009). Limb-salvage surgery might also be a problem in the developing world (Marulanda et al., 2008), although low-cost limb-salvage surgery is seemingly possible to achieve in several developing countries today (Agarwal et al., 2007). Moreover, rotationplasty is a procedure that can be used in patients with, for example, remaining growth potential whose tumours are located in the distal femur or the proximal tibia (Kotz, 1997; Badhwar et al., 1998) or in cases of failed limb-salvage surgery in adults after resection and reconstruction (Brigman et al., 2003). Rotationplasty of the hip joint can also be performed to avoid exarticulation in order to resect tumours of the proximal femur (Winkelmann, 1986). However, this surgical procedure is barely used anymore due to advanced expandable and adjustable endoprostheses (Mangat et al., 2011).

Patients have different ways of coping with the loss of a limb or impaired function due to cancer. Changes in, for example, body image after surgery may affect their self-realisation, especially in terms of their social life (Fauske et al., 2016). According to a previous review
article (Barr et al., 2009), no consensus has been reached regarding whether limb-sparing surgery improves functional outcomes and health-related quality of life (HRQoL) compared to amputation. Nevertheless, most bone tumour survivors manage well once they have adjusted to their physical limitations, according to a study by the Scandinavian Sarcoma Group (SSG), although limb-salvage surgery preserves more function than does amputation (Aksnes et al., 2008). Another study states that psychosocial adjustment after bone cancer treatment was determined based on the age of the patients at diagnosis and not by the type of procedure (amputation or limb-sparing surgery) or resultant physical function (Felder-Puig et al., 1998).

*Metastatic disease:* Patients with metastatic disease, most common in the lungs with bone being the second most common site of metastasis (Bielack et al., 2002), are treated with the same treatment approaches as patients with localised disease (Luetke et al., 2014). Therefore, pulmonary metastasectomy (Rusch, 2002) remains an essential and effective adjunct to multi-agent chemotherapy (Luetke et al., 2014). Surgical resection is considered if all lung nodules can be removed and a sufficient amount of pulmonary tissue can be saved to maintain adequate pulmonary function (Kempf-Bielack et al., 2005; Carrle et al., 2009). Repeated metastasectomies are acceptable for patients with resectable lung metastases (Saeter et al., 1995; Ferrari et al., 2003; Kempf-Bielack et al., 2005; Bielack et al., 2009c; Briccoli et al., 2010). The impact of surgery for other locations of metastases is not well established, but surgery appears to be the treatment of choice if a complete removal of all lesions is feasible (Bielack et al., 2009c; Errani et al., 2011; Vijayamurugan et al., 2014; Hattinger et al., 2015).

*Chemotherapy*  
The rationale for neoadjuvant therapy is that chemotherapy should be introduced as early as possible when the metastatic tumour load is minimal (Rosen et al., 1979). Preoperative chemotherapeutic treatment also allows time for planning limb-salvage surgery and reconstructive procedures, to reduce the likelihood of viable tumour tissue spreading during surgery, to evaluate the histological effect of the preoperative chemotherapy and hopefully shrinkage of the primary tumour (Jeon et al., 2010). This strategy allows postoperative (salvage) chemotherapy to be modified accordingly (Jeon et al., 2010).

Currently, doxorubicin, cisplatin, high-dose methotrexate with leucovorin rescue and ifosfamide are considered the most active agents against high-grade OS (Ta et al., 2009;
Hogendoorn et al., 2010; Anninga et al., 2011; Luetke et al., 2014; Hattinger et al., 2015). A variety of different pre- and postoperative combinations are used, but the ideal combination scheme and the optimal treatment duration, both for neoadjuvant and adjuvant chemotherapy, are yet not defined (Hogendoorn et al., 2010; Luetke et al., 2014). Moreover, several trials have demonstrated that four-drug regimens do not result in a significant difference in survival compared to three-drug regimens, such as MAP\(^4\) chemotherapy (Anninga et al., 2011; Gill et al., 2013; Ferrari et al., 2015).

Before the start of the prospective SSG clinical trials in the early 1980s (Bruland et al., 2009a), several patients were treated according to the ‘CAMOS’ regime (Harvei et al., 1981) in Norway. Since 1982, seven consecutive chemotherapy protocols were running in this country: SSG II (Saeter et al., 1991), SSG VIII (Smeland et al., 2003), ISG\(^5\)/SSG I (Ferrari et al., 2005), ISG/SSG II (Boye et al., 2014), SSG XIV (Smeland et al., 2011), Euroboss 1 (Carrle et al., 2006) and EURAMOS-1 (Marina et al., 2009; Bielack et al., 2015; Whelan et al., 2015). These protocols are now closed and the main results from all the studies, except the long-term outcome in the Euroboss 1 study, have been reported.

Patients not eligible for inclusion in the protocols were considered for individualised chemotherapy adjusted for age and toxicity. Acute toxicities such as alopecia, myelosuppression, mucositis and nausea and vomiting are common complications of most cytotoxic chemotherapy regimens (Janeway et al., 2010). However, OS patients may also experience long-term treatment sequelae, such as cardiac toxicity, chronic nephrotoxicity, neurotoxicity, hearing loss and infertility (Janeway et al., 2010). Therefore, reducing the complications of OS medical therapy without sacrificing the possibility of cure is still an important goal in the current treatment of OS.

Patients presenting with metastases at time of diagnosis are usually given the same first-line chemotherapy as those with localised disease (Bruland et al., 1997; Picci, 2007).

**Other treatment**

The role of radiotherapy in OS is limited but may be appropriate in highly select cases, such as local treatment of unresectable OS or following marginal or intralesional surgery (Ozaki et

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\(^4\) High-dose methotrexate with leucovorin rescue, doxorubicin and cisplatin.

\(^5\) The Italian Sarcoma Group
Radiotherapy is also commonly used as palliation of metastases (Schwarz et al., 2009). The bone-seeking radioisotope samarium-153-EDTMP can be combined with radiotherapy, bisphosphonates and/or chemotherapy to synergistically improve palliation of painful osteoblastic bone metastases that show up on bone scans (Bruland et al., 1996; Anderson, 2006; Schwarz et al., 2009).

Supplemental therapeutic approaches, such as radiofrequency ablation or cryotherapy, are experimental (Errani et al., 2011; Luetke et al., 2014).

**Recurrent disease**

The choice of treatment in recurrent disease should be decided on an individual basis after considering a number of factors, including site of recurrence, the number of new lesions, the length of the disease-free interval between the initial treatment and the time of relapse and the type of previous treatments (Bacci et al., 2001b). Nevertheless, the role of second-line chemotherapy for resectable recurrences is still controversial (Jaffe, 2014; Luetke et al., 2014), and there is no standard regimen for recurrent OS (Hogendoorn et al., 2010; Vijayamurugan et al., 2014; Xiao et al., 2014; Hattinger et al., 2015).

7.5.2 Low-grade osteosarcoma

POS and LGCOS are variants with less malignant potential that are treated by surgery only (Hogendoorn et al., 2010). However, both subgroups have the potential to dedifferentiate to high-grade malignant lesions (DLGOS) upon recurrence (Kurt et al., 1990; Okada et al., 1994; Schwab et al., 2008; Malhas et al., 2012; Hang et al., 2014) or as areas of high-grade OS within a large primary tumour (Fletcher et al., 2013). Such patients should then be treated as having conventional OS (Chapter 7.5.1) due to the increased risk of subsequent metastasis (Takeuchi et al., 2006; Schwab et al., 2008; Fletcher et al., 2013).

7.5.3 Extraskeletal osteosarcoma

The traditional treatment of ESOS has usually been restricted to local intervention, including surgery alone or a combination of surgery and radiotherapy (Patel et al., 1995). ESOS tends to respond poorly to chemotherapy (Sordillo et al., 1983; Bane et al., 1990; Lee et al., 1995; Patel et al., 1995; Ahmad et al., 2002). However, two recent studies (Goldstein-Jackson et al., 2005; Torigoe et al., 2007) have shown that multi-agent chemotherapy may yield good
results. As of today, ESOS may be treated according to the chemotherapy regimens of high-grade soft tissue sarcomas or OS schedules (Hogendoorn et al., 2010). There is no consensus amongst experts on this point (Hogendoorn et al., 2010).

7.6 Prognosis and prognostic factors
7.6.1 High-grade osteosarcoma

Localised disease
Multimodal treatment, including multi-agent chemotherapy, has been essential to improve the survival of high-grade OS patients (Pratt, 1997; Whelan et al., 2006; Ferrari et al., 2009; Jaffe, 2009; Geller et al., 2010; Isakoff et al., 2015). The cure rates improved from less than 20% prior to the 1970s (Chapter 7.4) to the current levels of 60–70% among patients with extremity localised, non-metastatic disease (Luetke et al., 2014; Friebele et al., 2015). However, further improving the cure rate for OS continues to be challenging, as discussed in numerous reports (Geller et al., 2010; Luetke et al., 2014; Hattinger et al., 2015; Anderson, 2016), and the prognosis is still grave for other subgroups of high-grade OS (discussed further in Chapter 10.2.4).

The prognostic factors for high-grade OS are well known (Hogendoorn et al., 2010; Luetke et al., 2014), and the most commonly cited ones are mentioned below.

Age: The unfavourable prognosis in older patients (at least 40 years old) has been linked to a higher rate of axial tumours and/or more frequent metastases at presentation (Grimer et al., 2003; Longhi et al., 2008; Hogendoorn et al., 2010; Luetke et al., 2014). Older people might also have decreased tolerance for high-dose chemotherapy (Bruland et al., 1997; Grimer et al., 2003) or reduced histologic response to chemotherapy (Jeon et al., 2006; Song et al., 2010).

Gender: Currently, there is seemingly no unanimous answer regarding the relationship of sex on the survival of high-grade OS patients (Bielack et al., 2002; Bacci et al., 2004; Jawad et al., 2011; Sampo et al., 2011; Whelan et al., 2012b; Collins et al., 2013).

Tumour sites: Axial tumour localisation results in worse outcomes than primary disease location within the limb (i.e. appendicular skeleton) (Saeter et al., 1996; Bielack et al., 2002; Jawad et al., 2011). This is partly explained by a higher rate of axial tumours in older patients (Whelan et al., 2012a) or the lack of local tumour control (Chapter 9.2).
**Tumour size/volume:** Larger size/volume is an adverse prognostic factor (Bieling et al., 1996; Hogendoorn et al., 2010; Luetke et al., 2014) that may be partly associated with increased tumour size among patients with primary metastatic disease (Bacci et al., 2002a).

**Pathologic fracture:** The development of a pathologic fracture is a negative prognostic factor in OS, according to two recent meta-analyses (Salunke et al., 2014; Sun et al., 2015).

**Primary metastatic disease:** Up to one fifth of all patients have metastasis at presentation (Luetke et al., 2014). Metastatic disease predicts poor prognosis despite aggressive treatment (Meyers et al., 1993; Harris et al., 1998; Bielack et al., 2002; Bacci et al., 2003; Mialou et al., 2005; Curry et al., 2006; Wu et al., 2009). The poor outcome may relate to primary chemotherapy resistance (Bruland et al., 1997), partly due to the different biologic characteristics of these tumours (Bacci et al., 2002a) and the number of metastases at diagnosis (Kager et al., 2003), in addition to the absence of complete surgical remission (Kager et al., 2003). Patients who present with skip metastases may have poor prognoses (Sajadi et al., 2004) although they can achieve long-term survival when aggressively treated with combined chemotherapy and surgery (Kager et al., 2006).

**Histological response:** The extent of the histological response to preoperative chemotherapy offers important prognostic information (Rosen, 1985; Bielack et al., 2002; Bakhshi et al., 2010; Friebele et al., 2015). Currently, most cancer hospitals define a good histologic response as less than 10% viable tumour cells remaining at the time of surgery (Friebele et al., 2015).

**Dose-intensity:** The prognostic relevance of dose intensity is still under discussion (Meyers et al., 1998; Bacci et al., 2001a; Eselgrim et al., 2006; Bakhshi et al., 2010; Kushnir et al., 2014; Luetke et al., 2014).

**Histological subgroups:** The histologic subtype of high-grade OS (Chapter 7.1) might affect survival because the chemotherapy response is not equal among subgroups (Bakhshi et al., 2010; Anderson, 2016). Currently, however, there is seemingly no significant relationship between subtype and prognosis (Hauben et al., 2002).
Surgical resection margin: The surgical resection margin is closely related to the rate of local recurrence in OS (Andreou et al., 2011; Luetke et al., 2014).

Serum alkaline phosphatase (ALP) and serum lactate dehydrogenase (LDH): There are no specific laboratory tests or biomarkers for OS (Hogendoorn et al., 2010; Sampson et al., 2015), despite that elevated levels of ALP (associated with the osteoblastic activity of the tumour cells) and/or LDH (as an unspecified marker of tumour load) correlate with adverse outcomes (Bacci et al., 2002b; Bacci et al., 2004; Ren et al., 2015).

Recurrent disease
Both local recurrence and metastatic relapse are associated with poor prognosis (Saeter et al., 1995; Grimer et al., 2005; Kempf-Bielack et al., 2005; Bacci et al., 2007; Carrle et al., 2009), although the longer the disease-free interval between primary treatment and relapse, the better the survival (Ferrari et al., 2006; Anderson, 2016). Nevertheless, patients with relapse after several years may also succumb to the disease (Strauss et al., 2004; Hauben et al., 2006). The site of first recurrence is related to survival (Gelderblom et al., 2011).

7.6.2 Low-grade osteosarcoma
LGOS patients have excellent long-time survival when surgically resected with a wide margin (Kurt et al., 1990; Malhas et al., 2012). Transformation de novo from low-grade into high-grade lesions occurs more commonly in relation to unsatisfactory margins for both POS (Sheth et al., 1996; Hang et al., 2014) and LGCOS (Kurt et al., 1990; Schwab et al., 2008; Malhas et al., 2012).

7.6.3 Extraskeletal osteosarcoma
In general, ESOS is associated with poor prognosis (Chung et al., 1987; Bane et al., 1990; Lee et al., 1995) despite that two previous studies have shown good results following multi-agent chemotherapy (Goldstein-Jackson et al., 2005; Torigoe et al., 2007). Therefore, both local recurrences and/or distant metastases during follow-up are common among this group of cancer patients (Allan et al., 1971; Sordillo et al., 1983; Bane et al., 1990; Lee et al., 1995; Lidang Jensen et al., 1998).

7.7 Previous nationwide studies
To our knowledge, only seven nationwide studies on the clinical presentation and prognosis
of OS have been published (Larsson et al., 1974; Harvei et al., 1981; Curry et al., 2006; Stiller et al., 2006; Sampo et al., 2008; Sampo et al., 2011; Whelan et al., 2012a) in addition to one nationwide study on ESOS (Lorentzon et al., 1979). All these studies are based on National Cancer Registries. Only the Finnish studies (Sampo et al., 2008; Sampo et al., 2011) included a mandatory histological re-evaluation of all cases, in addition to a detailed description of clinical features, treatment and prognosis. The main results from each of the seven studies are briefly presented below.

Larsson and Lorentzon retrospectively studied OS, chondrosarcoma and Ewing sarcoma in a study of 832 malignant primary bone tumours in relation to age, sex and site reported to the National Cancer Registry in Sweden during 1958 and 1968 (Larsson et al., 1974). The peak incidence for OS (242 cases) occurred at a mean age of 12 for females and 16 for males. The preponderance of disease in males over females was only valid for locations in the long bones of the lower limb, the pelvis and the spinal column. Harvei and Solheim conducted a nationwide retrospective review of 240 patients reported to the CRN for OS diagnosed between 1953 and 1977 (Harvei et al., 1981). No significant improvement in survival was seen during 1953–1975. However, the introduction of adjuvant chemotherapy in 1975 was the most likely cause for the improvement of the 48-month survival rate from 25% for the period 1953–1974 to 47% during the next three years.

All patients in New Zealand with OS from 1994–1999 (84 cases) were compared with a previous study covering the period 1981–1987 (96 cases) (Curry et al., 2006), and the five-year survival improved from 32% to 44% for these cohorts. Data on a population-based series of 1,349 patients with OS were compiled from regional and national cancer registries in Great Britain during 1980–1994 (Stiller et al., 2006). Five-year survival rates during 1980–1984, 1985–1989 and 1990–1994 were 42%, 54% and 53%, respectively.

Sampo et al. presented two comprehensive nationwide studies for the decades 1970–1990 (net 139 OS) and 1991–2005 (net 144 OS) (Sampo et al., 2008; Sampo et al., 2011).6 The overall five-year survival for the first study was 58%, with an improvement in survival during 1981–

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6 It was not possible to retrieve original specimens for histological re-examination in 103 cases (34% of the gross material) in the first report and 14 cases (8% of the gross material) in the second report. An additional 33 cases in the first study and 8 cases in the second study were not verified as OS. Another 27 cases were excluded for various reasons during the period 1970–1990 compared to 10 cases from 1991–2005.
1990 (65%) compared to the previous decade (47%) (Sampo et al., 2008). In the latter study, the 10-year SSS was 63% for all patients and 58% for patients with high-grade tumours of any stage during 1991–2005 (Sampo et al., 2011). No significant improvement in SSS was documented between 1991 and 2005. The mean annual incidence was 1.8 OS per million during 1991–2005, and no incidence analyses were given in the first report.

Whelan and co-workers gave a five-year survival rate of 43% for all OS patients in England during 1979-2007, based on a patient registry from the National Cancer Intelligence Network (Whelan et al., 2012a). They identified no further improvement in five-year survival since the mid-1980s; it remained at just under 45% for all patients. The annual age-standardised incidence rate per million was about 3.0 in males and 2.2 in females during the study period, with no clear time trends. The authors documented a clear ‘second’ peak in the annual incidence of OS among the elderly, which is in line with the literature (Mirabello et al., 2009a). Patients with non-extremity OS had inferior survival rates compared to patients with OS in the appendicular skeleton. Patients over 40 also had worse outcomes compared to their younger counterparts.

Lorentzon et al. identified four cases of ESOS among 242 cases recorded as OS in the Swedish Cancer Register during 1958–1968 (Lorentzon et al., 1979). The tumours occurred in middle-aged and elderly patients. Three of the tumours were situated in the proximal part of the thigh and one was in the scapular region.

7.8 The SEER programme

The Surveillance, Epidemiology and End Results (SEER) programme is to our knowledge the largest non-nationwide cancer registry in the world. The data covers approximately 30% of the US population and consists of 17 geographically defined central cancer registries (Duchman et al., 2015; SEER, 2015). It comprises population-based data from 1973–2012 and offers the opportunity to perform detailed analyses of the incidence and survival rates associated of rare neoplasms (SEER, 2015).

It is also possible to merge information from the SEER programme with the corresponding data from other cancer registries in the US to achieve larger epidemiological studies. Such an approach was, for example, used in a previous study by Duong and co-workers in which they identified 7,104 cases of primary OS from approximately 90% of the US population during
1999–2008, which represented an annual incidence of 2.7 for all patients included (Duong et al., 2013). The authors found an appendicular-to-axial ratio of 3.1. The peak incidence was observed in the second decade. Among the cases of axial OS, the frequency was distributed almost equally by gender, whereas among total and appendicular OS cases, the incidence was higher among males compared to females. Age-adjusted incidence rates for appendicular and axial OS were higher among blacks compared to other racial groups, which is in line with a previous report that used SEER data (Mirabello et al., 2009b).

7 Interestingly, the corresponding ratio is 2.4 in the present thesis (Table 3, Paper I).

7.9 The Scandinavian Sarcoma Group
The SSG was founded in 1979 with the aim of encouraging sarcoma surgeons, oncologists, pathologists and radiologists to work in multimodal teams to optimise sarcoma treatment and research in line with international recommendations (Alvegard et al., 2009). The goal was to improve the care of patients with tumours in connective tissues and to increase knowledge of all aspects of tumour biology, including pathology and clinical research. The SSG secretariat is located in Lund, Sweden.

The SSG provides treatment recommendations and clinical protocols for different sarcoma types and participates in different multinational clinical trials, including OS (Bruland et al., 2009a). All specimens from all patients included in the various SSG protocols, such as for OS (Chapter 8.5), have previously been re-examined by the SSG panel of pathologists (Bjerkehagen et al., 2009). The Central SSG Register was established in 1986 as a multicentric, prospective study for the evaluation of treatment results and prognostic factors in patients with sarcomas (Zaikova et al., 2015).
8. Materials and methods

8.1 Study population

This thesis is based on a nationwide cohort comprising all histologically confirmed OS and ESOS patients from 1975–2009 in Norway. The inclusion criteria required Norwegian residence and a Norwegian personal identification number. The cohort comprises all subgroups of OS/ESOS, including post-mortem diagnoses and cases with OPF (Chapter 7.2). Patients with synchronous or metachronous OS (Bacci et al., 1996; Jaffe et al., 2003; Brandal et al., 2009) were only counted once.

All cases were extracted from the CRN using the International Classification of Disease for Oncology second edition (ICD-0-2). We also considered spindle cell non-OS cases arising from bone tissue (SCS) reported to the CRN and more unspecific bone tumours to capture any OS cases wrongly classified among these ICD codes (Paper I). However, we were not able to scrutinise the very high number of spindle cell non-OS arising from soft tissue (close to 3,000 cases) as part of this work. From 1975–2009, a total of 544 cases were reported as OS/ESOS to the CRN, 59 cases were reported as SCS and 29 were reported as more unspecific bone tumours (Figure 1, Paper I). This cohort was complemented with data from all hospitals involved in sarcoma management in Norway, namely The Norwegian Radium Hospital (NRH), Haukeland University Hospital (HUH) and the SSG registry regarding Norwegian OS protocol patients (Paper I). Thus, the gross study material amounted to 702 cases, including 586 OS/ESOS cases and 116 SCS cases (Figure 1, Paper I).

Histologically confirmed OS (473 cases) and ESOS (36 cases) cases were included in the net population (Figure 1, Paper I). One additional ESOS case from a previous study (Brustugun et al., 2005) was not included in our gross material of 702 patients. This case was initially reported as spindle cell non-OS arising from soft tissue (see above) and was re-classified as ESOS as part of the latter study by Brustugun and co-workers. Therefore, the total net population of the cohort was 510 cases (Figure 1).

All excluded cases due to wrong diagnoses of OS are presented in Table 1 of Paper I.
Paper III also included four high-grade OS cases from Paper II and one low-grade ESOS case from Paper IV.

Figure 1. Flow chart showing the inclusion of all osteosarcoma patients in the present thesis and the distribution between skeletal osteosarcoma (OS) (Paper I), high-grade OS (Paper II), low-grade and dedifferentiated OS (Paper III) and extraskeletal osteosarcoma (ESOS) (Paper IV).
8.2 Histopathology

We have histologically confirmed the present cohort of OS and ESOS patients according to the current World Health Organization (WHO) criteria (Fletcher et al., 2013), with minor modifications, supplemented with clinical records (Paper I). Most of the cases were re-evaluated based on the histological reports only, more detailed discussed in Paper I. However, all questionable cases were histologically re-examined as part of this project in addition to all cases not previously examined by at least two senior pathologists at a university hospital, preferably by NRH and/or HUH, the SSG panel of pathologists (Chapter 7.9) or by second opinion at the Mayo Clinic. In total, 128 cases were retrieved from files and re-examined histologically; of these, 26 were LGOS/DLGOS cases and 30 were ESOS cases. Another two cases, with an obvious clinical and radiologically OS diagnosis, were confirmed by fine-needle aspiration cytology; previously reported to be adequate for an OS diagnosis (Sathiyamoorthy et al., 2012). We retrieved histological specimens from all questionable cases. Nevertheless, we received no representative biopsy in three cases, and another three cases were evaluated as unclassified sarcoma (Table 1, Paper I). We did not retrospectively review radiographic images as part of this work.

Two patients that had been reported as having LGOS in Paper I received chemotherapy due to small areas with documented grade III malignancy. Consequently, these cases were classified as ‘DLGOS at diagnosis’ in Paper III.

8.3 Data sources and management

Demographic, clinicopathological and treatment variables during primary treatment and follow-up were retrospectively validated based on multiple and partly overlapping data and registry sources. The following sources of information were used:

- Data from the CRN. It is mandatory to report clinical information on new cases of cancer to the CRN no later than two months after the diagnosis has been determined (CRN, 2014). The CRN receives its information from several sources comprising clinical notifications, pathology reports, autopsy documents, death certificates and radiation therapy documentation (CRN, 2006; Larsen et al., 2009). In those cases where the clinical notification is missing for a cancer case identified in one of the other sources, a reminder is sent to the hospital/ward/physician responsible for the
treatment. Since 2002, the CRN has also received data files from the Patient Administrative Data System (PAS) used in all Norwegian hospitals (CRN, 2014). These files contain information about patients treated for premalignant and malignant conditions since 1998, and therefore PAS has been a key source for information on unreported cases.

- Of the net population of 510 patients (Figure 1), 68% were also registered in a separate prospective sarcoma database at the NRH. This database contains all sarcoma patients referred to the NRH from 1980 onwards, including clinical information during treatment and follow-up (Aksnes et al., 2006).
- We also received relevant information from clinical records (electronic and/or paper) from all hospitals involved in sarcoma management, mostly university hospitals, in addition to information from general practitioners in a few cases.
- Information regarding all Norwegian OS patients in the SSG registry (Chapter 7.9) was also registered.
- The database was matched to the National Registry on vital status and migration.

8.4 Demographic and tumour-related variables
The current database was constructed to include information relevant to this study: gender; age at diagnosis of OS/ESOS; metastasis at the time of diagnosis; anatomic localisation of primary tumour; histological subtype of OS; malignancy grade; morphological phenotype; patients with OPF; pathological fracture; previous cancer treatment; follow-up status; date and cause of death; tumour size; symptom duration; ALP; LDH and immunohistochemistry results (Paper IV). Most of these variables are discussed below.

Time of diagnosis: This variable was based on data from the CRN and is dependent on several aspects, first of all the time the biopsy was performed. We have controlled these figures against corresponding results from the NRH database. Here, the date of diagnosis to a large degree equals time for biopsy report. We accepted up to 60 days difference between these two sources. All larger deviations (approximately 20 cases) were further re-examined.

Primary metastatic disease: We defined metastasis within six weeks after primary diagnosis as overt metastasis at the time of diagnosis. Information regarding metastasis was based on radiographic images and/or biopsy or fine-needle aspiration cytology.
**Histological subtype:** All tumours were classified according to current WHO criteria (Fletcher et al., 2013), with minor modifications (Paper I).

**Malignancy grade:** All OS/ESOS cases were graded histologically according to a four-grade malignancy scale (Bjerkehagen et al., 2009). We dichotomised between low-grade (grades I–II) and high-grade (grades III–IV) tumours (Bjerkehagen et al., 2009).

**Morphological phenotype:** The dominating morphological phenotype was determined in connection with the histologically re-examination of the 128 cases in the present study (Chapter 8.2). Correspondingly, the phenotype of the remaining cases was decided based on the histological reports, both from primary biopsies and from successive surgical specimens. The term ‘mixed phenotype’ was used when there was no obviously dominating phenotype.

‘Secondary’ OS/ESOS: This group comprises ROS/RESOS and patients with other OPF (Chapter 7.2). We also included a more unclassified group with an indication of a hereditary predisposition for OS, such as LFS-like, but not a genetically confirmed syndrome. We refer to Papers I and IV for a more detailed presentation of cases with OPF.

**Date and cause of death:** Information was primarily retrieved from the Cause of Death Registry in Norway (CDR). We stratified between death due to OS, including treatment-related death associated with OS, and other causes/unknown causes.

**Tumour size:** Tumour size was measured using surgical specimens and/or radiographic images at time of diagnosis and recorded as the maximum length of the tumour in cm.

**Duration of symptoms:** This was the interval in months between the first symptom and the time of the biopsy. We could not dichotomise this interval into ‘patient delay’ and ‘doctor delay’ (Kim et al., 2009) due to the lack of accurate information in too many of the clinical case records.

**ALP and LD:** The normal ranges for ALP and LDH were measured in international units at the time of diagnosis, in line with the common Nordic Reference interval from May 2003 (Rustad et al., 2004), that is ALP: 0–17 years, <400 U/L; >17 years, <105 U/L; LDH: 0–10 years, <400 U/L; 11–70 years, <205 U/L; >70 years, <255 U/L. Analyses before May 2003
needed a 60% and 50% reduction in ALP and LDH values, respectively, to be compatible with the above-mentioned thresholds.

Molecular markers: Ten different antibodies were used for the immunohistochemistry of ESOS in Study IV.8

8.5 Treatment variables
8.5.1 High-grade osteosarcoma
Adequate primary treatment was defined as a patient having received both adequate chemotherapy and radical surgery. All treatment had to be given before the appearance of metastatic disease or local relapse was confirmed for patients without primary metastatic disease.

Adequate chemotherapy was defined as patients having received at least six courses of chemotherapy containing a minimum of two of the following drugs: high-dose methotrexate (at least 8 g/m3), doxorubicin, cisplatin or ifosfamide (Saeter et al., 1998). We did not consider the CAMOS regimen (Harvei et al., 1981) as adequate chemotherapy in this study, as compared to the current standard.

Adequate surgery implied surgical removal of primary tumour during primary treatment with wide or marginal margins (Enneking et al., 1980). This was always attempted and defined by the surgeon and/or pathologist. We dichotomised between amputation (last surgery performed) and limb-sparing surgery, including rotationplasty. Patients with metastatic disease at the time of diagnosis were in need of a complete surgical removal of both the primary tumour and metastases, mostly to the lungs, to be classified as having received adequate surgery. However, we accepted adequate surgery just for primary tumours when preoperative chemotherapy had resulted in complete remission of all visible lung metastases present at time of diagnosis and with no thoracotomy performed.

8.5.2 Low-grade and dedifferentiated osteosarcoma
The margins after the last surgery for the primary tumour were classified as free (wide or marginal) or positive margins (intralesional or residual macroscopic tumour) (Enneking et al.,

8 Ki67, P53, E cadherin, P cadherin, N cadherin, PCD68, AE1/AE3, BCL-2, Desmin, Caspase 3
1980). All patients treated with curettage as the last surgical procedure were assumed to have a positive margin. The other assumptions regarding both surgery and adequate chemotherapy were in line with the treatment for high-grade OS (see above). Radiotherapy in patients treated with curative intent was defined as fractionated radiotherapy following surgery, either for the primary tumour or a local recurrence, otherwise considered as palliative treatment.

8.5.3 Extraskeletal osteosarcoma
Adequate surgery was equally defined as for high-grade OS (see above). Chemotherapeutic agents and the cumulative doses for each patient were recorded. All patients treated with radiotherapy as curative intent were presented in this study.

8.6 Statistical analyses
8.6.1 The database
The database was created using Microsoft Office Excel 2010 and Microsoft Office Access 2003. The statistical analyses were conducted using SPSS version 21 (SPSS, Inc., Chicago, IL, US) and Stata version 13.1 (Stata Corporation, College Station, TX, US).

The closing date for the cohort was set as November 2013 regarding date of death. The endpoint for survivors was correspondingly set as July 2013 due to a common registration delay at the CDR. The mean and median follow-up time for survivors was 18 and 17 years, respectively. All clinical follow-up data were updated as close to the closing date as possible. One emigrated patient was censored at the date of the last follow-up.

8.6.2 Incidence calculations
The incidence calculations presented in Paper I were based on the WHO age standard incidence rate per million per year, and the results were presented as three-year moving averages, in line with the recommendation of the CRN.

8.6.3 Survival analyses
Survival analysis using Kaplan–Meier estimates (Kaplan et al., 1958) and log-rank tests (Mantel, 1966) were used to analyse overall survival, SSS and event-free survival (EFS). Overall survival was calculated as being from the date of diagnosis until death from any cause, while sarcoma-specific death or treatment-related death were the endpoints of SSS. EFS was calculated as being from the date of diagnosis until the date of first metastasis, local
recurrences or SSS, whichever occurred first. Patients with primary metastatic OS were not included in the analyses regarding EFS.

Multivariate cox regression analyses (Cox, 1972) were only attempted in Paper II. The cox proportional hazard assumption was evaluated using Kaplan–Meier plots, and the potential effect of missing values was evaluated using multiple Markov chain Monte Carlo imputation (Gilks et al., 1996).

8.7 Ethical approval
The Regional Ethical Committee was informed, although the study did not require a formal ethical approval because the data registration and handling was in line with the legitimate mandate and legislation of the CRN (Larsen et al., 2009).
9. Summary of results

9.1 Paper I. Skeletal osteosarcoma, all subgroups

Incidence rates: The mean annual age-standard incidence amounted to about 3.8 per million for males and 2.8 per million for females for the period 1975–2009, with no clear time-trends (Figure 2a, Paper I). The male to female ratio was 1.4: 1.7 for age under 40 and 0.9 for older patients. The peak incidence was observed in the 10–20 year old age group for both genders, with hardly any second peak among the elderly (Figure 2b, Paper I). The incidence of OS was independent of geographic residence in Norway.

Histopathology: The conventional type of OS was the most common and comprised 80% of all high-grade OS cases and 71% of all OS cases (Table 2, Paper I). ROS represented 7% of all OS cases in the cohort. We observed about the same relative incidence of fibroblastic and chondroblastic phenotypes, with the highest frequency being the osteoblastic variant followed by the mixed subgroup (Table 2, Paper I).

Anatomical localisation: The axial-to-appendicular ratio increased with age (Figure 3, Paper I), and the average rate was 0.4 for all patients. The most common primary sites of OS were in the distal femur and the proximal tibia (Table 3, Paper I). OS of the mandible/maxilla accounted for 7% of all skeletal OS cases, with a low- to high-grade ratio of 32%.

Survival analyses: The overall 10-year survival rate for the whole study population did increase from about 30% during the late 1970s to around 50% 20 years later, with no subsequent improvement during the last two decades (Figure 4a, Paper I). Axial tumours, age above 40 and overt metastatic disease at the time of diagnosis were all negative prognostic factors (Table 4, Paper I).

Conclusion: No further improvement in the overall survival rate for OS since the 1990s was documented. The survival rates are still poor for elderly people, patients with axial disease and patients in the primary metastatic stage. The average incidence rate of OS in Norway was in line with international figures (Chapter 7.1).
9.2 Paper II. High-grade skeletal osteosarcoma

Patient characteristics: Only 48% of all high-grade OS patients (203 cases) had classical OS, that is, < 40 with a tumour in the extremities and without metastasis at diagnosis. The remaining 221 patients (non-classical OS) had primary metastatic disease, non-extremity localisation, age > 40 at the time of diagnosis or had various combinations of these features (Figure 2).

![Figure 2. Non-classical high-grade osteosarcoma (221 patients), with an illustration of the overlaps between the subgroups.](image)

We identified no significant differences in the percentage of patients with primary metastatic disease dependent on OS in the axial versus appendicular skeleton (p = 0.953) or due to age (p = 0.109). However, patients over 40 had a significantly higher rate of axial OS than did younger patients (p < 0.001).

Treatment: Twice as many classical OS patients (144 patients) received adequate treatment (surgery and chemotherapy) compared to non-classical OS patients (72 patients). The main reasons for inadequacy among the latter group were incomplete surgery or the absence of surgery in patients with primary metastatic disease or axial tumours, followed by inadequate chemotherapy in elderly patients and inadequacy of both modalities in patients with several factors (Table 3, Paper II).

Time trend: Interestingly, the percentage of adequate primary treatment increased from 50%
during the 1980s to 64% since 2005 (Figure 2a, Paper II). The non-classical OS group had just above 50% adequate treatment since 1995, in contrast to slightly above 95% for the classical OS group since the millennium. The percentage of amputations among extremity OS patients decreased from 89% during the first half of the 1980s to 18% since the millennium; this is in line with the international trend (Chapter 7.5.1). Amputation remains a valid procedure in select OS cases. We observed an increase in the percentage of OS patients with adequate chemotherapy since 1980, and we also identified an improved time trends of chemotherapy use in elderly patients and those with primary tumours in the axial skeleton during the last three decades (Figure 2c, Paper II). No corresponding trend was seen among patients with primary metastatic disease who received chemotherapy.

Primary metastatic disease was most common in the lungs (approximately 80%); otherwise, it developed in bones and in the lungs (six cases) and in bones only (10 cases). There was one case with a soft tissue metastasis. Patients with metastatic disease had larger primary tumours than patients with localised disease (Table 2, Paper II). We observed a fluctuation in primary metastatic disease among high-grade OS patients between 1975 and 2009 (Figure 3).

![Figure 3. Number and percent of skeletal osteosarcoma patients with metastasis at the time of diagnosis. Annual figures during 1975–2009.](image)

Local recurrence and metastases: We identified a higher rate of local relapses among patients
with non-extremity tumours than among patients with extremity OS, but no corresponding differences regarding the first metastatic event were documented. Forty-five percent of the patients with local recurrence (17 patients) were never diagnosed with metastasis during follow-up, and 94% of these cases (16 patients) were related to relapses in the axial skeleton. Twelve of these patients died due to their local relapse and without metastasis.

Survival: A considerable discrepancy in survival rates between classical and non-classical OS patients was observed: 61% versus 26% 10-year SSS (Figure 3a, Paper II). However, ‘less adequate’ treatment could not explain the entire discrepancy in SSS between these two groups (Figure 4d\(^9\), Paper II). The latter observation may be due to chemoresistance in primary metastatic disease (Chapter 10.2.4) and a higher rate of local relapses among patients with axial tumours (see above). We observed just minor differences in relative risk (RR) between all patients with primary metastatic disease (RR = 3.1) and those in this group that received adequate treatment (RR = 2.9). Patients with either axial OS or with age >40 had a larger difference in RR, but the observed difference was not statistically significant (with uncertainty due to the limited number of observations).

Prognostic factors. Adequate primary treatment, elevated LDH and decade of diagnosis were all significant factors for overall survival and EFS (Table 5, Paper II). Primary metastatic disease, age > 40 and increased tumour size were also adverse factors for overall survival, whereas axial tumour localisation did not reach significance (Table 5, Paper II). Further, non-classical OS was an adverse factor for overall survival.

Conclusion: We documented a dramatic difference in outcomes between classical and non-classical high-grade OS patients, but treatment variables could only partly explain the dismal outcome of the latter.

9.3 Paper III. Low-grade and dedifferentiated osteosarcoma

Incidences: Fifty-four patients with LGOS and DLGOS were identified. Twelve patients had DLGOS, including six patients at primary diagnosis. The annual incidence for all patients was 0.3 per million, with the peak incidence in the third decade of life (Figure 1, Paper III).

\(^9\) Separate survival analyses for patients with primary metastatic disease, non-extremity localisation or age > 40 at the time diagnosis among adequately treated high-grade OS patients are presented in Figure 1a-c, Chapter 14.2.
Clinicopathological data: The LGCOS group comprised 29 patients, only 12 of which had tumours in long bones. Ten cases had tumours in the mandible and maxilla (Table 2, Paper III). With one exception, all 20 cases of POS were located in long bones. Four of the remaining five patients were classified as having secondary LGOS, while the fifth case was the only low-grade ESOS. We observed a wide range in duration of symptoms prior to diagnosis but with approximately half a year in median length for both LGOS and DLGOS (Table 1, Paper III). The median tumour size among DLGOSs was 11 cm, nearly twice as large as that of the rest of the LGOS cohort. About one-third of all patients had elevated ALP at diagnosis, and 20% had increased levels of LDH.

Treatment: All 54 patients underwent at least one operation (Table 4, Paper III). Only five patients were treated with amputation, including three with amputations as part of their primary treatment. Adjuvant chemotherapy was mainly reserved for patients with DLGOS (Table 4, Paper III). Six patients received postoperative radiotherapy with curative intent.

Local recurrence and metastases: Fifteen patients experienced local relapses during follow-up and 10 developed metastatic diseases, including three at primary diagnosis (Table 3, Paper III). Local relapses were uncommon in cases of adequate (16%) versus inadequate (67%) surgery. Six cases of LGOS showed transformation to high-grade malignancy at the time of local recurrence, including five patients at the time of the first local relapse. Four of these six patients had received inadequate surgery, while the other two were reported to have received primary surgery with a marginal margin.

Survival: The five-year SSS was 91%, with no documented improvement over time (Figure 2b, Paper III). Free margins following surgical resection of the primary tumour had a positive impact on survival (Figure 2c, Paper III). As expected, both local relapse and metastasis at diagnosis or during follow-up were associated with an unfavourable outcome (Table 5, Paper III). In addition, radiotherapy as a treatment modality predicted inferior SSS, probably due to the selection of high-risk patients in need of such treatment. Chemotherapy had no significant impact on outcome. Neither higher age nor axial tumour localisation were adverse prognostic factors.
**Conclusion:** Patients with LGOS had an excellent prognosis when surgically resected with a free margin. However, LGOS has the potential to dedifferentiate and metastasise with an unfavourable outcome.

9.4 *Paper IV. Extraskeletal osteosarcoma*

**Incidence:** Thirty-seven patients were classified as having ESOS, most of whom were elderly. ESOS accounted for 7% of all OS cases in Norway from 1975–2009. The average annual incidence for all ESOSs was roughly 0.2 per million during this period.

**Histopathology:** Thirty-six cases were of high-grade malignancy. Several ESOSs were heterogeneous in terms of varying expression of the major OS phenotypes (Figures 1 and 2, *Paper IV*). ESOS did not reveal any characteristic immunophenotype except for Ki67, which was significantly correlated to an unfavourable outcome (Table 6, *Paper IV*).

**Clinicopathological data:** A dominant peak of ESOS among the elderly was observed (Figure 3, *Paper IV*). The median age was 68 years for all patients. Seventy-six percent (28 cases) had an axial tumour (Table 3, *Paper IV*), including all patients (nine cases) with RESOS. The gender balance was equal in all cases of ESOS, in contrast to a male to female ratio of only 0.1 among patients with RESOS (Table 1, *Paper IV*). The median tumour size and symptom length were 10 cm and 4 months, respectively, for all patients. About one-third of all patients had elevated ALP at diagnosis, in contrast to 29% who had increased levels of LDH.

**Metastases and local recurrence:** Metastases were present in eight patients at diagnosis, including four patients with RESOS. Among the patients without primary metastatic disease, 52% (13 cases) developed metastases, and 28% (7 cases) experienced local relapse during follow-up. Five patients in the former group were also represented in the latter.

**Treatment:** Twenty-nine patients underwent at least one operation (Table 4, *Paper IV*). Eleven patients received chemotherapy with a curative intent in nine of these cases and palliative intent in the remaining two cases. Six patients received radiotherapy with a curative intent as part of their multimodal treatment.

**Survival:** The five-year SSS was only 16%. A free margin following surgical resection had a positive impact on SSS, in contrast to chemotherapy and radiotherapy (Table 6, *Paper IV*).
Primary metastatic disease, large tumour size and elevated ALP, LDH and Ki67 all predicted a poor outcome.

**Conclusion:** The relatively poor prognosis of ESOS may relate to both primary chemotherapy resistance and the different biological characteristics of these tumours compared to conventional OS. Therefore, new predictive molecular markers and therapeutic approaches to the treatment of ESOS are needed.

9.5 *Previously unpublished data*

9.5.1 Radiotherapy – High-grade skeletal osteosarcoma

One hundred and sixty patients received radiotherapy as part of their treatment for high-grade OS. Radiotherapy was only considered as curative in 11% of these cases (17 patients). Here, curative treatment intent was defined as fractionated radiotherapy following marginal or intralesional surgery for a primary tumour before metastatic disease or local relapse was confirmed. The remaining group (143 cases) received radiotherapy for various reasons, such as towards local relapses and/or metastasis, including total lung irradiation in a palliative setting.

Four of the 17 patients with curative intent were treated for OS in the long bones—that is, the proximal humerus (two cases), the distal femur (one case) and the distal ulna (one case). The other patients received radiotherapy for OS in the mandible/maxilla (nine cases), columna (two cases) and skull/facial bones (two cases). Among the group of patients treated with radiotherapy with a curative intent, 13 received chemotherapy during primary treatment, but it was only adequate in six of these cases.

Radiotherapy predicted inferior SSS (Figure 4), probably because of poor marginal status among most of the patients given such treatment. In a few cases, treatment-related deaths also contributed (Lia *et al.*, 2013). OS is also known to be relatively resistant to such therapy (Chapter 7.5.1). Based on a significance level of 5%, curative treatment intent did not have a significant positive impact on survival compared to the remaining group of patients treated with radiotherapy (SSS; RR = 1.7, CI 0.9–3.1, p = 0.073, overall survival; RR = 1.7, CI 0.9 – 2.9, p = 0.084). This latter result might be due to few observations with curative intent (and hence wide confidence intervals). We might well have achieved a different result with an alternative definition of curative intent.
9.5.2 Osteosarcoma in different long bones of the extremity

Seventy percent of all OS patients (332 cases) had OS arising in extremity long bones (Table 1). Forty-three percent of these patients (142 cases) had a primary tumour in the proximal part of the extremity long bones. This result is in line with the literature (Huvos, 1991; Unni et al., 2010).

Table 1: Distribution of osteosarcoma in different long bones of the extremity, 1975–2009.

<table>
<thead>
<tr>
<th>Location of long bones</th>
<th>Humerus (%)</th>
<th>Radius (%)</th>
<th>Ulna (%)</th>
<th>Femur (%)</th>
<th>Tibia (%)</th>
<th>Fibula (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>30 (83)</td>
<td>1 (50)</td>
<td>1 (25)</td>
<td>22 (12)</td>
<td>71 (85)</td>
<td>17 (85)</td>
<td>142 (43)</td>
</tr>
<tr>
<td>Diaphysis</td>
<td>2 (6)</td>
<td>11 (6)</td>
<td>2 (2)</td>
<td>153 (82)</td>
<td>11 (13)</td>
<td>3 (15)</td>
<td>175 (53)</td>
</tr>
<tr>
<td>Distal</td>
<td>4 (11)</td>
<td>1 (50)</td>
<td>3 (75)</td>
<td>186 (100)</td>
<td>84 (100)</td>
<td>20 (100)</td>
<td>332 (100)</td>
</tr>
</tbody>
</table>

Total: 36 (100) 2 (100) 4 (100) 186 (100) 84 (100) 20 (100) 332 (100)

*Forty-three percent of all high-grade cases (303 cases) also had a primary tumour arising in the proximal part of the extremity long bones. Data is not shown.
10. General discussion

10.1 Methodological considerations

10.1.1 Study material

The patient material is based on a nationwide, unselected and longitudinal observational study comprising all Norwegian OS and ESOS patients within a time frame of more than three decades (Chapter 8.1). The main strength of the present study is the large number of patients included within such a context and the consistency of the database with multiple and partially overlapping data and registry sources (Chapter 8.3).

All clinical follow-up data were updated as close to the closing date (November 2013) as possible. Termination date for all survivors set at July 2013 (Chapter 8.6.1) prevented bias (systematic error) due to non-identical follow-up of patients with few or frequent appointments.

Our main source was data retrieved from the population-based registry of the CRN. Since 1953, the CRN has collected data on cancer incidence, mortality and survival in Norway based on the mandatory reporting of all new cancers (Larsen et al., 2009). The completeness has been reported to be higher than 95% during the registration period 1987–96 for ovarian cancer (Tingulstad et al., 2002) and approximately 98.8% on average for most neoplasms reported to the CRN during the period 2001–2005 (Larsen et al., 2009). Importantly, our approach using different data sources increased the gross number of OS/ESOS patients by 7.2% (42 cases) added to the 544 cases reported to the CRN (Figure 1, Paper I). Of these patients, 28 were nevertheless reported to the CRN but not correctly as OS, ESOS or SCS ICD-0-2 codes.10

Many countries have cancer registries, but few have the advantage of the Nordic countries with mandatory cancer reporting being combined with a unique personal identification number, high-quality registers on a national level, a social structure with limited migration and the opportunity to follow individuals through their entire life. These advantages enable the CRN not only to count the number of cancers accurately but also to estimate cancer rates precisely, including how cancer incidence changes over time.

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10 The remaining 14 cases had residency abroad and were correctly excluded from the CRN.
The data collected from the CRN contains mainly demographic characteristics, histologic type, tumour stage and cause of death. Accordingly, information concerning treatment and the recurrence of OS from the CRN has been insufficient, as it is for most other population-based registries. We have therefore received most of the missing information from the sarcoma database at the NRH and from clinical records at hospitals involved in sarcoma management (Chapter 8.3).

Our knowledge concerning OS/ESOS are also based on institutional series (Ferrari et al., 2009; Aggerholm-Pedersen et al., 2015), experiences from cooperative sarcoma societies like the Cooperative Osteosarcoma Study Group (COSS) (Bielack et al., 2009b) or various clinical trials, as outlined in a previous review (Luetke et al., 2014). Hospital-based cancer registries offer the advantage of detailed patient information, although, for example, inconsistent follow-up data and administration of a variety of treatments might limit the usefulness of these sources (Travis, 2006). Moreover, cancer data from institutional series are normally relatively small sized, although they often are larger than clinical trial databases. Therefore, hospitals/organisations might cooperate like e.g. the COSS or the SSG to increase the study population (Alvegard et al., 2009; Bielack et al., 2009b). Nevertheless, neither institutional series nor studies from cooperative sarcoma societies are population-based, and these studies may be biased according to the referral pattern and treatment profile of the hospital(s). For instance, nearly all participants in the COSS are in the paediatric or adolescent age group.

Data from protocol-based trials are usually collected prospectively in a uniform manner, thus allowing detailed data on treatment to be acquired (Laake et al., 2007). The results from controlled clinical trials typically have high internal validity (Chapter 10.1.3) due to the study design based on randomisation and blinding (Laake et al., 2007). However, the drawbacks with clinical trials are often their relatively small sizes and the fact that patients are often followed for a relatively short period. There can also be a selection bias due to the selection of patients according to the inclusion criteria, which implies that research results cannot necessary be generalised; this limit the external validity (Chapter 10.1.3).

As expected, we have not reached full completeness regarding all clinical information for all patients in the present database. Nor has it been possible to obtain the same degree of details regarding clinical variables as compared to institutional registries, cooperative sarcoma societies and/or clinical trials. Still, we believe that our key variables (Chapter 8.4–5) ensure
an adequate amount of detail in order to expand our knowledge regarding the whole OS and ESOS population, including those with non-classical OS and LGOS/DLGOS. As of today, the present literature covering this field of research has been both fragmented and sparse.

10.1.2 Reliability
Reliability is understood as consistency of the recorded data on measurements (Laake et al., 2007). A measure is said to have high reliability if it produces similar results under consistent conditions for the same test subjects. For example, measurements of people’s height and weight are often extremely reliable (Carlson et al., 2009). We also assume that data from clinical trials are more reliable than the corresponding variables from most other studies, as the former are collected prospectively in a uniform manner.

The methodology in Papers I-IV was based on the review of each of the different data sources used as part of this thesis (Chapter 8.3). The reliability of the obtained data is strictly dependent on the quality and consistency of each of these data sources. Not surprisingly, we received more detailed clinical information from the NRH and HUH than from the other hospitals in Norway. Further, the amount of clinical data has increased from all hospitals involved in sarcoma management during the last three or four decades and in particular since the late 1980s, probably due to the improved computer technology. In general, we cannot draw any conclusion regarding the reproducibility of the study material received from each hospital in connection to this research project. Nevertheless, the information from NRH and HUH has been crucial for the whole study because the majority of all patients were only treated at these two hospitals.

The reliability of the recorded data is dependent on the variables that have been analysed (Chapter 8.4–5). The retrieved data based on general guidelines like the subclassification of OS or the level of ALP has probably improved reproducibility as compared to, for example, data on the symptom duration before a diagnosis is reached. However, histological examination is still subjective and hampered by poor intra- and inter-reproducibility (Mangrud et al., 2014). The biological reference interval might also depend on each laboratory’s procedures and measurements, etc. (Rustad et al., 2004).

We have tried to improve the data quality of the cohort using the following three principles. First, we aimed to achieve high internal consistency of the data that was analysed. The review
of the different data sources was done by the same person(s) using the same guidelines for all cases in the study. For instance, the third and last author (Paper I) re-evaluated all histological reports from not previously re-examined OS/ESOS cases according to the current WHO criteria (Chapter 8.2); there is a more detailed presentation in Paper I. Nevertheless, we cannot rule out that the quality could have been even better with a full uniform histological re-examination of all cases in the cohort; this is discussed further in Chapter 10.1.4. The lack of a retrospective review of radiographic images as part of this project (Chapter 8.2) may also have biased our results.

Second, we included defined variables in the cohort, such as ‘adequate’ treatment (Chapter 8.5). Information regarding the latter variable was based on a retrospective collection and review of the documented treatment data, performed by first author, according to defined guidelines. Overall, we experienced a high degree of consistency between the different sources used for this purpose, mainly information from medical records and different treatment protocols. Therefore, we assume that the reproducibility of this clinical information is high. We also omitted variables with assumed low reliability in this study, such as treatment intent. This is because it might be a possible grey zone between curative and palliative intent prior to primary treatment and/or later relapses/metastases, at least in a retrospective study. This is confirmed in several medical records used in relation to this project.

Our last principle was to define variables with high consistency between the different data sources. The decision to use, for example, the maximum length of a tumour in cm as a proxy of tumour size made it possible to compare the estimates from reports about surgical specimens and/or radiographic images in addition to data from the sarcoma database at the NRH. The latter source included only this variable as an estimate of tumour size. A more accurate measure of actual tumour size, such as tumour volume (Bacci et al., 2002a; Smeland et al., 2011), could not be performed. Tumour sizes based on tumour volume would also have increased the number of missing values considerably in the current database.

11 Professor Bruland was consulted in some difficult cases.
12 Treatment intent was still presented in a few cases, especially regarding radiotherapy, in Paper III-IV (Chapter 8.5).
No defined test method was applied to estimate the reliability of the obtained data/variables in Paper I–IV. However, we first obtained information from all Norwegian OS patients included in the various formal SSG trials (Chapter 7.5.1) from the SSG secretariat after finishing our review process concerning the patient base. From our perspective, an additional 70 Norwegian OS patients were identified as SSG protocol patients due to this information. With one exception, no discrepancies were found regarding the patient population comprising the current study (from our review process) and the ‘new’ information from the SSG. Therefore, we consider the reproducibility of the population base to be high.

10.1.3 Validity

Internal validity is defined as the degree to which a study is representative of the particular group of individuals being studied (Laake et al., 2007). Internal validity may be weakened by sample selection bias, information bias and statistical confounding (Laake et al., 2007). Our nationwide approach has in our opinion minimised the sample selection bias. We have also, as previously mentioned, tried to reduce the information bias through multiple and partly overlapping data and registry sources, but we have no guarantee that all clinical information reported in, for example, medical notes is correctly reported or if information registered in the cohort is flawed in other ways. Nor have we reached full completeness regarding clinical information for all patients in the present study, which might bias the results.

A confounder is a variable whose presence affects the variables being studied so that the results do not reflect the actual relationship. There are various ways to exclude or control for confounding variables. Statistical models like multivariate regression analyses can, for example, eliminate the effects of confounders. We analysed the prognostic factors presented in Paper II based on such a statistical method. However, these analyses are still uncertain due to the lack of (a) ‘large’ dataset(s) as part of this project. This was confirmed through expanded confidence intervals in many cases in this study and is discussed further in Chapter 10.1.7. In the present thesis we also present new multivariate analyses based on the dataset from Paper I (Chapter 14.1), which confirm all conclusions from our first study. Multivariate cox regression analyses were not attempted in Papers III and IV, as there were relatively few cases in connection to these studies.

One OS patient treated at HUH was registered with incorrect ICD-O-2 codes at the CRN (Figure 1, Paper I) and was therefore first ‘discovered’ as eligible in our cohort based on the information from the SSG registry.
External validity refers to our ability to generalise the results of our study to other settings and individuals (Laake et al., 2007). This is a complex challenge because generalisation depends on such things as study design, population and the statistical model used. All situational observations of a particular study like time interval, treatment conditions, study population, country etc. potentially limit generalisation. Therefore, we cannot in principle generalise our results to, for example, other countries. Still, we believe that nationwide unselected patient materials, like in the present thesis, are highly warranted especially in the international research community in order to highlight relevant clinical challenges, e.g. non-classical OS, in particular.

10.1.4 Classification of osteosarcoma
A definitive OS diagnosis requires histopathological examination with access to additional analyses like immunohistochemistry and genetic analyses, when needed. To our knowledge, no major adjustments in the OS classification were introduced between 1975 and 2009 according to the WHO criteria. Other subdivisions of OS are commonly used at the MD Anderson Cancer Center or the Mayo Clinic (Raymond et al., 2009a). However, there have been some changes in the classification of other primary bone sarcomas during the study period as the result of new molecular tests (Fletcher, 2014). For instance, this is the case for SCS (Chapter 8.1) because some fibrosarcomas, according to current WHO criteria, have been re-classified as leiomyosarcomas (Fletcher et al., 2013). Further, the entity malignant fibrous histiocytooma is currently classified as undifferentiated pleomorphic sarcoma (Matushansky et al., 2009; Fletcher, 2014). SCS resembles OS in biological behaviour and radiological findings and is therefore generally treated as OS.

As mentioned above, we have histologically confirmed all OS/ESOS cases as part of this project, but only about 20 % of all cases were formally re-examined (Chapter 8.1-2). We cannot exclude that the quality could have been even better with a uniform histological re-examination of all 702 cases in terms of the gross study material analysed and a retrospective review of the relevant radiographic images (Chapter 10.1.2). However, a significant disadvantage of such an approach is the potential lack of histological specimens available for re-examination. This might be an even larger problem in nationwide studies than in studies based on, for example, an institutional series. The dropout rate in a Finnish study was as high as 34% due to missing original histological specimens (Sampo et al., 2008), which is an
unavoidable pitfall in national studies. We assume that the dropout rate in our study would have been lower because most cases were already diagnosed and treated at NRH and/or HUH.

Like most malignant tumours, OSs are morphologically heterogeneous with poor intra- and inter-reproducible histological examination (Chapter 10.1.2). We also observed that many of the histologically re-evaluated tumours in the present study were heterogeneous; two examples of tumour heterogeneity are presented in Paper IV (Figure 1-2). Despite variation in histological appearance, consensus in determining dominating phenotype was achieved in most cases, otherwise reported as mixed phenotype (Table 2, Paper I and Table 1, Paper IV).

We cannot guarantee that ‘all’ OS and ESOS cases in Norway are included in the present thesis. Many conditions may mimic OS/ESOS and the differential diagnosis includes benign and malignant tumours, infections and inflammatory processes arising from the musculoskeletal system (Raymond et al., 2009b). Cases not confirmed upon re-evaluation as OS in Study I were excluded and are presented in Paper I (Table 1).

10.1.5 Selection of clinical variables
Our main principles regarding the selection of relevant variables in the cohort were previously discussed in Chapter 10.1.2. In the present section, we elaborate on our decisions concerning the design and choice of certain important clinical variables (Chapter 8.5) and why other relevant variables were omitted from the present study.

*High-grade osteosarcoma*
Our treatment variables for high-grade OS in Paper II comprised treatment of primary tumour at time of diagnosis in addition to patients with primary metastatic disease. This decision was based on the fact that ‘adequate’ primary treatment is essential for long-term survival because high-grade OS is considered a systemic disease characterised by the non-detectable spread of micrometastases at primary diagnosis in a majority of patients (Chapter 7.4). Therefore, adjuvant chemotherapy is given in addition to surgery, with the intention to either eradicate occult, micro-metastases or to delay the time to relapse (Chapter 7.4-5). To facilitate the impact of surgery and chemotherapy, the regimens in the present study were divided into ‘adequate’ versus ‘inadequate’ treatment (Chapter 8.5). Radiotherapy was not included in Paper II because OS is considered a radio-resistant neoplasm (Chapter 7.5.1), which is confirmed in the present thesis (Chapter 9.5.1).
Supplementary analyses regarding the treatment of local recurrence or metastases for high-grade OS were not performed in Paper II, essentially due to increased uncertainty of the received treatment in connection to each local and/or systemic relapse. Further, we have only dichotomised between adequate (free or marginal) and inadequate margins in the statistical analyses in the present thesis (Chapter 8.5) and not a more detailed classification of surgical margins, due to our nationwide approach (Chapter 10.1.1-2).

Correspondingly, we have not attempted to analyse the prognostic effect of dose intensity chemotherapy treatment of high-grade OS (Luetke et al., 2014) as part of this project. The prognostic relevance of dose intensity has not yet been clarified in the OS literature (Chapter 7.6), perhaps partly due to methodological ‘challenges’. For instance, it is important to obtain precisely recorded data about received chemotherapy and treatment duration (Eselgrim et al., 2006) in such a context, including handling ‘practical’ problems like treatment compliance and chemotherapy toxicity in a satisfactory manner. Such study populations should preferably be treated in a uniform way and with adequate follow-up, ideally as prospective patient cohorts, in order to obtain data with high reliability.

Moreover, we have not included histologic response to preoperative chemotherapy as a separate variable in the present thesis. This variable may also have affected our multivariate analyses regarding the prognostic factors in Paper II (Chapter 10.2.9). Our decision was based on the different systems for screening prospective chemotherapy used from 1975 to 2009 (Bruland et al., 1997) and on the fact that the preoperative multidrug combinations have also changed considerably, as the concept was introduced by Rosen and co-workers in the 1970s (Rosen et al., 1975). More active drugs have also been added to the preoperative schedules, and the timeline from start of chemotherapy to surgery has been extended (Bruland et al., 1997). Moreover, histological responses to chemotherapy have already been well-documented in the reports of the consecutive trials of the SSG and the ISG/SSG (Chapter 7.5.1). In addition, we could not evaluate a histological response to chemotherapy without a complete and uniform histological re-examination of all cases in the cohort (Chapter 10.1.4).

Last, we have not included proximal versus distal localisation in extremity long bones (now presented in Chapter 9.5.2) as a potential prognostic factor in the statistical analyses in the present thesis. OS of the proximal femur and humerus was associated with a worse outcome.
than other long bone extremity OS tumours in a previous large study by COSS (Bielack et al., 2002).

Low-grade osteosarcoma

LGOSs are usually treated by surgery alone due to a lower malignancy potential than for high-grade OS (Chapter 7.5.2). In Paper III, the surgical result, used as a prognostic variable in the survival analyses, was dichotomised between adequate margin and positive margin after the last surgery of primary tumour (Chapter 8.5.2). Therefore, this definition comprises, unlike the definition of high-grade OS (see above), also surgical treatment of local recurrence(s). Our decision was based on the fact that several patients in the cohort first received their primary OS diagnosis at the time of the first local relapse after histological re-evaluation of their primary specimens, for example, re-classification from exostosis. Margins from the last surgery for localised disease were, in our opinion, also the most relevant approach in the survival analyses because none of the LGOS or DLGOS patients in the study had proven metastases at the time of the last surgery for the primary tumour, except for three patients with primary metastatic disease (Paper III). This assumption also eliminated a potential bias in the dataset regarding surgery due to local relapse versus re-operation as a consequence of positive margins.

10.1.6 Causes of death

Date and cause of death were primarily retrieved from the CDR (CDR, 2015). All deaths are reported by doctors who are required to complete a death certificate. These reports are also prepared in accordance with the ICD codes. The aim is to identify the underlying cause of death (and not only the acute reason for death) according to the rules set by the WHO. However, the exact underlying cause of death might be difficult to assess without an autopsy (Alfsen et al., 2012), for instance, in cases of multiple primary malignancies when death from a second cancer might have been misinterpreted as death from the first cancer and vice versa, especially when there is short gap between the diagnosis of the first and second primary malignancies.

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14 ICD-8 was used for cases diagnosed from 1975 to 1978, ICD-9 for cases from 1979 to 1994 and ICD-10 for cases from 1995 onwards.
We have attempted to verify the cause of death stated in the CDR by scrutinising all relevant clinically available information (Chapter 8.3). Overall, there were relatively few discrepancies between our findings and the cause of death stated in the CDR. In total, we found that an additional 22 patients died due to OS than was reported in the CDR, as of November 2013 (Paper I), and the revised number was used in the survival analyses in Papers I-IV. These discrepancies occurred for three main reasons. The first is that the CDR had not received cause of death for all dead patients in the cohort by November 2013, in most cases because the deaths were recent. The second is that incorrect or unspecific ICD codes were used in a few cases; for example, the CDR reported ‘malignant tumour in jaw’ instead of OS of the jaw. Another example is the use of the following code ‘question about brain cancer’ when these patients actually had brain metastases due to OS in a palliative setting. The last reason is that three patients were incorrectly reported to have died of their first malignant cancer when they actually died from the secondary one, that is, ROS in all three cases.

10.1.7 Statistical analyses
The majority of our results in all papers were based on descriptive analyses of the investigated cohort, combined with cox regression (Chapter 8.6). The confidence intervals for several survival estimates presented in this thesis were rather wide, showing the uncertainty due to the small number of cases. In Papers III and IV in particular, datasets were small, even on the national level, due to the infrequency of the disease under study. Multivariate cox regression analyses were only applied for high-grade OS patients in Paper II. Despite the fact that this study included 424 patients, the results were still encumbered with uncertainty (Table 5, Paper II), partly as a consequence of missing values. Additional multivariate analyses based on the dataset from Paper I are also included in the present thesis (Chapter 14.1). We identified no missing variables in the latter analyses due to other covariates included in Paper I compared to Paper II.

We observed a contradictory result in terms of the treatment effect dependent on decade of diagnosis between the univariate (Table 4) and the multivariate analyses (Table 5) in Paper II. The former documented no significant improvement in OS survival during the last two decades of the study, whereas the latter only confirmed improved survival since the 1980s. The results of the univariate analyses from Paper II were in line with our findings from Paper

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15 Missing values were related to LDH and tumour size (169 cases).

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I, including the additional multivariate analyses presented in Chapter 14.1. Numerous reports have also reached a similar conclusion regarding the survival trends for OS during the modern chemotherapy era (Chapter 7.6.1). Therefore, the results of the multivariate analyses in Paper II may be due to chance, perhaps partly as a consequence of missing values (see above).

10.2 Discussion of main results

10.2.1 Incidence

OS is the most common primary high-grade sarcoma of the skeleton, particularly among children and adolescents (Mirabello et al., 2009a; Fletcher et al., 2013). The average incidence rates of all OS in our cohort, about 3.3 per million (Paper I), were in line with the literature (Mirabello et al., 2009a) but substantially higher than in a previous nationwide study (Sampo et al., 2011). The latter result is partly due to methodological differences between the Finnish study (Chapter 7.7) and our approach (Chapter 8.1-2). Sampo and co-workers experienced an increased drop-out ratio due to the lack of histological specimens for re-examination compared to our results (Chapter 8.2). They also excluded ESOS and post-mortem diagnosis in their nationwide study (Sampo et al., 2011) compared to the present study (Chapter 8.1). Correspondingly, our incidence rate was slightly higher than in a recent report by Duong and co-workers regarding most of the US population (Chapter 7.8). However, direct comparison is difficult to evaluate due to multifactorial differences between our study and that study. We observed no significant change in the incidence across all groups since 1975 (Figure 2a, Paper I), which is in line with a previous nationwide study from England (Whelan et al., 2012a).

We confirm that OS is rare in younger children, while there is a significant increase in the incidence among teenagers, coinciding with pubertal growth (Mirabello et al., 2009a; Ottaviani et al., 2009a). The peak among females is earlier than in males (Mirabello et al., 2009a), which is also in line with our results (Figure 2b, Paper I). The occurrence of OS is most frequent in the metaphyseal area adjacent to the growth plate of long bones, such as the distal femur, proximal tibia and proximal humerus (Savage et al., 2011), which are the sites of particularly rapid growth during the adolescent growth spurt. This reinforces the relationship between bone growth and OS formation. There may be an increased vulnerability at these growth plates due to the high cell turnover during puberty (Savage et al., 2011). Several studies have also suggested an association between bone cancer risk and height at diagnosis.
The tendency of OS to occur in the appendicular skeleton also decreases with age (Chapter 7.1), as confirmed in our study (Figure 3, Paper I).

OS among children and adolescents is more common in males than females (Mirabello et al., 2009a), which is confirmed in the present thesis (Figure 2b, Paper I). A possible explanation of these sex differences in OS may be attributed to hormonal differences between genders (Duong et al., 2013). Moreover, we could not evaluate a potential difference in the incidence of OS being dependent on ethnicity in the present thesis, as documented in the literature (Ottaviani et al., 2009a; Mirabello et al., 2009b). This is due to the very high percentage of Caucasian inhabitants in Norway and limited migration.

We did not verify the clear bimodal distribution (Figure 2b, Paper I) found in previous studies (Mirabello et al., 2009a; Whelan et al., 2012a). This may be related to a low incidence of Paget disease in Norway (Table 2, Paper I) compared to several other countries with increased incidence of this predisposing condition (Chapter 7.2). This is despite a somewhat higher percentage of ROS in the present thesis (Table 2, Paper I) than in earlier publications (Unni et al., 2010; Fletcher et al., 2013). Mirabello and co-workers also reported a comparatively lower second incidence peak among elderly people in Europe, excluding the United Kingdom (Mirabello et al., 2009a).

We observed a slightly higher percentage of ESOS in % of all OS (7%) than previously presented (Chapter 7.1). This may be due to a higher percentage of RESOS in our cohort (Chapter 9.4) compared to previous findings (Chapter 10.2.8), in addition to a broader patient base (Chapter 8.1). Most patients were elderly (Figure 3, Paper IV) in line with the literature (Chapter 7.1).

10.2.2 Histological subtypes of osteosarcoma
Most OSs are of high-grade malignancy (Fletcher et al., 2013). However, LGOS (POS and LGCOS) accounted for more than 10% of all OSs in our study (Table 2, Paper I), which is

Mirabello et al. (2009a) discussed several reasons for the great variations observed in elderly patients throughout the world, such as diagnosis and/or classification differences among countries, differences in completeness of cancer registration, differences in environmental exposures that could alter risk of primary OS and/or increase the chance of a secondary OS and different genetic components of OS in the elderly.
higher than in other reports (Okada et al., 1994; Andresen et al., 2004; Malhas et al., 2012). Correspondingly, we reported a somewhat lower incidence of conventional and telangiectatic types of OS in the present thesis (Table 2, Paper I) compared to what was previously reported (Fletcher et al., 2013). Some of these discrepancies may be explained by our minor modifications of the current WHO criteria (Fletcher et al., 2013), which are presented in Paper I. Further, our results may be due to a broader patient base than normally studied, both as a consequence of the nationwide approach and because we have included, for example, head and neck OS and secondary OS in the present cohort.

10.2.3 Sex distribution and effect on survival
We confirmed a higher male to female ratio in extremity high-grade OS (rate 1.6) compared to non-extremity tumours (rate 1.0) (Saeter et al., 1996; Duong et al., 2013). In addition, elderly OS patients and/or patients with primary metastatic disease had approximately an equal sex distribution in Paper II. Although gender had a significant effect on survival for adequately treated high-grade patients in the current study (Table 6, Paper II), we identified no corresponding effect for all patients (Table 4, Paper II). This is in line with previous findings in the literature (Chapter 7.6.1). The gender balance was equal among LGOS and ESOS in the present thesis, with no significant differences in survival. This is discussed further for ESOS in Paper IV.

10.2.4 Non-classical osteosarcoma
A major goal of this project has been to identify patient characteristics and treatment results for non-classical OS (Chapter 4.1). To our knowledge, no previous nationwide studies have analysed in detail the non-classical group, which represents just over half of the patients in our cohort (Figure 2, Chapter 9.2). This is even higher than previously reported (Bruland et al., 1997).

As previously mentioned, a considerable discrepancy in survival between classical and non-classical OS was observed, and treatment variables could not explain the entire discrepancy in outcomes between these two groups (Chapter 9.2). One important reason for the latter observation is the poor prognosis of patients with primary metastatic disease, independent of given treatment (see below). Further, non-extremity tumours have been associated with a

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17 The male to female ratio was 1.4 for all high-grade OSs.
higher rate of local recurrence than extremity OS, while no corresponding differences regarding the first metastatic event were documented (Saeter et al., 1996). We confirmed these results in the present study (Chapter 9.2). Both local recurrence and metastatic relapse are associated with rather poor prognosis, even though post-relapse survival seems to be higher among patients with late relapse (Chapter 7.6.1).

Non-extremity localisation of primary tumours
Axial primary tumour localisation was not of significance in the multivariate analyses (Table 6, Paper II). Nor did we report any significant difference in tumour site among adequately treated patients (Figure 1a, Chapter 14.2). Nevertheless, it is well established that axial localisation has a less favourable outcome than primary disease arising in the appendicular skeleton (Chapter 7.6.1). This is partly explained by a higher rate of axial tumours among elderly patients (Whelan et al., 2012a), which was also confirmed in our study (Figure 3, Paper I). However, we have far too many cases with inadequate surgery among our patients with axial OS (Table 3, Paper II), with local relapse as a frequent consequence.

Metastasis at the time of diagnosis
Primary metastatic disease was a strong negative and independent prognostic factor (Tables 4–6, Paper II), in line with the literature (Chapter 7.6.1). Interestingly, we also observed large discrepancies in the survival rate among ‘adequately’ treated patients with metastasis at the time of diagnosis compared to the other patients in the present cohort that where ‘adequately’ treated (Figure 1b, Chapter 14.2).

Compared with patients with localised disease, patients with detectable metastases at the time of diagnosis had larger primary tumours (Table 2, Paper II). Results similar to ours have been reported by the Rizzoli Institute (Bacci et al., 2002a), COSS (Bielack et al., 2002) and a recent SEER study (Miller et al., 2013). The latter two studies also documented an increased risk for metastatic disease among patients with axial OS. This is in contrast to our findings (Table 2, Paper II). We found no significant differences in the duration of symptoms between patients with primary metastatic versus localised disease (Table 2, Paper II), and there seems to be no unanimous answer regarding this issue in the literature (Bielack et al., 2002; Bacci et al., 2002a).
Patients presenting with overt metastases at the time of diagnosis are usually given the same first-line chemotherapy as those without overt metastases (Chapter 7.5.1), despite poor response to this treatment, even when all macroscopic tumour tissue is surgically eliminated (Meyers et al., 1993). Nevertheless, there is still a lack of consensus regarding effective second-line chemotherapy for metastatic OS (Chapter 7.5.1).

COSS has previously stated that the main reason for the low success rate in primary metastatic disease is not that chemotherapy is less effective than in localised disease but that complete surgery is difficult to accomplish (Bielack et al., 2002). As imaging is not sensitive enough to detect all lung metastases (McCarville et al., 2001), careful and thorough manual palpation of the lungs has become the standard intraoperative approach in pulmonary metastasectomy for, for example, OS (Rusch, 2002; Picci, 2007; Carrle et al., 2009). A previous study also suggested surgical explorative thoracotomy despite the seemingly complete disappearance of lung lesions after chemotherapy on preoperative CT scans due to the limitations of imaging pulmonary metastases (Kayton et al., 2006). Therefore, thoracotomy apparently plays a critical role in any curative approach for primary pulmonary metastatic OS and should always be attempted when there is a possibility of complete resection (Kager et al., 2003; Carrle et al., 2009).

Better understanding of tumour heterogeneity for patients with primary metastatic disease is important. The biology of the primary tumour might differ significantly from that in metastases (Janku, 2014). This may be caused by a different environment or because metastases may arise from expansion of an aggressive subclone within the primary tumour (Khanna, 2008). Molecular heterogeneities between disparate metastatic sites, such as differences in cell cycle regulations, DNA repair mechanisms or specific fusion proteins, may all contribute to nonuniform treatment responses between the primary tumour and metastasis in patients receiving (preoperative) chemotherapy.

In summary, the poor results in primary metastatic disease are most probably due to a combination of incomplete surgery and reduced sensitivity/resistance to chemotherapy as a consequence of the different biological characteristics of these tumours.

*Age at diagnosis*

Being older than 40 at diagnosis was an adverse prognostic factor (Tables 4–5, Paper II),
which is in line with previous reports (Chapter 7.6.1). It has earlier been advocated that patients over 40 should, whenever possible, be treated similarly to those in the younger age group (Grimer et al., 2003). Still, more than 70% of all elderly patients in the present cohort (with non-metastatic limb-localised OS) received inadequate primary treatment, mainly due to inadequate chemotherapy (Table 3, Paper II). Interestingly, there were no significant differences in survival based on age among adequately treated patients (Figure 1c, Chapter 14.2).

A recent retrospective study from Japan stated that adequate surgery significantly improved survival in elderly OS patients, whereas chemotherapy did not influence survival (Iwata et al., 2014). Two previous studies from Korea also documented inferior histologic response to chemotherapy among elderly patients (Jeon et al., 2006; Song et al., 2010). However, another study at the Rizzoli Institute confirmed an advantage for the use of neoadjuvant chemotherapy among elderly patients with high grade non-metastatic OS of the extremities (Bacci et al., 1998b). Multimodal therapy also increased the survival compared to surgery alone in patients older than 40, according to a previous study from Memorial Sloan Kettering Cancer Center (Manoso et al., 2005).

To conclude, there seems to be no unanimous answer regarding the treatment effect of chemotherapy among elderly patients in the available literature. Therefore, more information regarding elderly OS patients, including their tolerance and sensitivity to chemotherapy, is warranted, like the forthcoming mature results from the Euroboss 1 protocol (Carrle et al., 2006), which was closed for inclusion at the end of 2014.

10.2.5 Survival of patients with high-grade osteosarcoma - time trends
Major improvements in the long-term outcome of OS patients have been achieved since the 1970s (Chapter 7.6.1). This development was due to the introduction of multidisciplinary teams and multimodal treatment, including the introduction of complex surgical procedures and the discovery that several chemotherapeutic agents were active in the disease (Chapter 7.5.1). Despite major discoveries in the chemotherapeutic pantheon having been essential for the improved outcome of OS patients as compared to the pre-chemotherapy era (Chapter 7.4), the survival rate seems to have reached a plateau over the last two to three decades (Chapter 7.6.1).
Sadly, the huge effort to come up with a more effective chemotherapeutic regimen like, for example, the recent EURAMOS-1 trial, has unfortunately not further improved the long-term survival of OS patients (Bielack et al., 2015; Hattinger et al., 2015). Therefore, the increasing intensity of chemotherapy, with a greater burden of both acute and late toxicity, has not been followed by a corresponding improvement in survival rates (Whelan et al., 2006). We confirm this fact in the present thesis based on overall survival for all age groups with OS (Figure 4a, Paper I), which is in line with two other nationwide studies (Sampo et al., 2011; Whelan et al., 2012a). Correspondingly, the SSG registry observed no improvement in the survival of high-grade OS patients in Norway and Sweden since the millennium (Zaikova et al., 2015), although the latter is not a strictly population-based register (Chapter 10.1.1). This development may be because the available effective chemotherapeutic agents have not changed substantially since the 1980s.

Moreover, the overall treatment results for high-grade OS patients are less impressive than widely assumed because most previous clinical trials only involved the classical patients (Bruland et al., 1997; Bruland et al., 2009a) and not all high-grade OS patients, as studied in this thesis (Chapter 10.2.4). The prognosis is also poor for patients with metastatic relapse or recurrence because OS tumours may be inherently resistant to chemotherapy agents or may become unresponsive to these drugs during the chemotherapeutic treatment (Chou et al., 2006). The treatment of relapsed OS patients is based on different therapeutic approaches, including second-line chemotherapy (Chapter 7.5.1), which, however, have not provided satisfactory results (Hattinger et al., 2015). A delayed surgical removal of tumours that do not respond to neoadjuvant chemotherapy also increases the possibility of the systematic spread of drug-resistant tumour cells (Bacci et al., 1998a).

Multidrug resistance appears to be mediated by various mechanisms in OS, including drug uptake and transport, detoxification in the cell, apoptosis inhibition and repair of DNA damage (He et al., 2014; Li et al., 2015). Nevertheless, the question of when chemotherapy resistance emerges is still unanswered (Luetke et al., 2014; Hattinger et al., 2015), in line with several other aspects regarding OS tumour biology. The challenges ahead may not only include defining which agents need priority for further clinical investigation but also how we measure their success (Gill et al., 2013). Therefore, new therapeutic strategies and improvements are warranted. This is discussed further in Chapter 13.
10.2.6 Stage migrations
The Will Rogers phenomenon (Feinstein et al., 1985) is a possible cause of systematic distortions when the results of clinical studies are being interpreted, for example, if new or improved imaging tools like a spiral CT of the thoracic area allows the detection of OS metastases before they became visible on conventional chest X-rays. As a consequence, more patients are classified into the overt metastatic disease stage from the less severe localised disease. Such a stage migration results in an improved survival rate of patients in both the less and the more severe disease stages, which may be incorrectly interpreted as treatment effects (Sormani, 2009).

In the present thesis, we could not confirm any stage migration over time due to better imaging, where CT of the chest now is the standard diagnostic technique (Chapter 7.3). We observed a fluctuation of patients with primary metastatic disease from high-grade OS between 1975 and 2009, as demonstrated in Figure 3 (Chapter 9.2). This result may due to the limitations of CT scans in OS patients with pulmonary metastases because not all lung nodules found during surgery are evident on the CT (Chapter 10.2.4), and not all minor nodules seen on the CT scan are true metastatic lesions (Bacci et al., 2003). Further, the ‘true’ incidence of primary metastatic disease could also have been confounded by the low numbers of patients in the present study, approximately 3 cases annually within this time frame.

10.2.7 Low-grade and dedifferentiated osteosarcoma
The anatomical distribution of LGCOS in our study, with the mandible/maxilla as the single most frequent site, differed substantially from that of previously published studies in which long bones were most commonly affected (Unni et al., 1977; Andresen et al., 2004). This discrepancy may be due to chance, partly as a result of the relatively small sample size (Paper III). The anatomical sites of POS were, with one exception, all located in long bones. This is in line with the literature (Schwab et al., 2008; Hang et al., 2014). We observed a wide range in average duration of symptoms prior to diagnosis, probably due to the indolent tumour biology of LGOS. Such tumour growth may also explain why patients with low-grade

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18 The Will Rogers ‘concept’ also has applications in other academic fields (under different names), such as economics, for example the concern with the optimal production and distribution of scarce resources (economic efficiency).
histology had smaller tumour size at diagnosis (Table 1, Paper III) than patients with high-
grade OS (Table 1, Paper II).

Local relapses were uncommon following wide local excision or amputation compared to
curettage or excision with positive margins (Chapter 9.3). Transformations to DLGOS upon
recurrence, in most cases due to unsatisfactory margins, were in line with previous reports
(Chapter 7.6.2).

We confirmed that patients operated with a wide margin had excellent long-term survival, as
previously reported in the literature (Chapter 7.6.2). Chemotherapy and radiotherapy are not
routinely required for patients with LGOS (Chapter 7.5.2), as documented in this study.

10.2.8 Extraskeletal osteosarcoma
To our knowledge, this is the first large study (Paper IV) addressing clinicopathological
features of ESOS patients in a nationwide setting.\(^19\) We confirm that ESOS is typically a high-
grade neoplasm that commonly affects an older age group compared to OS (Chapter 7.1). Low-grade ESOSs are extremely rare (Umiker \textit{et al.}, 1953; Sabatier \textit{et al.}, 2010), and we
identified only one patient in our cohort.

Interestingly, nearly one quarter of all patients (nine cases) received radiotherapy to the area
where their ESOS developed. This is a substantially higher proportion than previously
presented (Lee \textit{et al.}, 1995; Lidang Jensen \textit{et al.}, 1998; Torigoe \textit{et al.}, 2007; Choi \textit{et al.}, 2014)
and is perhaps due to our nationwide approach. Although the poor prognosis of radiation-
induced soft-tissue sarcomas is well known (Gladdy \textit{et al.}, 2010), we were unable to
document any significant difference in the outcomes of our patients with ESOS (Figure 4b,
Paper IV), which is in line with a recent study (Choi \textit{et al.}, 2014).\(^20\) Interpretations are
challenging because of the small sample size.

Approximately three fourths of all patients (28 cases) had an axial tumour, including all
RESOS cases. The distribution of anatomical sites also differed substantially from that

\(^19\) Recently, however, a large study from the SEER database with 256 ESOS patients was published
(Thampi \textit{et al.}, 2014).
\(^20\) Unfortunately, the latter study was incorrectly cited in Paper IV (see the fourth sentence in the first
paragraph, second column, page 2137).
reported in the available literature (Fletcher et al., 2013), with one exception (Thampi et al., 2014); where the ESOSs were reported to be most commonly located in the lower extremity, with the thigh being the single most frequent site.

About one quarter of all patients presented with primary metastatic disease, just slightly above that in a previous study (Bane et al., 1990) but substantially higher than in other reports (Lee et al., 1995; Goldstein-Jackson et al., 2005). A large number of the patients experienced local recurrences and/or distant metastases during follow-up (Chapter 9.4), which is in line with the available literature (Chapter 7.6.3).

We observed a dismal outcome among most of the ESOS patients included in the present study, with only 16% five-year SSS. Previous literature states 25–50% five-year survival (Chung et al., 1987; Lidang Jensen et al., 1998; Mc Auley et al., 2012). Nevertheless, two recent studies of ESOS have described overall survival rates of 66–77% (Goldstein-Jackson et al., 2005; Torigoe et al., 2007). The poor prognosis for ESOS in Norway may be related to a relatively high median age at diagnosis (Chapter 9.4) and the fact that nearly one quarter of all patients had primary metastatic disease and/or RESOS. Moreover, only about half of the patients received adequate surgery (Table 4, Paper IV). The use of chemotherapy did not appear to influence survival, perhaps partly due to the limited number of patients and the broad spectrum of drugs used (Table 5, Paper IV). In general, the poor tolerance to adjuvant chemotherapy among the elderly represents another hurdle (Chapter 10.2.4). The use of radiotherapy did not appear to influence survival in our study, which is in line with the other OS subgroups in the present thesis.

To conclude, the poor prognosis for ESOS in our study may relate to both primary chemotherapy resistance and the different biological characteristics of these tumours compared to conventional OS.

10.2.9 Prognostic factors

*High-grade skeletal osteosarcoma*

Overall, we confirm primary metastatic disease, elevated tumour size, elevated LDH and old age as adverse prognostic factors for high-grade OS (Paper II), which is in line with the literature discussed in Chapter 7.6.1. Adequate primary treatment, including surgery and chemotherapy, had a positive impact on survival. We also documented an improved survival
rate for OS since the 1970s, further discussed in Chapter 10.2.5.

Neither elevated ALP nor the axial primary tumour site were significant adverse factors in the multivariate analyses in Paper II. This is in contrast to the previous findings discussed and presented in Chapter 7.6.1. Nevertheless, differences in risk assessment might occur due to inequalities in, for example, the methods adopted to identify and analyse the various prognostic factors in different studies. The results may also depend on the clinical and demographic characteristics of the OS population analysed, as well as the number of covariates included in the statistical analyses.

**Low-grade and dedifferentiated osteosarcoma**

The prognostic factors presented in Papers III–IV were only based on univariate analyses due to the relatively small datasets. Malignancy grade, local recurrence and metastases at diagnosis or during follow-up were all negative prognostic factors. Interestingly, neither primary tumour site nor age > 40 were significant adverse prognostic factors in these subgroups of OS compared to high-grade OS (Chapter 7.6), probably because LGOSs are characterised by slow growth, low metastatic potential and prolonged survival after adequate surgery. The same considerations relate to elevated ALP and LDH at diagnosis. Further, we found no improvement in long-term survival since 1975 for this group of patients, unlike for high-grade OS, most likely for the same reasons mentioned above but also because chemotherapy is not routinely required for patients with LGOS.

**Extraskeletal osteosarcoma**

Adequate surgery had a positive impact on survival, in line with a previous report (Goldstein-Jackson et al., 2005). Correspondingly, increased tumour size was a negative prognostic factor, which is in line with a recent SEER study (Thampi et al., 2014). Primary metastatic disease, elevated ALP and elevated LDH were adverse prognostic factors, as in high-grade OS (Chapter 7.6.1). Neither chemotherapy, radiotherapy nor the anatomical site of the primary tumour reached significance in our analyses; this is discussed further in Paper IV. Moreover, we did not find any obvious characteristic immunophenotypes of ESOS, which is in line with the literature (Hasegawa et al., 1991; Lidang Jensen et al., 1998; Fanburg-Smith et al., 1999). However, an elevated Ki67 score was significantly associated with a dismal outcome, as reported in a previous study of OS (Hernandez-Rodriguez et al., 2001)
11. Conclusions

The main goal of this project was to study epidemiological and treatment outcomes in a truly nationwide cohort of histologically confirmed OS and ESOS patients treated during the modern chemotherapy era. We particularly focused on patients with OS in the axial skeleton, those with metastases at the time of diagnosis and elderly patients.

The average incidence rate of OS in Norway was in line with international figures. However, we did not confirm the clear bimodal distribution found in previous studies, probably because of a very low incidence of Paget disease among the elderly in Norway compared to several other countries.

Patients with non-classical OS represented just over half of all high-grade OS cases in the present cohort, which is higher than previously reported. A considerable discrepancy in survival rates between classical and non-classical OS patients was observed. Twice as many of the former received both adequate surgery and chemotherapy compared to the latter. This could only partly explain the differences in survival due to inherent chemoresistance in primary metastatic disease and a higher rate of local relapse among patients with axial tumours. Overall, the prognostic factors for high-grade OS were in line with the research literature.

LGOS is associated with an excellent prognosis when surgically resected with a free margin, although LGOS has the potential to dedifferentiate and metastasise with a poor outcome. Correspondingly, patients with ESOS typically had a dismal outcome. This may relate to both primary chemotherapy resistance and the different biological characteristics of these tumours as compared to OS.

In our opinion, the strength of the present study is the high reliability of the database validated by using multiple and partially overlapping data and registry sources supplemented with clinical data from all Norwegian hospitals involved in sarcoma management. As expected, we have not reached full completeness regarding clinical information for all patients in the present database. Nor has it been possible to obtain the same degree of details regarding clinical variables as compared to institutional series and/or clinical trials. Furthermore, we cannot rule out that the quality could have been even better with a uniform histological re-
examination of all 702 cases in the gross study material, as well as a retrospective review of the radiographic images in relevant cases. Nevertheless, a significant disadvantage of such an approach is the potential lack of histological specimens or radiographic images available for re-examination. Hence, we believe the potential disadvantage will exceed the potential gain by such an approach, and that our key variables ensure an adequate amount of information to expand our knowledge regarding this family of rare neoplasms.
12. Forthcoming studies

We plan to address the following issues in the near future, in most cases based on data already included in our database.

• Patients with primary metastatic disease still have a poor outcome (Chapter 10.2.4). These tumours may be inherently resistant to standard chemotherapy agents or may rapidly become unresponsive to these drugs during the treatment. Therefore, we want to study the response to preoperative chemotherapy in pulmonary metastases by OS patients undergoing primary metastatic disease based on the present cohort from Paper II.

• SCS resembles OS in biological behaviour and radiological findings and is therefore commonly treated as OS. We have already defined the patient base for SCS as part of this study (Paper I), and we plan to describe the epidemiological and clinical characteristics related to the treatment outcomes of this cohort in a separate article.

• One hundred and sixty patients received radiotherapy as part of their treatment of high-grade OS in the present thesis (Chapter 9.5.1). We will expand this analysis into a separate research article.

• We will attempt to evaluate the prognostic impact of OS arising in the proximal part of the long bones of the extremities compared to the distal part (Chapter 9.5.2).

• OS of the mandible is often analysed together with OS originating at other head and neck sites (Kassir et al., 1997; Thariat et al., 2012), despite the fact that the prognosis may differ from that for extragnathic sites and the maxilla (Jasnau et al., 2008). Therefore, we plan to analyse the prognostic factors of mandible OS compared to other anatomical sites in a separate article based on the present cohort.

• As a result of improved outcomes for many cancers, an increasing number of cancer survivors are able to return to work after their treatment (Spelten et al., 2002; Taskila et al., 2007). However, as of today, we have limited information regarding this topic
for OS survivors. Therefore, we hope to shed light on this issue in a forthcoming project, partly through register linkage research.

- We will retrieve data on the prevalence of OS from the CRN as part of one of our forthcoming projects.
13. Future perspectives

Understanding OS biology still remains a complex challenge. It is a rare disorder that displays considerable heterogeneity, high genetic instability of tumour cells and clinicopathological variability (Hattinger et al., 2015). The aetiology of this aggressive cancer with an early metastasising potential is still unknown, which may reflect the great differences in prognosis.

Various studies have shed light on molecular targets in OS, for example, molecules involved as regulators of cell proliferation and apoptosis, growth factors, regulators of angiogenesis and cell migration and invasion (Clark et al., 2008; Gill et al., 2013; Yang et al., 2013). Despite a huge number of potential molecular markers that appear to be of clinical interest in terms of OS, published evidence is limited by contradictory results so far. This might be due to variability between tumours of the same histology and the different techniques used in the studies. Moreover, no molecular markers have so far been prospectively validated to predict the response to chemotherapy and outcome (Gill et al., 2013). Therefore, among, for example, patients with poor response to neoadjuvant chemotherapy, no markers have been validated to help delineate patients who are likely to be cured with the current chemotherapy regimens compared to those who may benefit from investigational drugs or escalating the dose intensity of drugs already in common use.

Immunotherapy still appears to remain in its infancy for sarcoma patients (Fagioli et al., 2008; Jaffe et al., 2013; Roberts et al., 2015; Bishop et al., 2016), despite the fact that this treatment concept was launched as ‘Coley’s Toxin’ during the late 19th century (Coley, 1891; McCarthy, 2006). Nevertheless, we see some hope for the future, for example, the use of mifamurtide. This drug is designed to activate macrophages, induce tumoricidal monocytes and increase levels of cytokines and other inflammatory molecules with a significant anti-tumour effect (Fidler, 1994). Several randomised clinical trials using mifamurtide for the treatment of OS have also reported improved outcomes for sarcoma patients (Kleinerman et al., 1993; Meyers et al., 2008; Kager et al., 2010), and the drug has been approved in Europe for patients < 30 with completely resected localised OS (Hogendoorn et al., 2010).

Efforts to develop a more effective chemotherapeutic regimen seem to have failed in further improving patient survival. There is great variability in treatment response between patients (Hattinger et al., 2015). Each metastatic lesion may contain different clones of genetically
unstable cancer cells that undergo further random mutations and develop chemotherapy resistance (Lerman et al., 2015). Consequently, due to the intra- and inter-tumour heterogeneity in OS, it is unlikely that a single therapeutic drug, such as the use of imatinib in gastrointestinal stromal tumours (GIST) (Joensuu et al., 2001)21, will be successful for all OS patients (Botter et al., 2014; Hattinger et al., 2015).

Tailored treatment approaches in OS are not currently available (Hattinger et al., 2015). Therefore, continuing research to understand the OS pathogenesis, novel therapeutic modalities beyond current treatment regimens and more personalised medicine are urgently needed.

21 The optimal treatment strategy for metastatic GIST patients is still under discussion (Hompland et al., 2015).
14. Appendix

14.1 Multivariate analyses – data from Paper I

Multivariate analyses based on the dataset from Paper I are presented in Table 1. These results confirm all conclusions from the univariate analyses in that study (Table 4, Paper I).

Table 1: Multivariate Cox regression analyses of prognostic factors for overall survival and sarcoma-specific survival (SSS). All skeletal osteosarcoma (OS), 1975–2009.

<table>
<thead>
<tr>
<th>Variables a</th>
<th>Overall survival</th>
<th></th>
<th>SSS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR b (95% CI c)</td>
<td>p d</td>
<td>RR b (95% CI c)</td>
<td>p d</td>
</tr>
<tr>
<td>Time of diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1975–1979</td>
<td>2.9 (2.0–4.4)</td>
<td>&lt;0.001</td>
<td>3.4 (2.3–5.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1980–1989</td>
<td>1.9 (1.4–2.6)</td>
<td>&lt;0.001</td>
<td>2.0 (1.4–2.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1990–1999</td>
<td>1.3 (0.9–1.8)</td>
<td>0.143</td>
<td>1.4 (0.9–1.9)</td>
<td>0.095</td>
</tr>
<tr>
<td>2000–2009</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Primary metastatic disease</td>
<td>3.6 (2.7–4.9)</td>
<td>&lt;0.001</td>
<td>4.0 (3.0–5.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age &gt; 40</td>
<td>2.3 (1.8–3.0)</td>
<td>&lt;0.001</td>
<td>2.0 (1.5–2.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Axial primary tumour</td>
<td>2.0 (1.5–2.7)</td>
<td>&lt;0.001</td>
<td>2.0 (1.5–2.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Type malignant bone lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional OS</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Low-grade OS</td>
<td>0.4 (0.3–0.6)</td>
<td>&lt;0.001</td>
<td>0.2 (0.1–0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Secondary OS</td>
<td>1.4 (1.0–2.2)</td>
<td>0.061</td>
<td>1.5 (1.0–2.3)</td>
<td>0.040</td>
</tr>
<tr>
<td>Other</td>
<td>0.6 (0.4–1.0)</td>
<td>0.050</td>
<td>0.6 (0.3–1.0)</td>
<td>0.050</td>
</tr>
</tbody>
</table>

a Variables from Table 4, Paper I, with adequate Cox proportional hazard assumption evaluated using Kaplan–Meier plots.

b Relative risk.

c Confidence interval.

d p-value.

14.2 Survival among adequately treated high-grade osteosarcoma patients

As previously mentioned in Chapters 9.2, we documented a significant difference in survival among adequately treated classical versus non-classical patients (Figure 4d, Paper II).

Separate survival analyses for patients with primary metastatic disease, non-extremity localisation or age > 40 at the time of diagnosis among adequately treated OS patients (216 cases) are presented in Figure 1a-c below. Some of these patients are presented in more than one of these figures due to the overlaps between the three subgroups (Figure 2, Chapter 9.2).

There are no significant differences in survival among adequately treated patients with axial OS (Figure 1a) or elderly people (Figure 1c) compared to the other high-grade OS patients. However, adequately treated patients with primary metastatic disease still have a significantly
inferior outcome compared to adequately treated patients without metastasis at the time of diagnosis (Figure 1b). This topic is further discussed in Chapter 10.2.4.

Figure 1a: Sarcoma-specific survival among adequately treated high-grade skeletal osteosarcoma (OS) patients. Extremity versus non-extremity OS.

Figure 1b: Sarcoma-specific survival among adequately treated high-grade skeletal osteosarcoma (OS) patients. Patients with and without metastasis at the time of diagnosis.
Figure 1c: Sarcoma-specific survival among adequately treated high-grade skeletal osteosarcoma (OS) patients. Patients over and under 40 years of age at the time of diagnosis.
15. Errata

- Chapter 6, first sentence: ‘…a complete nationwide study’ is changed to ‘…a complete nationwide cohort’.

- Chapter 14.1, Table 1: Heading above and not below Table 1.

- Chapter 14.1, Table 1: Second column, second line from bottom ‘1.4 (1.0-0.2)’ is changed to ‘1.4 (1.0-2.2)’.
16. References


Grimer, R. J., Cannon, S. R., Taminiau, A. M., Bielack, S., Kempf-Bielack, B., Windhager, R., Dominkus, M., Saeter, G., Bauer, H., Meller, I., Szendroi, M., Folleras, G.,


17. Papers I–IV