Pediatric Oncology
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Sarcomas of Bone and Soft Tissues in Children and Adolescents

Springer

Pediatric Oncology

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ISSN 1613-5318 ISSN 2191-0812 (electronic)
Pediatric Oncology
ISBN 978-3-030-51158-6 ISBN 978-3-030-51160-9 (eBook)
https://doi.org/10.1007/978-3-030-51160-9

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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Philip J. Lupo, Logan G. Spector, Schuyler O'Brien, Joshua D. Schiffman, and Simone Hettmer

1.1 Introduction

Bone and soft tissue sarcomas are relatively rare cancers that collectively account for approximately 1% of adult solid tumors and 12% of all pediatric malignancies (Burningham et al. 2012; Ries et al. 1999). Major bone tumors include osteosarcoma and Ewing sarcoma, whereas soft tissue sarcomas are largely categorized by rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue sarcomas. In spite of their relative infrequency, these tumors remain a leading cause of cancer death in individuals <20 years of age in developed countries and are a significant source of cancer-related morbidity (Ries et al. 1999). Given the suboptimal outcomes seen among those with bone and soft tissue sarcomas, these malignancies have a substantial impact on public health as measured by the average number of productive years of life lost due to cancer (Burningham et al. 2012). Thus, it is important to characterize their clinical/biological behavior and their etiologies. Epidemiologic studies help this endeavor in two ways. Descriptive studies reveal the incidence of these sarcomas and their associated mortality and survival rates with respect to histologic subtype and demographic characteristics. Analytic studies compare the risk of bone and soft tissue sarcomas in people with and without certain characteristics (cohort studies) or compare the histories of people with and without sarcomas (case-control studies) to identify and assess a wide range of possible risk factors, including exposures to radiation and hereditary cancer predisposition syndromes. Combined consideration of epidemiologic insights and progress in the molecular classification of these tumors provides greater insight into the role of tumor biology in disease progression and sensitivity to radiation treatment and chemotherapy, ultimately providing a framework for improved assessment of sarcoma risk, early tumor identification, and eventually strategies for individualized prevention and treatment of sarcomas.

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C. A. S. Arndt (ed.), *Sarcomas of Bone and Soft Tissues in Children and Adolescents*, Pediatric Oncology, https://doi.org/10.1007/978-3-030-51160-9_1

1.2 Descriptive Epidemiology

1.2.1 Osteosarcoma

Osteosarcoma (OS) in the young is rare worldwide, with rates between 4 and 7 cases per million children and adolescents <25 years of age, and shows minimal international variation (Mirabello et al. 2009). In the USA, an estimated 400 cases of OS are diagnosed each year among children 0–19 years of age and accounted for about ~3% of total cancer in this age group (Ries et al. 1999; Howlader et al. 2015).

The overall rate for OS is 5.3 cases per million children ages 0–19 years in the USA (Howlader et al. 2015), but this obscures substantial variation in incidence of the tumor by age (Fig. 1.1). Notably OS is very rare in early childhood but has a markedly peaked incidence in adolescence. Males have a somewhat higher incidence than females, and, interestingly, the peak incidence in females appears 2 years earlier than in males. Incidence among young adults (20–24 years) is also appreciable but less than at the peak (Wu et al. 2003). The incidence of OS in Surveillance, Epidemiology, and End Results (SEER) program data rose significantly by 1.6% per annum between 1975 and 1993 but remained steady between 1994 and 2012 (Ries et al. 1999). OS has a distinctive pattern of tumor location, with almost 80% of OS cases occurring in the long bones of the lower limbs and only about 5% in the central axis (Ries et al. 1999).

1.2.2 Ewing Sarcoma

The incidence of Ewing sarcoma is 1 case per million for all ages and increases to 3 cases per million for those under the age of 20 years. Estimates vary, but approximately 80–90% of individuals who develop Ewing sarcoma are under the age of 24 years (Cotterill et al. 2000). For this reason, Ewing sarcoma is largely considered a childhood cancer, although it can also occur in adults. The most common primary site for Ewing Sarcoma is the pelvis and lower extremities (Cotterill et al. 2000). Males are at a slightly increased risk for developing Ewing sarcoma in comparison to females. The incidence of

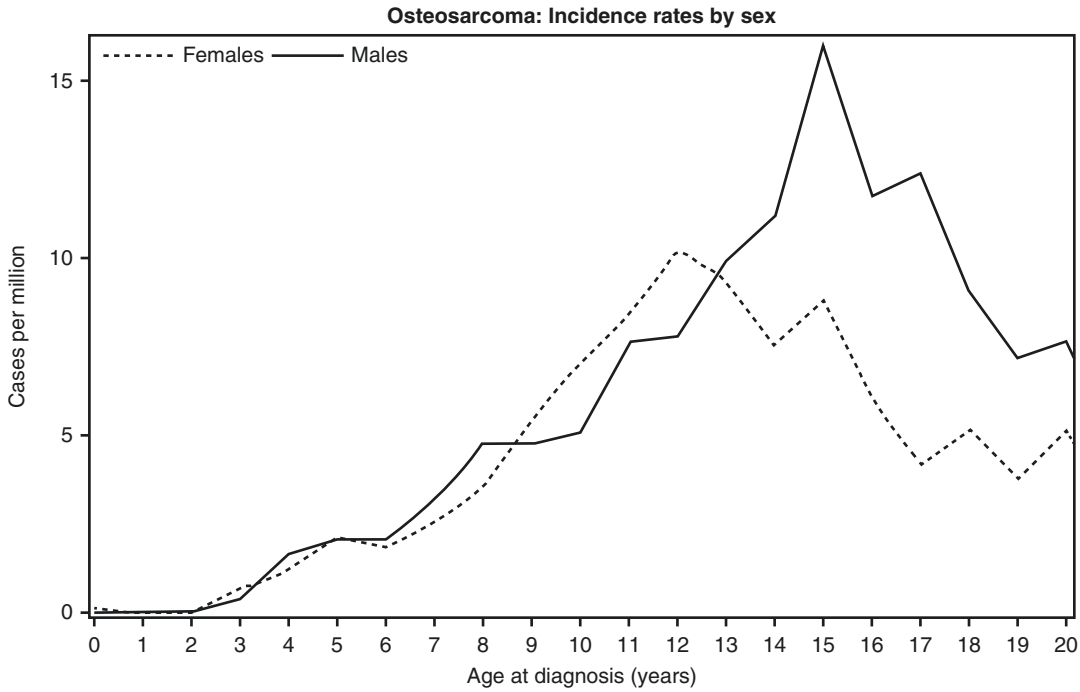

Fig. 1.1 Incidence of OS by age and sex (SEER 2000–2012)

Ewing sarcoma also varies significantly by race. Caucasians have the highest incidence, followed by Asians/Pacific Islanders, and African Americans, with incidence rates of 0.155, 0.082, and 0.017 per 100,000 individuals, respectively (Fig. 1.2) (Jawad et al. 2009). The fact that Caucasians are 9-times more likely to Ewing sarcoma compared to African Americans is a very important observation. In populations worldwide, those of European ancestry exhibit the highest incidence rates, regardless of geography. Furthermore, those with African ancestry on different continents still exhibit the lowest incidence, suggesting racial disparities in incidence are due to differences in genetics (Jawad et al. 2009; Fraumeni and Glass 1970).

1.2.3 Rhabdomyosarcoma (RMS)

RMS is the most common soft tissue sarcoma in children and adolescents, with an overall incidence rate of 4.5 cases per million among those <20 years of age. In the USA, this equates to approximately 350 new cases per year. While adult cases of RMS make up 40% of all RMS diagnoses, these tumors are considered rare in adults and are often characterized by a different histologic subtype (pleomorphic) (Sultan et al. 2009). In fact, information regarding the clinical and biologic characteristics of RMS in adults is very limited. Large, multi-institutional trials have not been performed, and only reports from single institutions have been published.

Based on data from the SEER Program, we know the incidence of RMS differs both by age and histology (Fig. 1.3). Specifically, the incidence of embryonal RMS (eRMS) has a bimodal peak, with each peak occurring during key developmental periods (i.e., early development and puberty). This is not seen with alveolar RMS (aRMS), where the incidence remains constant throughout childhood and adolescence. Also using data from SEER (Ognjanovic et al. 2009), Ognjanovic et al. reported the incidence of eRMS for the period 1975–2005 has remained relatively stable. However, there has been a significant increase in the incidence of aRMS (annual per-

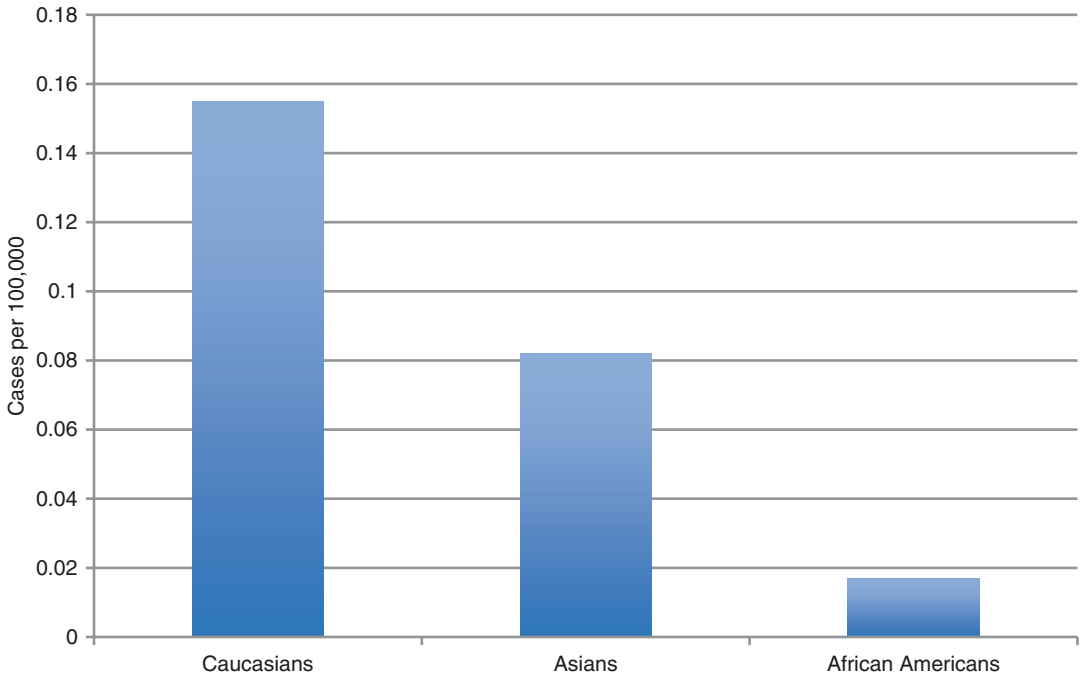

Fig. 1.2 Incidence of Ewing sarcoma by race (Jawad et al. 2009)

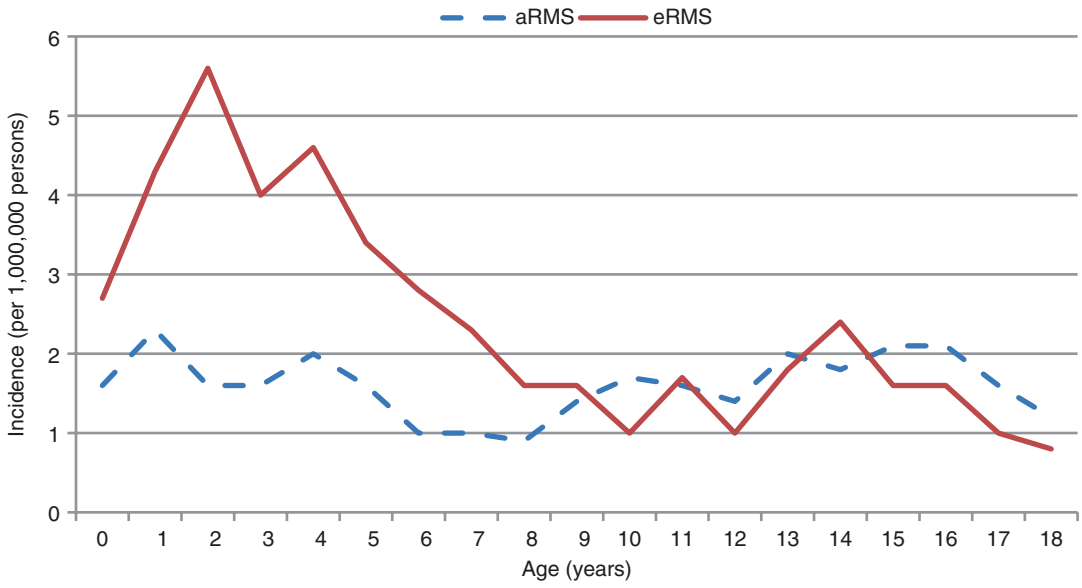

Fig. 1.3 Incidence of RMS by age and histology (SEER 1975–2011)

centage change (APC) = 4.20%, 95% confidence interval (CI): 2.60–5.82) for the same period. The authors noted that this might be attributable to changes in diagnostic criteria over time. There are also differences in incidence by gender. Males had a higher incidence of RMS compared to females, but this predominance was mainly among those diagnosed with eRMS (male/female ratio = 1.51, 95% CI: 1.27–1.80). Overall, there were no notable differences in incidence by race/ethnicity. This is consistent with a study pooling cancer registry data across five states (California, Minnesota, New York, Texas, and Washington), which indicated there were no significant differences in the risk of RMS by parental race (Chow et al. 2010). The only exception was the risk of RMS in offspring was significantly lower when both parents were of Hispanic ethnicity (OR = 0.65, 95% CI: 0.48–0.88).

1.2.4 Non-rhabdomyosarcoma Soft Tissue Sarcoma (NRSTS)

Non-rhabdomyosarcoma soft tissue sarcoma (NRSTS) incidence rates range between 1.8 and 5.0 per 100,000 individuals per year (Gustafson 1994; Levi et al. 1999; Ross et al. 1993; Storm 1994; Toro et al. 2006; Zahm and Fraumeni Jr. 1997). Differences in incidence rates between countries (e.g., 1.8 per 100,000 in Sweden (Gustafson 1994) and 4–5 per 100,000 in the USA (Toro et al. 2006; Zahm and Fraumeni Jr. 1997)) are likely due to racial differences, discrepant Kaposi sarcoma rates, and changing pathology definitions. Several reports documented increasing STS incidence rates over time (Toro et al. 2006; Zahm and Fraumeni Jr. 1997; Lahat et al. 2008), which are likely explained by improved registry systems and diagnostic tools, as well as the marked rise in Kaposi sarcoma incidence rates from 0.5 per 100,000 in 1975 to 3.3 per 100,000 during the HIV/AIDS epidemic of the late 1980s/early 1990s (Eltom et al. 2002). Incidence rates per year of specific NRSTS subtypes in the general population are summarized in Table 1.1 (Toro et al. 2006; UK CR 2008–2010).

There is a modest peak in the incidence of NRSTS among young children under the age of 5 years with a larger peak observed at approximately 60 years of age (Burningham et al. 2012; Wibmer et al. 2010). Mean age of diagnosis is 58 years, and mean age of death is 65 years (Burningham et al. 2012). However, STS

Table 1.1 Incidence rates per year of specific NRSTS subtypes

NRSTS	Yearly incidence rates per 100,000
GIST	0.68
Leiomyosarcoma	0.81–1.23
Liposarcoma	0.59–0.62
Synovial sarcoma	0.11–0.13
MPNST	0.12–0.19
Fibrosarcoma	0.18–0.65
Malignant fibrous histiocytoma	0.88
Sarcoma NOS	0.65–0.85

accounts for 7% of all cancer diagnoses in the pediatric age group and <1% of all malignancies in adults (Pappo and Pratt 1997). There are marked differences between histologies diagnosed in different age groups. Frequent NRSTS in the pediatric age group include synovial sarcomas (accounting for up to 42% of NRSTS (Dillon et al. 1992)); and fibroblastic/myofibroblastic/fibrohistiocytic tumors. In the adolescent and young adult (AYA) population, synovial sarcomas are relatively more common compared to the general adult NRSTS population and account for approximately 33% of AYA NRSTS (Herzog 2005).

Data on gender differences in STS risk are inconsistent, with male/female ratios ranging from 0.80 to 1.42 in different populations (Levi et al. 1999; Ross et al. 1993; Zahm and Fraumeni Jr. 1997; Wibmer et al. 2010). The highest NRSTS incidence rates were noted among black women, followed by black men, white men, and white women (Toro et al. 2006). Differences in black-white incidence rates were observed at all ages except childhood and driven largely by higher rates of leiomyosarcomas and dermatofibrosarcomas among non-Hispanic blacks (Toro et al. 2006). Race/ethnicity differences in STS incidence rates were also noted in another report, with incidence rates of 5.1 per 100,000 in blacks, 4.5 per 100,000 in whites, and 2.8 per 100,000 in Asians/Pacific Islanders (Burningham et al. 2012).

The epidemiology of certain NRSTSs is notable for specific features: classic gastrointestinal stromal tumors (i.e., GIST,which harbor *C-KIT* or *PDGFRA* pathogenic variants (Miettinen et al. 2005)) typically presents with a male/female ratio of 1.5 and affects individuals over 50 years of age (Tran et al. 2005), whereas its wild-type form (*C-KIT*/*PDGFRA*-negative) more commonly occurs in younger age groups and females (Nannini et al. 2013; Pappo and Janeway 2009). Finally, EBV-associated leimyosarcomas in immunodeficient individuals occur at any age, with a distinct age peak in children aged 0–9 years, whereas leiomyosarcoma rates in the general population increase with age and peak over the age of 50 years (Bhatia et al. 2012).

1.3 Environmental (Non-genetic) Risk Factors

1.3.1 Osteosarcoma

1.3.1.1 Growth and Development

The descriptive epidemiology of osteosarcoma strongly suggests an etiology related to growth and development. The age-incidence curve of OS closely follows that of the childhood growth curve (Fraumeni 1967), especially with respect to peak incidence in females reflecting their earlier growth spurts. Moreover, OS occurs most frequently at the metaphyseal region of long bones which are sites that contribute the most to vertical growth during the adolescent growth spurt (Price 1958).

Consequently, many studies have attempted to ascertain whether patients with OS attain greater height, grow faster, or reach puberty earlier than the general pediatric population. The largest analysis of height at diagnosis of OS pooled 1067 cases from seven studies to find that people in the 51st to 89th percentiles and those with ≥90th percentile of height had significantly increased risk of OS (odds ratio [OR] = 1.35, 95% confidence interval [CI]: 1.18–1.54 and OR = 2.60, 95% CI: 2.19–3.07, respectively) (Mirabello et al. 2011a). The same analysis pooled birth weight data from four studies to find that birth

weight ≥4046 g resulted in a greater risk of OS (OR = 1.35, 95% CI:1.01–1.79), although a subsequent, population-based, fairly sizable Scandinavian study did not replicate the finding (Troisi et al. 2014).

Some investigations have sought to quantify the rate of growth in OS cases and controls (Buckley et al. 1998; Gelberg et al. 1997; Operskalski et al. 1987), while others have examined the age at appearance of secondary sexual characteristics (Buckley et al. 1998; Gelberg et al. 1997). No correlation has been found with risk for OS.

The epidemiology of canine OS also points to a role for growth in the etiology of the tumor. It has been known since the 1960s that OS is more common in large dog breeds than in small ones (Tjalma 1966). One study in Rottweilers indicated that endogenous hormone exposure, and not simply growth, drives OS (Cooley et al. 2002). Investigators found that earlier age of gonadectomy raised the risk of OS significantly both male and female dogs, independent of size.

1.3.1.2 Exogenous Exposures

Most investigations of exogenous exposures and OS have been unrevealing, with previous treatment for cancer being a prominent exception. Radiation and alkylating agents each raise risk of OS independently with risk increasing by dose (Hawkins et al. 1996; Tucker et al. 1987). However, only about 1% of childhood cancer survivors developed OS within 20 years of their primary diagnosis in one cohort (Hawkins et al. 1996).

Because fluoride and radium are deposited in the bones, and especially because the latter is radioactive, several ecologic or case-control studies have examined exposures to these elements, especially in drinking water, in relation to OS. One case-control study that comprehensively reconstructed fluoride exposure across the lifespan found risk increased at higher levels of exposure among males, but not females (Bassin et al. 2006); direct measurement of fluoride in bone samples from OS patients in this study showed no difference compared to controls with other tumors (Kim et al. 2011), suggesting the previous finding was by chance. Moreover, other studies of OS and fluoride have been mostly null (Moss et al. 1995; Blakey et al. 2014; Levy and Leclerc 2012; Gelberg et al. 1995; McGuire et al. 1991). Although high doses of ingested radium caused OS in an historical cohort of radium dial painters (Fry 1998), there is little evidence that radium at the levels present in drinking water raises the risk of disease (Moss et al. 1995; Finkelstein 1994; Finkelstein and Kreiger 1996; Guse et al. 2002).

Few population studies have directly addressed the topic of OS and infections although some evidence indirectly supports an association. Simian virus 40 (SV40), JC, and BK viruses comprise the polyomavirus family and have in common a T antigen which interferes with the function of the *TP53* and *RB1* tumor suppressor genes (Fanning 1998; Barbanti-Brodano et al. 1998), which are the frequent targets of somatic mutation in OS. SV40 induces OS in hamsters (Diamandopoulos 1973) and has been detected in OS tissue (Yamamoto et al. 2000; Mendoza et al. 1998; Carbone et al. 1996). On the other hand, studies in cohorts of children who received early batches of poliovirus vaccine contaminated by SV40 have not supported an increased risk of OS (Engels et al. 2003; Carroll-Pankhurst et al. 2001; Fisher et al. 1999; Strickler et al. 1998; Olin and Giesecke 1998). One case-control study of OS did not indicate an association with childhood or maternal infections. Little evidence supports the spatiotemporal clustering of OS cases (Ross et al. 1999; Silcocks and Murrells 1987; McNally et al. 2006, 2012; Basta et al. 2010), which can occur in the presence of a causative infection.

Lastly there have been several exploratory analyses of putative exogenous risk factors, which have generated few positive associations with OS. Risk factors examined to date include parental smoking (Hartley et al. 1988a), medications taken by mother or child (Hartley et al. 1988a), and in utero or postnatal diagnostic X-rays (Operskalski et al. 1987; Hartley et al. 1988a). One study suggested an association with bone fracture, particularly at the tumor site (Operskalski et al. 1987), but this was not replicated in another study (Buckley et al. 1998).

Studies of parental occupation and OS have also produced only isolated reports of associations with OS (Buckley et al. 1998; Gelberg et al. 1997; Operskalski et al. 1987; Hartley et al. 1988a; Hum et al. 1998).

1.3.2 Ewing Sarcoma

To date, there are no well-documented environmental risk factors for Ewing sarcoma. A handful of environmental association studies have found a correlation between farming-related environments and an increased risk for developing the disease. Specifically, parental exposure to pesticides and other chemicals associated with agriculture have been linked to Ewing sarcoma (Holly et al. 1992; Stiller et al. 1991; Valery et al. 2002). A few studies have associated both inguinal and umbilical hernias with an increased risk for the disease (Cope et al. 2000; Valery et al. 2003, 2005). The possible association between these two factors makes analyzing them in the context of Ewing sarcoma somewhat challenging. Ewing sarcoma and hernias do share common embryological pathways. Therefore, it is possible that the two may share or have an overlapping genetic predisposition. However, this has not been demonstrated in a molecular context. More work is clearly needed to determine how farming related environments, and/or hernias may contribute to the development of Ewing sarcoma.

Patient stature and pubertal growth patterns have also been investigated as risk factors for Ewing sarcoma. Studies have been somewhat conflicting with no clear trends emerging (Cotterill et al. 2000; Fraumeni 1967; Bacci et al. 1992; Pui et al. 1987; Winn et al. 1992). Part of the difficulty in evaluating these variables likely comes from alterations in normal development due to treatment (Cotterill et al. 2000).

1.3.3 RMS

Several environmental exposures have been explored in relation to RMS risk among children, including paternal cigarette smoking (Grufferman et al. 1982), prenatal X-ray exposure (Grufferman et al. 2009), advanced maternal age (Grufferman et al. 2009), maternal antibiotic use (Grufferman et al. 1993), and parental recreational drug use (Grufferman et al. 1993). The majority of these assessments were based on the largest epidemiologic case-control study of RMS to date. Specifically, cases were collected from patients enrolled on the IRS-III study, coordinated by the Intergroup Rhabdomyosarcoma Study Group, whose protocols enrolled 80–85% of all childhood RMS cases in North America (Grufferman et al. 1984). Cases (n = 322) were 0–20 years old at the time of their RMS diagnosis from April 1982 to July 1988. Central expert pathology review confirmed all RMS diagnoses, as well as histologic subtype (i.e., embryonal, alveolar, or other). Controls (n = 322) were identified by random-digit dialing during the same period (1982–1988) (Grufferman et al. 1993, 2009). Controls were pair-matched to cases on race, sex, and age. Key findings from this study are presented in Table 1.2. While some notable associations were reported, few have been confirmed in

Table 1.2 Review of factors evaluated in IRS-III case-control study of RMS

Factor (reference)	OR (95% CI)
Birth defects (Yang et al. 1995)	2.4 (0.9–6.5)
Prenatal X-ray exposure (Grufferman et al. 2009)	1.9 (1.1–3.4)
Parental drug use (Grufferman et al. 1993)	
Maternal	3.1 (1.4–6.7)
Paternal	2.0 (1.3–3.3)
Allergies (Lupo et al. 2014a)	0.6 (0.4–0.9)
Maternal and birth characteristics (Lupo et al. 2014b)	
Fertility medications	0.7 (0.2–2.3)
Vaginal bleeding during pregnancy	1.8 (1.1–2.7)
Premature birth	2.5 (0.7–8.5)
Family history of cancer (Lupo et al. 2015)	
First-degree relative (eRMS)	2.4 (1.5–3.9)[a]
First-degree relative (aRMS)	1.0 (0.3–3.5)
Paternal exposure to agent Orange (Grufferman et al. 2014)	1.7 (0.6–5.4)

OR odds ratio, *CI* confidence interval
[a]Combined with data from the Utah Population Database

independent assessments. Unfortunately, germline DNA was not collected as part of this study, making the evaluation of genetic susceptibility of gene-environment interactions impossible. Other epidemiologic studies of RMS evaluating the role of environmental exposures have been relatively small (<100 cases). Novel and larger studies are needed to validate previous findings and explore other potential risk factors. Additionally, much work is needed to identify risk factors for RMS among adults.

1.3.4 NRSTS

NRSTS have been associated with a number of non-genetic risk factors, including virus infections conferring NRSTS susceptibility in immunodeficient patients. Elevated rates of Kaposi sarcomas and leiomyosarcomas in transplant recipients and individuals with HIV/AIDS first linked these sarcomas with immunosuppression (Bhatia et al. 2012). HIV/AIDS patients and transplant recipients also experience an excess of leiomyomas and leiomyosarcomas (Bhatia et al. 2012). The majority of leiomyosarcoma and leiomyoma cells in these patients contain EBV (Bhatia et al. 2012), which was also detected in smooth muscle tumors from individuals with congenital immunodeficiency syndromes (Hatano et al. 2006). Yet, EBV does not appear to be a general requirement for the development of leiomyosarcomas, because tumors arising in immunocompetent individuals do not contain the virus (Bhatia et al. 2012; Fernandez et al. 2010).

Most studies aimed at evaluating NRSTS-relevant environmental exposures have employed small case-control studies covering groups of NRSTS subtypes and a wide range of exposures/oncogenic factors (Burningham et al. 2012). Consequently, findings are prone to bias and should be interpreted with caution. Gardeners (Balarajan and Acheson 1984; Wingren et al. 1990), farmers (Balarajan and Acheson 1984), building caretakers, and military personnel (Pukkala et al. 2009) appear to experience an increased risk for developing NRSTS. More specifically, high-intensity occupational exposures to chlorphenol and cutting oils (Hoppin et al. 1999) and dioxin exposure from incinerators (Comba et al. 2003; Viel et al. 2000) were associated with STS risk. There is no known association between STS risk and exposure to solvents, wood dust, asbestos, DDT, or benzene (Burningham et al. 2012; Hoppin et al. 1999).

Finally, excess risk of developing NRSTS has been reported in cancer survivors in relation to therapeutic radiation (Burningham et al. 2012; Rubino et al. 2005; Menu-Branthomme et al. 2004; Virtanen et al. 2006). STS risk in a large cohort of survivors of childhood solid cancer was increased 19-fold compared to the general population after radiation alone (Menu-Branthomme et al. 2004). The same study reported a 113-fold increase in STS rates after radiation plus chemotherapy (Menu-Branthomme et al. 2004). Of note, there is no known association between STS risk and birth weight (Schuz and Forman 2007) or growth/development in early adolescence (Burningham et al. 2012).

1.4 Germline Genetic Risk Factors

1.4.1 Osteosarcoma

As with most childhood cancers a small proportion of cases are due to inherited, high-penetrance genetic variation. Li-Fraumeni (Li et al. 1988), hereditary retinoblastoma (Wong et al. 1997; Hansen et al. 1985), and Rothmund-Thomson syndromes (Leonard et al. 1996; Wang et al. 2003), which result from germline variants in *TP53, RB1*, and *RECQL4*, respectively, each raise the risk of OS substantially in carriers. Somatic mutations of the former two genes are commonly found in sporadic OS (Miller et al. 1996), while those in the latter are not (Nishijo et al. 2004). The advent of next-generation sequencing is now bringing the prevalence of these syndromes in OS into greater focus. One study in which 765 OS cases were sequenced at the *TP53* locus found that nearly 10% of harbored known or likely Li-Fraumeni variants (3.8%) or rare exonic

variants of unknown significance (5.7%) (Mirabello et al. 2015a). Another recent study which performed whole exome sequencing on 39 cases found that 7 (17.9%) had pathogenic or likely pathogenic variants in one of 21 known autosomal dominant cancer-predisposition genes (Zhang et al. 2015).

The role of common variation on OS risk has also been evaluated. There have been a number of candidate gene studies of OS (Musselman et al. 2012; Mirabello et al. 2011b; Ruza et al. 2003; Savage et al. 2006); however none appeared significantly associated with OS in the sole genome-wide association study conducted to date (Savage et al. 2013). Rather, the only two variants which reached genome-wide significance were rs1906953 near the *GRM4* gene and rs7591996 in an intergenic region of 2p25.2; the function of these variants has not been investigated to date. A second genome-wide study also identified the rs7034162 variant in the *NFIB* gene which more than doubled the likelihood of metastasis at diagnosis (OR = 2.43; 95% CI: 1.83–3.24); this finding was supported by in vitro experiments which showed cell lines with the variant behaved more aggressively (Mirabello et al. 2015b).

1.4.2 Ewing Sarcoma

At present, Ewing sarcoma is not considered to be part of any hereditary cancer syndrome. Given its rarity, the occurrence of Ewing sarcoma in siblings is slightly suggestive of an unknown genetic predisposition. Given this lack of evidence, the diagnosis of Ewing sarcoma cannot yet be considered to be part of a larger hereditary cancer syndrome requiring clinical genetic testing.

Parental age has been found to be a potential risk factor for some childhood cancers (Dockerty et al. 2001; Johnson et al. 2009; Olson et al. 1993; Yip et al. 2006). While unclear, the reason for this may be related to an increase in de novo germline mutations or epimutations (Johnson et al. 2009; Olson et al. 1993; Dryja et al. 1997). In the case of Ewing sarcoma, epimutations seem more likely given the paucity of mutations observed in tumor samples (Tirode et al. 2014).

A number of other studies have aimed at identifying a genomic region responsible for the racial disparities in incidence for Ewing sarcoma. One candidate that has been identified is an intron near a frequent breakpoint region in the *EWS/ETS* translocation. This region has been observed to be smaller in African American populations, making it an appealing possibility. Other work has identified polymorphic repeat region binding sites referred to as (GGAA) microsatellites for the EWS/ETS fusion protein as potential candidates (Beck et al. 2012; Gangwal et al. 2008; Monument et al. 2014; Zucman-Rossi et al. 1997). These regions also show variation that can be specific to races and therefore offer a potential explanation for Ewing sarcoma incidence. An important finding in recent years comes from the first genome-wide association study (GWAS) of Ewing sarcoma (Postel-Vinay et al. 2012). Two notable risk loci were identified. The first is located upstream of *TARDBP* ($P = 1.4 \times 10^{-20}$; (OR = 2.2) and the second upstream of *EGR2* ($P = 4.0 \times 10^{-17}$; OR = 1.7). Interestingly, *EGR2* also contains a (GGAA) microsatellite with a Ewing sarcoma-associated SNP that appears to alter *EWS/FLI1* binding (Grünewald et al. 2015). The authors also showed that *EGR2* knockdown induced regression of Ewing sarcoma xenografts, increasing its plausibility as a candidate for contributing to disease development. While it is somewhat unclear how these candidates would fit in with Ewing sarcoma development, the risk haplotypes were less prevalent in African Americans. Future studies will hopefully shed more light on these candidate genomic regions and their possible role in Ewing sarcoma development.

1.4.3 RMS

As opposed to OS (Savage et al. 2013) and Ewing sarcoma (Postel-Vinay et al. 2012), there has not been a genome-wide associations study (GWAS) of RMS. Additionally, while there have been whole exome and whole genome sequencing efforts to identify somatic mutations in RMS tumors (Shern et al. 2014), to date, there have few

studies characterizing the role of germline DNA on disease susceptibility, especially among seemingly sporadic cases. There is, however, a great deal of literature to support the hypothesis that genetic susceptibility plays a role in RMS development. Numerous reports consistently highlight the fact that children with certain genetic syndromes develop RMS more frequently than their unaffected peers. The syndromes that are most commonly seen among those with eRMS are Li-Fraumeni (Diller et al. 1995), NF1 (Hartley et al. 1988b; Yang et al. 1995), Costello (Estep et al. 2006; Kratz et al. 2011); Noonan (Kratz et al. 2011), and DICER1 (Doros et al. 2012). The genes and syndromes previously identified among RMS cases are included in Table 1.3. DICER1 is particularly notable as this is a recent discovery in terms of germline genetic susceptibility to RMS. Based on smaller clinic-based studies, only about 5% of RMS cases are thought to be associated with these syndromes (Plon and Malkin 2010). Additionally, cancer predisposition syndromes appear to be more frequent in eRMS cases compared to those with aRMS (Yang et al. 1995; Estep et al. 2006; Kratz et al. 2011; Ognjanovic et al. 2012). However, there have been no large-scale population-based efforts to systematically characterize the prevalence of these variants among children with RMS.

Table 1.3 RMS predisposition syndromes and genes

Syndrome	Gene
Li-Fraumeni	*TP53*
Neurofibromatosis type 1	*NF1*
DICER1	*DICER1*
Costello	*HRAS*
Noonan	*BRAF* *KRAS* *NRAS* *PTPN11* *RAF1* *SOS1*

1.4.4 NRSTS

Several well-described cancer-predisposing germline mutations confer STS susceptibility (Table 1.4): *NF1* germline mutations (associated with Neurofibromatosis type 1) confer a 10% cumulative lifetime risk of developing MPNST (Burningham et al. 2012; Pollack and Mulvihill 1997). *RB1* germline mutations have been linked to STS, most notably leiomyosarcomas (Kleinerman et al. 2007). *DICER1* germline mutations confer pleuropulmonaryblastoma susceptibility (Slade et al. 2011). *SMARCB1*/*SMARCA4* germline mutations are associated with rhabdoid tumor predisposition (Sredni and Tomita 2015). Finally, *TP53* germline mutations (associated with Li-Fraumeni syndrome (Gonzalez et al. 2009; Li and Fraumeni Jr. 1969)); and spontaneous chromosomal instability in Werner syndrome, an autosomal recessive disorder of premature age more commonly reported in Japan than elsewhere (Goto et al. 1996), are associated with a predisposition to develop various STS types, including RMS and NRSTS.

NRSTS arising in the setting of a cancer predisposition syndrome may exhibit specific features that distinguish them from similar, spontaneous sarcomas. Familial forms of GIST,

Table 1.4 NRSTS predisposition syndromes and genes

NRSTS	Syndrome	Gene
MPNST	Neurofibromatosis type 1	*NF1*
Leiomyosarcoma	Retinoblastoma syndrome	*RB1*
Pleuropulmonaryblastoma	Pleuropulmonaryblastoma syndrome	*DICER1*
Rhabdoid tumor		*SMARCB1*/*SMARCA4*
Wild-type GIST	Neurofibromatosis type 1	*NF1*
	Carney triad	
	Carney-Stratakis syndrome	*SDHB*
Various NRSTS	Li-Fraumeni	*TP53*
Various NRSTS	Werner syndrome	

accounting for approximately 85% of pediatric and 15% of adult GISTs (Nannini et al. 2013; Corless et al. 2004), typically lack *C-KIT* or *PDGFRA* mutations (wild-type GIST (Miettinen et al. 2005)). Familial GISTs develop in individuals with neurofibromatosis type 1 (Nannini et al. 2013; Bajor 2009) and Carney triad, first described in 1977 as a triad of gastric leiomyosarcoma, extra-adrenal paraganglioma, and pulmonary chondroma (Carney et al. 1977; Matyakhina et al. 2007). Of note, familial GISTs in Carney-Stratakis syndrome, a familial predisposition to develop multifocal GISTs and multifocal paragangliomas, have been linked to succinate dehydrogenase B (*SDHB*) germline mutations (Carney and Stratakis 2002; McWhinney et al. 2007).

1.5 Conclusion

Even when using large population-based registries, the sensitivity of identifying an elevated risk of uncommon cancers depends on the categorization of cancer types, the magnitude of the association, and the incidence of these cancers in the general population. The rarity and heterogeneity of bone and soft tissue sarcomas represents a major challenge in epidemiologic studies aimed at illuminating etiologic factors. Nevertheless, descriptive epidemiology has already yielded important insights into the origins and manifestations of bone and soft tissue sarcomas and continues to provide a window into the etiologies of these malignancies. It is likely that the greatest gains in understanding the etiology of bone and soft tissue sarcomas in the near future will come from "omics" studies seeking to understand innate and exogenous factors that contribute to susceptibility. It will also be important to identify interactions between genetic and environmental factors and to conduct studies that integrate germline and somatic tumor data to determine how germline variation influences tumor mutation profiles and prognosis. For progress in these areas to occur, a coordinated investment in systematic collection of clinically annotated biological specimens (both tumor and normal) from a large number of bone and soft tissue sarcoma cases should be an international priority since cancer is a leading cause of death in children and bone and soft tissue sarcomas have very high rates of mortality and morbidity.

Acknowledgments This chapter is dedicated to our dear friend and colleague, Schuyler O'Brien (1991-2019). Schuyler was a committed scientist who worked tirelessly with the conviction that all data had the potential to become an important stepping-stone in cancer research. He was not content to make just a small contribution to the field of cancer research. Rather, he was singularly intent on dedicating all of himself to finding a cure, specifically a cure for Ewing sarcoma, a cancer he began battling at 12 years old that relapsed multiple times during his short lifetime. Facing so many relapses gave Schuyler a unique perspective, and he enjoyed gently and insightfully challenging his peers to work harder and better to understand the origins of childhood cancer. Somehow, despite years of physical suffering and intellectual exertion, Schuyler maintained a bewildering optimism and steadfast confidence in the power of science and in his ability to push through any difficulty to achieve his goals. Even though Schuyler was often—silently and unflinchingly—in pain from his multiple cancer treatments, he masterfully conducted his work over the years with an obsessive and somehow joyful passion. Schuyler never hesitated to patiently listen or lend a helping hand to his colleagues and friends. We are honored to have shared in his good-natured scientific zeal and marvel at his ability to conduct his life, like most cancer patients do, with simultaneous cynicism and hope, agony and joy. We dedicate this chapter, which Schuyler helped to write, to this young scientist who continues to serve as inspiration to all who knew him.

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Sarcoma Pathology and Biology

2

Marielle Yohe, Javed Khan, and Erin Rudzinski

2.1 Rhabdomyosarcoma

2.1.1 Pathology

Rhabdomyosarcomas (RMS) are malignant soft tissue tumors with skeletal muscle differentiation. The World Health Organization Classification for Tumours of Soft Tissue and Bone includes four histologic subtypes: alveolar, embryonal (including botryoid), spindle cell/sclerosing, and pleomorphic RMS (Fletcher et al. 2013). The pleomorphic variant is largely confined to the adult population and will not be further discussed in this chapter.

2.1.1.1 Alveolar Rhabdomyosarcoma

Alveolar rhabdomyosarcoma (ARMS) is a prototypical pediatric small round blue cell tumor. In its classic pattern, malignant cells line delicate fibrous septae lending a resemblance to alveoli in the lung. ARMS may also occur in a solid pattern, however, in which alveolar spaces or fibrous septae are inconspicuous. Regardless of the overall architecture, these tumors are characterized by a monomorphic population of cells with round nuclei. Immunohistochemically, desmin expression confirms the tumor cells demonstrate muscle differentiation, and there is co-expression of skeletal muscle specific markers myogenin and MyoD1. ARMS specifically show strong and diffuse expression of myogenin, which is present in the majority (often nearly 100%) of tumor nuclei (Rudzinski et al. 2013).

2.1.1.2 Embryonal Rhabdomyosarcoma

Embryonal rhabdomyosarcoma (ERMS) is a primitive sarcoma that recapitulates embryonic skeletal muscle. In its typical form, this appears as alternating loosely and densely cellular areas with varying degrees of rhabdomyoblastic differentiation. In the botryoid pattern, tumor cells condense under the mucosal surface creating a so-called cambium layer; however, the morphology deep to the mucosal surface is that of typical ERMS. Some tumors may be composed entirely of densely cellular regions which lack cytologic differentiation and may be difficult to distinguish from the solid variant of ARMS (Rudzinski et al. 2013). The immunohistochemical profile of ERMS is similar to ARMS, although ERMS lacks the diffuse myogenin expression of ARMS and instead shows patchy nuclear staining.

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C. A. S. Arndt (ed.), *Sarcomas of Bone and Soft Tissues in Children and Adolescents*, Pediatric Oncology, https://doi.org/10.1007/978-3-030-51160-9_2

2.1.1.3 Spindle Cell/Sclerosing Rhabdomyosarcoma

Spindle cell/sclerosing rhabdomyosarcoma (SCSRMS) is a recently added subgroup of RMS (Fletcher et al. 2013). The spindle cell pattern consists of sheets or fascicles of spindled cells resembling smooth muscle tumors, e.g., leiomyosarcoma. In contrast, the sclerosing pattern is composed of undifferentiated round cells in cords or small nests, separated by abundant hyalinized or myxoid stroma.

SCSRMS was first described in children where it was commonly paratesticular, associated with an excellent outcome, and considered to be a variant of ERMS (Cavazzana et al. 1992; Leuschner et al. 1993). However, recent studies have suggested that other subsets of rhabdomyosarcoma with spindle cell morphology may have different clinical and molecular features. Infants may have tumors with pure spindle morphology that harbor recurrent non-*PAX* gene fusions and are associated with a favorable prognosis (Alaggio et al. 2016; Mosquera et al. 2013). In contrast, a group of spindle cell or sclerosing tumors most often arising in the head and neck region or the extremities of adolescents and young adults (Mentzel and Kuhnen 2006; Nascimento and Fletcher 2005) is associated with a uniformly poor outcome (Chiles et al. 2005; Folpe et al. 2002; Mentzel and Katenkamp 2000; Rubin et al. 1998). Both spindle cell and sclerosing patterns share a common immunophenotype, with strong and diffuse MyoD1 expression but weaker desmin and myogenin staining. In adults, morphologic SCSRMS is associated with a poor outcome, with a rate of recurrence and metastasis of approximately 40–50% (Stock et al. 2009); in one recent study restricted to *MYOD1* mutant SCSRMS, 68% of patients died of disease (Agaram et al. 2019). Pediatric studies are more limited, but they suggest that the overall behavior of SCSRMS is not significantly different than ERMS, except parameningeal spindle cell RMS which had a much poorer survival (Rudzinski et al. 2015). This likely reflects the heterogeneous biology of pediatric SCSRMS.

2.1.2 Biology

RMS is characterized by two distinct genotypes, defined by the presence or absence of a *PAX-FOXO1* gene rearrangement. Approximately 80% of ARMS have evidence of a *FOXO1* gene rearrangement, while the remaining 20% of ARMS are fusion negative. Fusion-negative ARMS are molecularly indistinguishable from ERMS, and both histologic subtypes are included in the discussion below (Davicioni et al. 2009; Williamson et al. 2010).

2.1.2.1 Fusion-Positive Rhabdomyosarcoma

PAX-fusion-positive rhabdomyosarcoma (FP-RMS) is associated in 73% of cases with a balanced chromosomal translocation, t(2;13)(q35;q14), that results in the fusion of two transcription factors, *PAX3,* a member of the paired box family, and *FOXO1,* a member of the Forkhead family. In the PAX3-FOXO1 fusion, the amino terminal DNA-binding domain of PAX3 is fused to the C-terminal transactivation domain of FOXO1. Other less common fusions associated with FP-RMS include PAX7-FOXO1 (23% of cases), in which the paired box family member, PAX7, is fused to FOXO1 (t(1;13)(p36;q14)); PAX3-NCOA1 (1% of cases), in which PAX3 is fused to the nuclear receptor coactivator, NCOA1 (t(2;2)(q35;p23)); and PAX3-INO80D (<1% of cases), in which PAX3 is fused to the chromatin remodeler, INO80D (t(2;2)(q35;q33)), as well as others (Kashi et al. 2015). The tumors that harbor these less common fusions retain the expression signature characteristic of the canonical *PAX3-FOXO1* fusion (Shern et al. 2014).

FP-RMS tumors have an extremely low overall mutation rate (0.1 mutations per megabase) and no recurrent single nucleotide point mutations (Shern et al. 2014). However, regions of focal genomic amplification are frequently observed in FP-RMS. The most commonly amplified genomic regions observed in FP-RMS tumors are 2p24, containing the *MYCN* oncogene and 12q13-q14, which includes *CDK4*. Loss of heterozygosity at 11p15.5, while more common

Table 2.1 Genomic alterations in PAX-fusion-positive rhabdomyosarcoma

Gene	Type of alteration	Chromosomal locus	Frequency (%)
PAX3-FOXO1	Translocation	(2;13)(q35;q14)	73
	with amplification		6.5[a]
PAX7-FOXO1	Translocation	(1;13)(p36;q14)	23
	with amplification		21[a]
PAX3-NCOA1	Translocation	(2;2)(q35;p23)	1
PAX3-INO80D	Translocation	(2;2) (q35;q33)	<1
CDK4	Amplification	12q13-14	5
MYCN	Amplification	2p24	8
IGF2	Loss of heterozygosity	11p15.5	16

Kashi et al. (2015)
[a]Extrapolated from Duan et al. (2012)

in *PAX*-fusion-negative RMS (FN-RMS), is also observed in FP-RMS. Genomic amplification of the *PAX-FOXO1* fusion gene is found in more than 90% of *PAX7-FOXO1*-positive tumors, but in less than 10% of *PAX3-FOXO1*-positive cases (Duan et al. 2012). These genomic alterations are summarized in Table 2.1.

In addition to the focal genomic amplifications described above, FP-RMS tumors also commonly exhibit whole genome duplication (Chen et al. 2015).

Transcriptional targets of PAX3-FOXO1 have been identified using a combination of gene expression studies and chromatin immunoprecipitation with massively parallel DNA sequencing (ChIP-seq) experiments with an antibody specific for the fusion transcription factor. A total of 1463 putative PAX3-FOXO1 binding sites were identified genome wide in the PAX3-FOXO1 expressing tumor-derived cell line, RH4. The vast majority of the identified binding sites were either intergenic or intronic, indicating that PAX3-FOXO1 likely functions by binding to enhancers regulating expression of its target genes (Cao et al. 2010). These targets include transcription factors (*MYOD1, MYOG, SIX1, SNAI2, MYCN, HEY1*) and receptor tyrosine kinases (*FGFR4, IGF1R, ALK*) as well as *JARID2*, which recruits the polycomb repressor complex 2, and *PIPOX*, a component of cellular metabolism (Ahn et al. 2013; Barber et al. 2002; Begum et al. 2005; Khan et al. 1999; Mercado et al. 2008; Walters et al. 2014). Additional studies, in which active and repressive histone marks were mapped in RH4 cells using ChIP-seq revealed that PAX3-FOXO1 was almost exclusively localized to super-enhancers, which are a small fraction of the active enhancers within a cell and are known to control expression of oncogenes in cancer. PAX3-FOXO1 collaborates with the core regulator transcription factors, MYCN, MYOD1, and MYOG, at super-enhancers to maintain expression of genes important for maintenance of a myoblast-like state. PAX3-FOXO1 also recruits the transcriptional cofactor, BRD4, to super-enhancers in FP-RMS, and requires BRD4 for function and stability, which provides a druggable vulnerability for FP-RMS (Gryder et al. 2017).

2.1.2.2 Fusion-Negative Rhabdomyosarcoma

PAX-fusion-negative RMS (FN-RMS) is associated with several familial cancer syndromes, most notably Li-Fraumeni (loss of function mutations in *TP53*) (Li and Fraumeni Jr. 1969), neurofibromatosis type I (loss of function mutations in *NF1*) (Sung et al. 2004), Beckwith-Wiedemann syndrome (loss of imprinting at the 11p15.5 locus, an area that includes *IGF2*) (Steenman et al. 2000), and Costello syndrome (gain of function mutations in *HRAS*) (Philip et al. 1999), leading to the hypothesis that the genes responsible for the phenotypes of these syndromes also play a role in RMS development. The large-scale next-generation sequencing studies of primary RMS tumors that have been reported recently (Chen et al. 2013; Kohsaka et al. 2014b; Shern et al. 2014) largely confirm this hypothesis.

In contrast to FP-RMS tumors, FN-RMS tumors a higher rate of single nucleotide variants (0.58 mutations per megabase). There is a linear correlation between the number of point mutations and the age at diagnosis of the patient. These tumors display a wide range of recurrent single nucleotide variants. These variants most commonly are found within one of the RAS genes, *NRAS, KRAS*, or *HRAS* (Shern et al. 2014). Other recurrently mutated genes include *FGFR4, ERBB2, NF1, PIK3CA*, *TP53*, *CTNNB1*; the ubiquitin ligase, *FBXW7*; the transcriptional repressor, *BCOR* (Paulson et al. 2011); and the myogenic master transcription factor, *MYOD1* (Szuhai et al. 2014). Many of the genes recurrently mutated in FN-RMS are genes upregulated by PAX3-FOXO1, including *FGFR4* and *MYOD1*. The prognostic significance of the different ERMS driver mutations is currently being investigated.

The majority of FN-RMS tumors display loss of heterozygosity (LOH) at the 11p15.5 locus (Scrable et al. 1989). This LOH involves allelic loss of the maternal allele and duplication of the paternal allele, resulting in paternal isodisomy. The maternal allele of 11p15.5 is unmethylated at the imprinting control region governing expression of *IGF2*, rendering the *IGF2* locus transcriptionally inactive. The *IGF2* locus of the paternal allele, in contrast, is methylated at the imprinting control region, allowing for expression of *IGF2*. Paternal isodisomy at the *IGF2* locus, then, results in overexpression of *IGF2*, which is observed in FN-RMS tumors (Zhan et al. 1994).

FN-RMS tumors also have a higher number of copy number changes than FP-RMS tumors. FN-RMS tumors have recurrent gains of chromosomes 2, 7, 8, 12, and 13, the functional consequences of which are incompletely described. Recurrent focal gains and losses are also a feature of FN-RMS tumors. Importantly, focal losses of 9q32-34, which includes *CDKN2A,* and 17p, which includes the *TP53* and *NF1* loci, are observed. In addition, gain of the 12q14-15 locus containing the *MDM2* gene, which is known to bind and inactivate *TP53*, is recurrently observed (Shern et al. 2014). These genomic alterations are summarized in Table 2.2. In addition, while not highlighted in large-scale sequencing studies of FN-RMS, *DMD*, the gene encoding dystrophin, is recurrently deleted in myogenic tumors including FN-RMS (Wang et al. 2014).

FN-RMS tumors and cell lines are arrested in an activated muscle satellite cell state; they express the master transcription factors MYF5 or MYOD1 but fail to express the myotube-specific target of these transcription factors, MYOG. Several signaling pathways, including the NOTCH, canonical and non-canonical WNT, Hippo, p38 MAP kinase, and RAS/RAF/MEK/ERK MAP kinase pathways, have been implicated in maintaining FN-RMS self-renewal and blocking myogenic differentiation in these tumors (Chen et al. 2014a; Crose et al. 2014; Hayes et al. 2018; Ignatius et al. 2017; Puri et al. 2000; Tremblay et al. 2014; Yohe et al. 2018). Pharmacologic manipulation of these pathways may provide therapeutic benefit in FN-RMS as differentiation therapy.

FP-RMS and FN-RMS, therefore, are tumors of myogenic precursor cells that are arrested at different stages of myogenic differentiation and driven by disparate genetic mechanisms: FP-RMS by translocation and FN-RMS by mutation.

Table 2.2 Genomic alterations in PAX-fusion-negative rhabdomyosarcoma

Gene	Type of alteration	Chromosomal locus	Frequency (%)
IGF2	Loss of heterozygosity	11p15.5	63
NRAS	Point mutation	1p13.2	12
KRAS	Point mutation	12p12.1	6
HRAS	Point mutation	11p15.5	5
NF1	Point mutation	17q11.2	6
PIK3CA	Point mutation	3q26.32	6
FBXW7	Point mutation	4q31.3	5
FGFR4	Point mutation	5q35.2	10
BCOR	Point mutation	Xp11.4	5
CTNNB1	Point mutation	3p22.1	4
MYOD1	Point mutation	11p15.1	1
MDM2	Amplification	12q15	10
TP53	Point mutation	17p13.1	6

Chen et al. (2013), Kohsaka et al. (2014a), Shern et al. (2014)

2.1.2.3 Spindle Cell/Sclerosing Rhabdomyosarcoma

One subset of SCSRMS is associated with a recurrent L122R mutation in *MYOD1*, which leads the mutant protein to act more like the *MYC* oncogene than a pro-myogenic differentiation factor (Agaram et al. 2014; Kohsaka et al. 2014a; Szuhai et al. 2014). Frequently, MYOD1 L122R mutations co-occur with activating mutations in *PIK3CA* (Agaram et al. 2019; Kohsaka et al. 2014a). A second subset of SCSRMS arising in infants is associated with recurrent *VGLL2*-related fusions including *VGLL2-CITED2*, *VGLL2-NCOA2*, *TEAD1-NCOA2*, and *SRF-NCOA2* (Alaggio et al. 2016). The remaining group of SCSRMS is indistinguishable from other FN-RMS.

2.2 Ewing Sarcoma

2.2.1 Pathology

Ewing sarcoma (EWS) is another prototypical small round blue cell tumor. EWS tumors are composed of sheets of primitive cells with varying degrees of neural differentiation. The most differentiated tumors include well-formed pseudorosettes (tumor nuclei surrounding central neuropil), hence the historic designation as peripheral primitive neuroectodermal tumor. Classic EWS have strong and diffuse membrane staining for CD99, although markers such as FLI1 and NKX2.2 are also expressed (Shibuya et al. 2014; Yoshida et al. 2012). Atypical cases, those with prominent nucleoli or unusual morphologic patterns, require molecular or cytogenetic confirmation.

2.2.2 Biology

EWS is not associated with any familial cancer predisposition syndromes, unlike *PAX*-fusion-negative rhabdomyosarcoma and osteosarcoma. EWS, like *PAX*-fusion-positive rhabdomyosarcoma and many of the non-rhabdomyosarcomatous soft tissue sarcomas, is driven by the presence of specific translocation resulting in the expression of a fusion protein. In the case of EWS, the driving fusion juxtaposes the N-terminus of an RNA binding protein of the FET family (*FUS, EWSR1, TAF15*) to the DNA-binding domain of an ETS family transcription factor (*FLI1, ERG, ETV1, ETV4, FEV*). The most common EWS fusion, found in 85% of EWS cases, is *EWSR1-FLI1*, which is the result of a t(11;22)(q12;q11.2) translocation. Three types of *EWSR1-FLI1* fusions have been identified: in type 1 fusions exon 7 of *EWSR1* is fused to exon 6 of *FLI1*; in type 2 fusions exon 7 of *EWSR1* is fused to exon 5 of *FLI1*, and in type 3 fusions exon 10 of *EWSR1* is fused to exon 6 of *FLI1*. The breakpoints observed in ES tumors are typically intronic and require the cellular splicing machinery to create an in-frame transcript. This splicing machinery has been identified as a vulnerability in cells expressing *EWSR-FL1* fusions (Grohar et al. 2016). The second most common *EWS* fusion, in 10% of cases, is *EWSR1-ERG* (Fisher 2014). *FUS* (also a member of the FET family) may rarely substitute for *EWS* in the fusion transcript and is not detected by classic FISH break-apart probes to detect *EWSR1* rearrangement.

Transcriptional targets of EWS-FLI1 have been identified by gene expression studies, functional studies, and most recently, ChIP-seq experiments. Transcriptional targets in which EWS-FLI1 directly binds to the gene promoter include transcriptional regulators, such as *EZH2, ID2*, *and EGR1*; kinases, such as *AURKA*; and genes important for cell cycle regulation, such as *CDKN1A* and *CCNE* (Erkizan et al. 2010). Genes activated by direct EWS-FLI1 binding in the promoter also frequently bind E2F3, a transcription factor important in driving cell proliferation (Bilke et al. 2013). Recent ChIP-seq analysis revealed that in addition to binding to gene promoters, EWS-FLI1 also binds to enhancer elements, both in EWS cell lines and primary tumors. These enhancers are either activated or repressed by binding the EWS-FLI1 fusion. Enhancers activated by EWS-FLI1 show enrichment for GGAA repeats, and this activation is achieved through

EWS-FLI1-dependent recruitment of the histone acetyltransferase complex, p300, and the chromatin remodeling complex, BAF (Boulay et al. 2017). Enhancers repressed by EWS-FLI1 show enrichment for canonical ETS family transcription factor consensus motifs, and repression is mediated by the displacement of ETS transcription factors from these enhancers by EWS-FLI1 (Riggi et al. 2014).

Next-generation sequencing studies have been conducted in EWS to identify tumor vulnerabilities in addition to *EWS-FLI1*. These studies showed that the mutation rate in EWS is low, like that of other fusion protein-driven pediatric tumors, with approximately ten coding variants per tumor. Common recurrently mutated genes include the cohesin component, *STAG2*, and *TP53*. The majority of *STAG2* and *TP53* mutations identified in EWS were loss-of-function mutations. Less commonly mutated genes include the histone methyltransferase, *EZH2*, and the BCL6 co-repressor, *BCOR* and *BRCA2*, which is involved in DNA repair processes. The most common somatic copy number alterations included gains of chromosomes 8, 12, and 1q, deletion 16q, and focal deletion of the 9p21.3 locus, which contains the *CDKN2A* gene. Gain of chromosome 1q is associated with poor prognosis, potentially due to the overexpression of *DTL*, which is part of a complex that mediates the ubiquitination and degradation of p21 (*CDKN1A*) (Mackintosh et al. 2012). *STAG2* mutation and *CDKN2A* deletion appear to be mutually exclusive, while *STAG2* and *TP53* mutations co-occur in highly aggressive tumors (Brohl et al. 2014; Crompton et al. 2014; Tirode et al. 2014). Recurrent genomic alterations in EWS are summarized in Table 2.3.

STAG2 is a component of the cohesin complex, a multiprotein complex composed of SMC1A, SMC3, RAD21, and STAG1 or STAG2 that is responsible for the cohesion of sister chromatids between DNA replication and mitosis, at which time the complex is cleaved. Cohesin complexes containing STAG2 are essential for chromatid cohesion at centromeres, while STAG1 containing cohesin complexes are usually associated with telomeres. A cohesin complex of SMC1B, SMC3, REC8, and STAG3 is found exclusively in germ cells. The cohesin complex ensures the faithful segregation of sister chromatids into daughter cells. Recurrent alterations of STAG2 have been reported in several cancer types in addition to EWS, including acute myeloid leukemia, urothelial carcinoma, and glioblastoma. In several of these cancer types, the oncologic mechanism of loss of STAG2 function is increased aneuploidy due to disrupted chromosomal segregation. In EWS, however, *STAG2*-mutated tumors do not show increased aneuploidy, although structural variants are more common in *STAG2*-mutated EWS tumors than in *STAG2* wild-type tumors (Tirode et al. 2014). The cohesin complex is also enriched at active transcriptional sites; therefore, altered transcriptional output could be an alternative mechanism by much STAG2 loss leads to tumorigenesis in EWS. Cohesin also plays a role in DNA repair, suggesting that *STAG2*-mutant tumors might be more sensitive to DNA cross-linking chemother-

Table 2.3 Genomic alterations in Ewing sarcoma

Gene	Type of alteration	Chromosomal locus	Frequency (%)	Reference(s)
EWSR1-FLI1	Translocation	(11;22)(q12;q11.2)	85	Fisher (2014)
EWSR1-ERG	Translocation	(21;22)(q22;q12)	10	
EWSR1-ETV1	Translocation	(7;22)(p22;q12)	1	
STAG2	Point mutation	Xq25	17	Brohl et al. (2014), Crompton et al. (2014), Tirode et al. (2014)
TP53	Point mutation	17p13.1	6	
EZH2	Point mutation	7q36.1	1	
BCOR	Point mutation	Xp11.4	1	
BRCA2	Point mutation	13q13.1	1	
ZMYM3	Point mutation	Xq13.1	1	
CDKN2A	Deletion	9p21.3	18	

apeutics than *STAG2* wild-type tumors, although this hypothesis has yet to be formally tested (Solomon et al. 2014). EWS, then, is driven primarily by the FET-ETS fusion transcription factor, with cooperation from loss-of-function mutations in *STAG2, TP53*, and *CDKN2A*.

Functional genomics studies have identified several vulnerabilities in EWS cells. First, EWS cells are particularly sensitive to drugs that induce DNA damage. One potential mechanism for this increased sensitivity is that wild-type EWSR1 interacts with RNA polymerase II (RNA Pol II) as well as the RNA splicing machinery. The interaction of EWSR1 with RNA Pol II prevents the phosphorylation of C-terminal domain (CTD) at serine 2 by CDK9/CDK12, which prevents transcriptional elongation. EWS-FLI1, in contrast, is unable to block CTD phosphorylation, resulting in increased transcription and increased creation of R loops, which are 3-stranded nucleic acid structures composed of a template DNA: nascent mRNA hybrid and non-template single-stranded DNA. BRCA1 is sequestered in these R loops, creating a functional BRCA1 deficiency, which results in EWS cells having a decreased capacity for homologous recombination and therefore an increased sensitivity to both DNA damage and PARP inhibition (Gorthi et al. 2018; Heske et al. 2017). The increase in global transcription conferred by expression of EWSR-FLI1 could also partially explain the sensitivity of EWS cells to CDK12 inhibition (Iniguez et al. 2018). Expression of genes important for DNA damage repair, including *PARP*, are regulated by both EWSR1-FLI1 and CDK12. In addition, EWSR1-FLI1 upregulates genes important in the serine-glycine biosynthesis pathway, such as *PHGDH*, and glutamine transport, such as *SLC1A5*, which provide additional targetable vulnerabilities in this tumor type (Sen et al. 2018; Svoboda et al. 2018; Tanner et al. 2017).

EWS, in contrast to other pediatric sarcomas, is rarely observed in association with cancer predisposition syndromes. However, EWS is more common in people of European ancestry and can run in families, suggesting a genetic component to the risk of developing EWS. Accordingly, genome-wide association studies (GWAS) have identified several EWS susceptibility loci, confirming that interactions between germline variations and the somatic EWS translocation are important for EWS development. In several cases, the susceptibility loci are proximal to known targets of EWS-FLI1, such as *EGR2* and *NKX2.2* (Machiela et al. 2018; Postel-Vinay et al. 2012).

2.2.3 Ewing-Like Sarcomas

A group of Ewing-like sarcomas, which resemble EWS histologically but are driven by different gene fusions, are emerging histologically and clinically. In contrast to typical EWS, these tumors have more nuclear atypia, more morphologic heterogeneity (including spindle cell patterns), and commonly show patchy or even negative CD99 expression. Like EWS, these tumors may arise in the bone or soft tissues, occur in children and young adults, and have an aggressive clinical course.

Non-FET/ETS fusions, such as *CIC-FOXO4* or *EWSR1-NFATc2*, have been identified in tumors diagnosed as EWS; however, these tumors do not resemble EWS at the transcriptome level, suggesting a difference from classic EWS (Brohl et al. 2014). Two specific examples of Ewing-like sarcomas with recurrent non-FET/ETS fusions are discussed below.

2.2.3.1 *CIC*-Rearranged Sarcomas

Undifferentiated sarcomas harboring a *CIC* rearrangement usually occur in the somatic soft tissues of young adults (Antonescu et al. 2017; Italiano et al. 2012; Kawamura-Saito et al. 2006; Richkind et al. 1996; Yoshimoto et al. 2009). *CIC-DUX4* fusion results from either a t(4;19) or a t(10;19) translocation, resulting in the fusion of *CIC*, an HMG-box family transcriptional repressor, to *DUX4*, a double homeobox transcription factor found on either chromosome 4 or 10. *CIC* fusions with *NUTM1* or *FOXO4* are also described (Watson et al. 2018). Some studies suggest *CIC* rearrangements may be found in two-thirds of *EWSR1*-negative Ewing sarcomas (Italiano et al. 2012). *CIC-DUX4* transcribes a

DNA-binding site that upregulates ETS family genes, which may also result in immunohistochemical expression of FLI1, ERG, WT-1, ETV4, and other ETS-family factors (Kawamura-Saito et al. 2006). Recent reports suggest these tumors harbor amplification of the *MYC* oncogene with differential expression of downstream targets including p21 and metadherin (MTDH) (Smith et al. 2015). Microscopically, these tumors have a more heterogeneous cytology compared to EWS, and immunophenotypically these tumors show variable expression of CD99 with frequent nuclear expression of WT1, ETV4, or DUX4. The overall survival of patients with *CIC*-rearranged sarcomas is worse than that of patients with Ewing sarcomas, owing to highly aggressive tumor behavior, a high metastatic rate and poor response to chemotherapy (Antonescu et al. 2017; Yoshida et al. 2016).

2.2.3.2 Ewing-Like Sarcoma with *BCOR* Rearrangements

Undifferentiated sarcomas harboring a *BCOR-CCNB3* rearrangement arise in bone or soft of children, typically less than 18 years of age, and show a male predominance (Cohen-Gogo et al. 2014; Pierron et al. 2012; Puls et al. 2014). A paracentric inversion on chromosome X results in a fusion of *BCOR,* an epigenetic modifier of histone methylation, with *CCNB3*. This event may occur in up to 14% of all undifferentiated sarcomas in children (Peters et al. 2015). Histologically, these tumors harbor a wide variety of morphologies from round to spindle cells and can resemble synovial sarcoma, osteosarcoma, or MPNST. The tumors are found within the bone or soft tissue but rarely affect visceral organs. The diagnosis may be confirmed by RT-PCR for the *BCOR-CCNB3* fusion transcript or by immunohistochemistry for BCOR or CCNB3, which show diffuse nuclear staining. By gene expression profiling, *BCOR-CCNB3* sarcomas appear distinct from Ewing sarcoma, with distinctive upregulation of the HOX gene family. The *BCOR-CCNB3* sarcomas have a better prognosis than the *CIC*-rearranged sarcomas, with outcomes similar to patients with ES (Kao et al. 2018; Pierron et al. 2012).

The spectrum of *BCOR*-mutated sarcomas is expanding to include *BCOR* internal tandem duplications (ITD) and the variant fusion *YWHAE-NUTM2B*. These *BCOR* alterations tend to occur in infants and include tumors with similar morphologies including primitive myxoid mesenchymal tumor of infancy and clear cell sarcoma of the kidney. Expression profiling shows that the *BCOR* family of tumors (including *BCOR*-ITD and *BCOR-CCNB3*) cluster together and are separate from other primitive sarcomas including EWS, *CIC-DUX4*, and synovial sarcomas (Kao et al. 2018).

2.3 Osteosarcoma

2.3.1 Pathology

Conventional osteosarcoma (OS) is a high-grade malignancy arising in the metaphysis of long bones, although any bone can be affected. High-grade OS is characterized by markedly pleomorphic cells with increased mitotic activity and varying degrees of tumor necrosis. A diagnosis of OS requires at least focal osteoid formation, although OS may also show varying degrees of cartilaginous differentiation. Other variants include fibroblastic, telangiectatic, and small cell OS. Immunohistochemically, SATB2 is reported to stain cells with osteoblastic differentiation and may be of use in some otherwise undifferentiated pleomorphic sarcomas (Conner and Hornick 2013; Davis and Horvai 2016).

Low-grade osteosarcomas (central or parosteal) and intermediate-grade osteosarcomas (periosteal) are rare, and these tumors are not discussed in detail in this chapter.

2.3.2 Biology

OS is an extremely complex tumor in which next-generation sequencing efforts have been unable to elucidate clear causative mutations. Genetic studies have yielded some hypotheses regarding OS development. First, patients with cancer predisposition syndromes, such as hereditary

retinoblastoma (*RB1* mutations), Li-Fraumeni syndrome (*TP53* mutations), Bloom syndrome (*BLM* mutations) and Rothmund-Thomson syndrome (*RECQL4* mutations), as well as the premature aging syndrome, Werner syndrome (*WRN* mutations), are at increased risk of OS development. These syndromes are all characterized by mutations in DNA helicases (*BLM*, *RECQL4*, and *WRN*) or proteins important for DNA repair (*TP53* and *RB1*), indicating a possible causative role for DNA repair pathways in osteosarcoma development. This hypothesis is also supported by the fact that therapeutic radiation for other cancers, which introduces DNA damage, is also a risk factor for OS. Second, tall stature and high birth weight are risk factors for OS, and OS most commonly develops during puberty, potentially implicating pathways that cause cellular growth in leading to OS. Finally, Paget disease of the bone, a disorder of bone remodeling, is also an OS risk factor. Recently a genome-wide association study (GWAS) identified an OS susceptibility locus at 6p21.3, which is located in an intron of *GRM4* (Savage et al. 2013). The protein product of this gene, the metabotropic glutamate receptor type 4, is involved in bone remodeling (Cowan et al. 2012).

Next-generation sequencing studies have revealed that OS tumors are heterogeneous and highly complex at the genomic level. OS has a relatively high mutation rate among pediatric cancers, with an average of 37 somatic non-silent mutations per tumor (1.2 mutations per megabase). Despite this high mutation rate, there are very few recurrently mutated genes. Consistent with the increased risk of OS in patients with cancer predisposition syndromes, *TP53* and *RB1* are recurrently mutated in OS. In addition to mutations in *TP53* and *RB1*, recurrent mutations in the tumor suppressor, *TSC2*, and the chromatin remodelers, *ARID1A* and *ATRX*, are seen in a small number of OS cases. However, in most OS cases, the high mutation rate is not related to recurrent mutations in known oncogenes or tumor suppressors but rather is due to the phenomenon of kataegis, a pattern of localized hypermutation that co-localizes with regions of structural variation in which the base mutations in the region are almost exclusively C→T in the context of a TpC dinucleotide (Chen et al. 2014b; Perry et al. 2014).

Structural variation is very common in OS and in fact contributes most of the functional genomic lesions in OS. Unlike EWS and RMS, OS lacks a recurrent fusion gene. However, OS tumors have an average of 230 chromosomal rearrangements per tumor, which is much higher than any other pediatric tumor or any tumor in the TCGA database. Chromothripsis, a phenomenon in which many chromosomal rearrangements occur in localized regions of single chromosomes, occurs in a small fraction of OS tumors. Structural variants are commonly found in intron 1 of *TP53* in OS. Most OS tumors exhibit aneuploidy, and chromosome arm level and focal amplifications and deletions are common. Significant focal deletions occur at the *RB1*, *TP53*, and *CDKN2A* loci as discussed above, while significant focal amplifications occur at the *COPS3, CCNE1, CDK4*, and *MYC* loci (Chen et al. 2014b; Perry et al. 2014). Genomic alterations in OS are summarized in Table 2.4.

In summary, genomic studies have confirmed that *TP53* is the most commonly altered gene in OS, with at least one allele mutated by somatic nucleotide variation, structural rearrangement or focal deletion in 90% of cases. Since the predominant mechanism of somatic *TP53* inactivation is through structural variation, it is currently unclear whether genomic instability is the cause or the result of *TP53* inactivation in OS.

Table 2.4 Genomic alterations in osteosarcoma

Gene	Type of alteration	Chromosomal locus	Frequency (%)
TP53	Point mutation, deletion, translocation	17p13.1	81
RB1	Point mutation, deletion, translocation	13q14.2	63
DLG2	Point mutation	11q14.1	23
TSC2	Point mutation	16p13.3	3
ARID1A	Point mutation	1p36.11	4
ATRX	Point mutation, deletion, translocation	Xq21.1	18

Chen et al. (2014b), Perry et al. (2014)

2.4 Non-rhabdomyosarcoma Soft Tissue Sarcomas

The non-rhabdomyosarcomatous soft tissue sarcomas (STS) are a diverse group of rare diseases. Relatively few deep sequencing projects have been conducted in this group of sarcomas, but often much is known about the biology of the disease despite the lack of genomic information in this heterogeneous group. The diagnoses described below are by no means inclusive of all types of STS, but they include the most common histologic types as well as those with the best characterized genomic changes. Many of these tumors are also associated with unique chromosomal translocations, the functional consequences of which have yet to be elucidated. The genomic alterations identified in the diverse group of STS tumors are summarized in Table 2.5.

2.4.1 Alveolar Soft Part Sarcoma

2.4.1.1 Pathology

Alveolar soft part sarcoma (ASPS) is a pathologically distinct tumor composed of nests of large epithelioid cells with abundant granular cytoplasm. In children, it most commonly arises in the head and neck region and presents as a slow-growing, painless mass. Lung and brain metastases are common. Immunohistochemical staining for TFE3 typically shows diffuse, nuclear expression (Tsuji et al. 2012).

2.4.1.2 Biology

ASPS is driven by an unbalanced chromosomal translocation, t(X;17)(p11.2:q25), that results in fusion of *ASPSCR1*, a gene whose protein product is known to play a role in the trafficking the glucose transporter type 4, with transcription factor E3 (*TFE3*). TFE3 is part of the microph-

Table 2.5 Genomic alterations in soft tissue sarcomas

Sarcoma	Gene	Type of alteration	Chromosomal location	Reference(s)
Alveolar soft part sarcoma	*ASPSCR1-TFE3*	Translocation	(X;17)(p11.2:q25)	Kobos et al. (2013)
Clear cell sarcoma	*EWSR1-ATF1*	Translocation	(12;22)(q13;q13)	Davis et al. (2006)
Desmoid tumor	*CTNNB1* *APC*	Point mutation	3p22.1 5q22.2	Crago et al. (2015)
Desmoplastic small round cell tumor	*EWSR1-WT1*	Translocation	(11;22)(p13;q12)	Gerald and Haber (2005)
Ewing-like sarcoma	*CIC-DUX4* *BCOR-CCNB3*	Translocation	t(4;19)(q25;q13) inv(X)(p14.4p11.2)	Italiano et al. (2012) Kao et al. (2018)
Infantile fibrosarcoma	*ETV6-NTRK3* Other *NTRK1-NTRK3* fusions	Translocation	(12;15)(p13;q26)	Miettinen (2006)
Myxoid liposarcoma	*TLS-DDIT3* *EWSR1-DDIT3*	Translocation	(12;16)(q13;p11) (12;22)(q13;q12)	Conyers et al. (2011)
Malignant peripheral nerve sheath tumor	*NF1* *SUZ12* *EED*	Deletion, point mutation	17q11.2 17q11.2 11q14.2	De Raedt et al. (2014), Zhang et al. (2014)
Rhabdoid tumor	*SMARCB1*	Deletion, point mutation	22q11.23	Lee et al. (2012)
Synovial sarcoma	*SS18-SSX1* *SS18-SSX2* *SS18-SSX4*	Translocation	(X;18)(p11;q11)	Joseph et al. (2014)

thalmia-TFE (MiT) subfamily of basic helix-loop-helix leucine zipper transcription factors, along with TFEB, TFEC, and MITF. Two types of fusions are found in ASPS, however, both fusions lead to the creation of an aberrant transcription factor by the fusion of the DNA-binding domain of TFE3 with the amino terminal portion of ASPSCR1. The ASPSCR1-TFE3 fusion is a stronger transcriptional activator than wild-type TFE3. Wild-type TFE3 promotes cell cycle progression by blocking activity of the tumor suppressor, RB. Similar fusions have also been identified in renal cell carcinoma (Kobos et al. 2013). Gene expression profiling studies of ASPS tumors showed enrichment for hypoxia and angiogenesis pathways; these tumors have high expression of several targetable receptor tyrosine kinases such as MET, the VEGF receptors, and EGFR (Soheilifar et al. 2018). In addition, exome sequencing of a limited number of ASPS cases demonstrated that although ASPS tumors have a low mutational burden and do not have a mutation in a known mismatch repair gene, the mutation pattern in ASPS is consistent with tumors that have a mismatch repair deficit (Lewin et al. 2018). This mismatch repair gene expression signature indicates that ASPS tumors might be more vulnerable to immune checkpoint inhibitors than other pediatric sarcomas.

2.4.2 Clear Cell Sarcoma of Soft Tissue

2.4.2.1 Pathology

Clear cell sarcoma (CCS), also known as malignant melanoma of the soft parts, is a soft tissue malignancy of tendons and aponeuroses. Most cases arise in the extremity, and these tumors present as a slow-growing mass in adolescents and young adults. These tumors may have a variety of histologic patterns and appearances, but the malignant cells express markers of melanocytic differentiation including S100, Sox10, and HMB45.

2.4.2.2 Biology

CCS, like melanoma, ASPS, and translocation-associated renal cell carcinoma, is driven by increased activity of one of the MiT family transcription factors. In the case of CCS, a reciprocal translocation, t(12;22)(q13;q13), results in fusion of the Ewing sarcoma-associated gene (*EWSR1*) to activating transcription factor 1 (*ATF1*). ATF1 is a member of the CREB family of transcription factors, which require phosphorylation for activity. The EWSR1-ATF1 fusion retains the DNA-binding domain of ATF1 but does not require phosphorylation for activity. The expression of MITF, which confers both melanocytic differentiation and proliferation in CCS cells, is driven by EWSR1-ATF1 (Davis et al. 2006), although it is unclear if EWSR1-ATF1 directly engages the MITF gene. The protooncogene, FOS, is also a target of EWSR1-ATF1 (Yamada et al. 2013). Gene expression profiling of CCS cell lines and tumors by microarray analysis revealed that CCS expression profiles are like those of melanoma and other tumors of neural crest origin (Schaefer et al. 2004); however, CCS uniquely overexpresses ERBB3, a member of the EGFR family, which might represent and important targetable vulnerability for this malignancy.

2.4.3 Desmoid Tumor

2.4.3.1 Pathology

Desmoid-type fibromatosis is a locally aggressive tumor with an infiltrative growth pattern and a high incidence of local recurrence. Histologically, the lesions are poorly circumscribed and invade into surrounding soft tissues. These tumors are composed of small, uniform spindled cells with minimal atypia and low mitotic activity. The immunophenotype of desmoid-type fibromatosis is non-specific, with the exception of nuclear β-catenin staining in up to 90% of pediatric tumors (Bo et al. 2012).

2.4.3.2 Biology

Desmoid tumors can be sporadic or associated with the cancer predisposition syndrome, familial adenomatosis polyposis coli (FAP). FAP-associated desmoid tumors are caused by germline mutations in the tumor suppressor, *APC*, followed by somatic inactivation of the wild-type *APC* allele by mutation or deletion. Loss of APC in these tumors results in aberrant β-catenin activity. The large majority (88%) of sporadic desmoid tumors are driven by aberrant activity of the Wnt/β-catenin pathway, either through activating mutations in the β-catenin gene, *CTNNB1*, or through loss of function mutations in *APC*. Recent whole exome sequencing and array comparative genomic hybridization studies in desmoid tumors showed that additional mechanisms by which these tumors achieve aberrant β-catenin activity include mutations in *BMI1*. In total, 95% of desmoid tumors studied had evidence for Wnt signaling alteration (Crago et al. 2015). FAP-associated desmoid tumors have a greater number of copy number alterations than sporadic desmoids, including recurrent losses of chromosomes 5 q and 6q and gains of chromosomes 8 and 20q (Robanus-Maandag et al. 2011).

2.4.4 Desmoplastic Small Round Cell Tumor

2.4.4.1 Pathology

Desmoplastic small round cell tumor (DSRCT) is a mesenchymal tumor with multi-phenotypic differentiation. Although DSRCT may have variable morphologic patterns, they are composed of primitive round to epithelioid cells in abundant extracellular material. The tumor cells are immunoreactive for epithelial, muscular, and neural markers and show nuclear expression of WT-1 (C-terminus).

2.4.4.2 Biology

DSRCT has yet to be fully genomically characterized. However, this sarcoma is driven by a specific chromosomal translocation, t(11;22)(p13;q12), that leads to the juxtaposition of the *EWSR1* gene with that of the transcription factor, *WT1*. The resulting fusion transcription factor retains the DNA-binding domain of WT1 and likely functions by binding WT1 targets. Key transcriptional targets of EWSR1-WT1 include *IL2Rβ*, which signals through the JAK-STAT pathway, PDGFRα, IGF1R, EGFR, MYC, WT1, and EGR1 (Gerald and Haber 2005). Molecular profiling of 35 DSRCT tumors using panel sequencing and immunohistochemistry showed DSRCT has a low frequency of actionable mutations and low immunogenicity; however, somatic mutations in *TP53* and *FOXO3* were observed. DSCRT has high expression of the androgen receptor, indicating that this malignancy may be vulnerable to androgen receptor antagonists (Bulbul et al. 2017).

2.4.5 Malignant Peripheral Nerve Sheath Tumor

2.4.5.1 Pathology

Malignant peripheral nerve sheath tumors (MPNSTs) may arise de novo, from malignant degeneration of neurofibromatosis type 1 (NF1)-associated plexiform neurofibromas or because of prior radiation therapy. MPNSTs are usually large tumors (>5 cm) which arise from a nerve, often resulting in fusiform swelling or distortion of the underlying nerve, and are usually deep to the superficial fascia. Histologically, these malignancies, whether sporadic, NF1-associated, or radiation-induced, are characterized by spindled to epithelioid cells with neural differentiation as confirmed by immunoreactivity for S100 and SOX10 (Pekmezci et al. 2015). Occasionally, MPNSTs show heterologous elements, particularly rhabdomyoblastic differentiation where it is termed a malignant triton tumor. MPNSTs may be low to high grade, with varying degrees of cellularity, mitotic activity, and tumor necrosis, but there is poor correlation with French Federation of Cancer Centers (FNCLCC) grade and prognosis as compared to other mesenchymal neoplasms (Coindre et al. 2001; Rodriguez et al. 2012). Atypical neurofibromas, which show nuclear atypia or hypercellularity but maintain neurofibroma architecture and a low mitotic index, are

thought to represent an intermediate in the malignant transformation of plexiform neurofibromas to MPNST (Miettinen et al. 2017).

2.4.5.2 Biology

Two recent studies highlight that the mechanisms responsible for the development MPNST may be epigenetic in nature. Benign neurofibromas develop in patients with neurofibromatosis 1 (NF1) when the *NF1* gene inherited from the unaffected parent acquires a somatic inactivating mutation. Next-generation sequencing of both NF1-associated and sporadic MPNSTs shows that these tumors have a relatively high mutation rate for pediatric tumors (60 somatic coding SNVs per tumor). In addition to biallelic loss of *NF1*, atypical neurofibromas have loss of *CDKN2A* (p16). Malignant transformation of atypical neurofibromas to NF1-associated MPNST is a result of loss-of-function mutation components of the polycomb repressive complex 2 (PRC2). PRC2 is the complex responsible for writing the H3K27me3 histone mark, which is associated with transcriptional repression. Recurrent mutations have been identified in several PRC2 components in sporadic, NF1-associated, and radiation-induced MPNST, including *SUZ12*, *EED*, *EPC1*, and *CHD4* (De Raedt et al. 2014; Zhang et al. 2014). Additional recurrent mutations in MPNST include monoallelic loss of the tumor suppressors, *TP53* and *RB1*. These tumors also have high levels of LOH with frequent chromosome gains (Longo et al. 2018; Miettinen et al. 2017).

2.4.6 Synovial Sarcoma

2.4.6.1 Pathology

Synovial sarcoma (SS) is a mesenchymal tumor which displays variable epithelial differentiation. Histologically, it may be biphasic with gland formation or monophasic with uniform spindle cells arranged in vague fascicles. Rarely, poorly differentiated synovial sarcomas by be composed of round cells and resemble other small round blue cell tumors. Immunohistochemistry confirms the presence of at least focal epithelial differentiation, and these tumors may also express novel markers such as TLE1 (Kosemehmetoglu et al. 2009; Terry et al. 2007). Definitive diagnosis may require cytogenetic confirmation of rearrangement of the *SS18* (also known as *SYT*) gene.

2.4.6.2 Biology

SS is also driven by alterations in chromatin homeostasis. SS is associated with a specific chromosomal translocation, t(X;18)(p11;q11), which results in the fusion of *SS18* with *SSX1* or, less commonly, with *SSX2* or *SSX4*. Whole exome sequencing of 7 SS tumors revealed both that the somatic mutation rate of SS is low (0.27 mutations per megabase) and SS tumors have very little loss of heterozygosity (Joseph et al. 2014), indicating that the SS18-SSX fusion is the major oncogenic driver in this tumor.

The protein product of *SS18* was recently shown to be a subunit of the mammalian SWI-SNF complex, which functions in gene activation by nucleosome remodeling, allowing transcription factors to access their cognate recognition sites. The SS18-SSX1 fusion integrates into the SWI-SNF complex in place of SS18, but the presence of the fusion alters the composition of the SWI-SNF complex. In the presence of SS18-SSX1, BAF47 (also known as INI1, gene symbol *SMARCB1*) is removed from the SWI-SNF complex and is subsequently degraded. In the case of SS, the aberrant SWI-SNF complex, with SS18-SSX1 incorporated in place of wild-type SS18 and BAF47, drives tumorigenesis by allowing the expression of genes important for tumor cell proliferation, such as *SOX2* (Kadoch and Crabtree 2013). Additional SS18-SSX targets include *CDKN2A*, *EGR1*, Wnt ligands and Wnt targets (Nielsen et al. 2015), as well as *PAX3*, *PAX7*, and other genes normally characterized by bivalent histone marks during myogenic differentiation (McBride et al. 2018). Functional genomics and mechanistic studies have revealed that SS18-SSX-containing BAF complexes are recruited to these bivalent loci, which also contain unmethylated CpG islands, through an interaction with the non-canonical polycomb repressive complex 1 (PRC1) component, KDM2B (Banito et al. 2018), which is able to bind chromatin directly.

2.4.7 Malignant Rhabdoid Tumor

2.4.7.1 Pathology

Malignant rhabdoid tumor (MRT) is a highly aggressive tumor affecting infants and young children. Histologically, it is composed of rhabdoid cells which have abundant eosinophilic cytoplasm mimicking rhabdomyoblasts. Tumor cells may show divergent lines of differentiation, including epithelial and neural markers, but skeletal muscle specific markers are negative. Most tumors show loss of nuclear INI1 expression by immunohistochemistry, although a minority may have aberrant BRG1 staining instead (Hasselblatt et al. 2011).

2.4.7.2 Biology

Genetic loss of *SMARCB1* is tumorigenic in MRT, although rare tumors may show loss of *SMARCA4* (*BRG1*) (Schneppenheim et al. 2010). Recent genomic characterization of MRT reveals that the only somatic copy number alteration is deletion of 22q11.23, the locus that contains the *SMARCB1* gene. In addition, the mean mutation rate of primary MRT is low (0.19 mutations per megabase), with the only recurrent mutations found in *SMARCB1*. The identified *SMARCB1* mutations are predicted to be loss of function. Recurrent tumors have a higher rate of somatic mutation than primary tumors (1.53 mutations per megabase), but again no recurrent mutations other than *SMARCB1* were observed in a small sample set (Lee et al. 2012). Germline *SMARCB1* mutations predispose patients to development of MRT at a young age, a condition known as rhabdoid tumor predisposition syndrome (Bourdeaut et al. 2011). The function of SMARCB1 in the SWI/SNF complex is largely unknown, but SMARCB1 loss leads to activation of several cell signaling pathways, including cyclin D1, SHH, and WNT/β catenin. These results suggest that loss of SMARCB1 induces changes in chromatin structure leading to changes in transcription that result in functional uncoupling of pathways from upstream signal transduction pathways, ultimately causing tumorigenesis (Kim and Roberts 2014). Other members of the SWI/SNF complex, including SMARCA4/BRG1 and SMARCA2BRM, have been implicated in rhabdoid tumors in special sites (e.g., atypical teratoid/rhabdoid tumor, SMARC4-deficient thoracic sarcoma, and small cell carcinoma of the ovary, hypercalcemic type) (Arnaud et al. 2018).

2.4.8 NTRK-Fusion Sarcomas

2.4.8.1 Pathology

The *NTRK*-fusion sarcomas consist of an emerging group of pediatric sarcomas linked by the presence of an *NTRK*-gene fusion. This group of sarcomas includes infantile fibrosarcoma (IFS) and congenital mesoblastic nephroma with the classic *ETV6-NTRK3* rearrangement, as well as increasingly common descriptions of similar pediatric mesenchymal tumors with variant NTRK fusions. In each of these sarcomas, the tumor cells are typically primitive, stellate mesenchymal cells, often with areas of spindled cells arranged into fascicles. Immunohistochemically, these tumors are positive for vimentin and show variable and focal positivity for smooth muscle actin, desmin, S100, or CD34 (Bourgeois et al. 2000). They are also uniquely positive for pan-Trk protein expression (Rudzinski et al. 2018), which differentiates them from other childhood tumors comprised of spindle cells.

2.4.8.2 Biology

The Trk family of receptor tyrosine kinases comprise the transmembrane proteins, TrkA, B and C, encoded by NTRK1, NTRK2, and NTRK3, respectively. These receptors are normally activated by a unique neurotrophin for each Trk, namely, nerve growth factor (NGF) for TrkA, brain-derived growth factor (BDGF) for TrkB, and NT3 for TrkC. Ligand engagement activates signaling through the RAS-ERK, PI3 kinase, and PLCγ pathways, resulting in neuronal differentiation and survival. The most common oncogenic mutations in the NTRK genes are translocation events in which the 3′ region of the NTRK gene, which encodes the kinase domain of the Trk protein, is fused to the 5′ end of a variable fusion protein gene, resulting in an aberrantly active and overexpressed receptor tyrosine kinase (Amatu

et al. 2016). The canonical fusion identified in IFS is *ETV6-NTRK3* (Table 2.5); however, additional NTRK fusions have been identified, including fusions of *NTRK1, NTRK2*, and variant partners with *NTRK3* (Davis et al. 2018; Pavlick et al. 2017). The presence of these fusions confers a druggable vulnerability for these malignancies (Laetsch et al. 2018).

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Staging and Imaging of Sarcoma

3

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3.1 Staging of RMS and NRSTS

Staging of rhabdomyosarcoma (RMS) to determine risk group assignment and therapy has undergone significant evolution since the original description of the modified TNM system (Rodary et al. 1989). *Clinical Group*, which depends on the amount of tumor remaining after initial surgery but prior to initiation of systemic therapy, remains prognostic and is one of the factors used to determine risk classification (Table 3.1). Clinical Group can be influenced by subjective and arbitrary variables such as surgeons' different levels of expertise and by different treatment strategies: the definition of IRS group III includes patients who are unresectable at diagnosis but may also include patients suitable for resection whose physicians opted for biopsy and then upfront chemotherapy. The same case may be classified as group I or group III, not in relation to its biological aggressiveness, but due to physicians taking a different approach to treatment (Sultan and Ferrari 2010). The modified TNM system described by Lawrence in 1997 which incorporates tumor site, node status, and tumor size (Table 3.2) is used to help stratify patients (Lawrence Jr. et al. 1997). While it is clear that tumor size is a prognostic factor, for risk grouping purposes tumor diameter is usually considered in absolute terms (the 5 cm cutoff) regardless of a patient's size, even though RMS can occur in

Table 3.1 Clinical group rhabdomyosarcoma

Group I	*Localized disease, completely resected*
	a. Confined to muscle or organ of origin
	b. Contiguous Involvement, infiltration outside muscle or organ of origin, as through fascial planes
Group II	*Total gross resection with evidence of regional spread*
	a. Grossly resected tumor with microscopic residual disease
	b. Regional disease with involved nodes, completely resected with no microscopic residual
	c. Regional disease with involved nodes, grossly resected, but with evidence of microscopic residual and/or histologic involvement of the most distal regional node (from the primary) in the dissection
Group III	*Incomplete resection with evidence of gross residual disease*
Group IV	*Distant metastatic disease present at onset*
	(Sites of distant metastases include lung, liver, bones, marrow, brain, distant muscle, peritoneal or pleural implants, distant nodes, and positive cytology from cerebro-spinal, peritoneal, or pleural fluid. Note: the presence of a pleural effusion or ascites is not sufficient evidence of metastases without implants or a positive cytology)

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C. A. S. Arndt (ed.), *Sarcomas of Bone and Soft Tissues in Children and Adolescents*, Pediatric Oncology, https://doi.org/10.1007/978-3-030-51160-9_3

Table 3.2 Staging of rhabdomyosarcoma

Stage	Sites*	T	Size	N	M
1	Orbit, head/neck (except parameningeal), non-bladder/prostate GU, biliary tract	T1 or T2	a or b	N_0 or N_1 or N_x	M0
2	Bladder/prostate, extremity, parameningeal, other (trunk, retroperitoneum)	T1 or T2	a	N_0 or N_x	M0
3	Bladder/prostate, extremity, parameningeal, other (trunk, retroperitoneum)	T1 or T2	a b	N_1 N_0 or N_1 or N_x	M0 M0
4	All	T1 or T2	a or b	N_0 or N_1	M1

Definitions

Tumor

$T(site)_1$—confined to anatomic site of origin

a. ≤5 cm in diameter in size

b. >5 cm in diameter in size

$T(site)_2$—extension and/or fixative to surrounding tissue

a. ≤5 cm in diameter in size

b. >5 cm in diameter in size

Regional Nodes

N_0 regional nodes not clinically involved

N_1 regional nodes *clinically* involved by neoplasm

N_x clinical status of regional nodes unknown (especially sites that preclude lymph node evaluation

Metastasis

M_0 no distant metastasis

M_1 metastasis present

patients of very different ages. It has recently been demonstrated that the risk associated with a given tumor size is not the same in patients of different body size: the mortality is higher for younger children (with a lower BSA) than for older adolescents (with a higher BSA). Consequently, the unfavorable prognostic effect of tumor size is stronger in smaller children: a 5 cm tumor in a patient with a BSA of 1.75 m^2 (as in adolescents, for example) may carry the same death risk as a tumor under 3 cm in diameter in a child with a BSA of 0.6 m^2 (as in a 1-year-old child) (Ferrari et al. 2009). These findings suggest that the use of an absolute cutoff for tumor size may be questionable.

Favorable tumor sites include non-bladder/prostate genital sites (paratestis and female genital), non-parameningeal head and neck sites, biliary, and liver primary sites, with all other sites being unfavorable (Crist et al. 2001). *Age at diagnosis* is also an independent prognostic factor, with infants under 1 year of age and children and adolescents 10 years and older having a worse outcome (Joshi et al. 2004). *Nodal positivity*, while prognostic in alveolar RMS has not been shown to affect outcome in embryonal RMS (Rodeberg et al. 2011). *Histology* has also been recognized as an important prognostic determinant, with alveolar histology having a worse outcome compared to embryonal histology. However, the definition of alveolar histology has changed over the course of studies of the COG. Tumors which contained even small amounts of alveolar histology were classified as "alveolar" until the most recent rhabdomyosarcoma study ARTS0531 (2006–2013) in which there had to be at least 50% alveolar to be considered alveolar. The next generation of COG studies for RMS will use *PAX-FOXO1* fusion status, replacing histology. This will be the first generation of cooperative group RMS studies to utilize molecularly defined staging for risk assignment. Williamson and colleagues demonstrated the importance of the *PAX-FOXO1* fusion status in determining prognosis (Williamson et al. 2010). However, their cohort spanned several decades of European RMS trials so patients were not treated uniformly. In addition, patients with alveolar histology were treated more aggressively than patients with embryonal histology, and the alveolar histology patients were heavily weighted with patients who had metastatic disease and a known poor outcome. Nevertheless this landmark study showed the importance of fusion status in determining outcome. Subsequently, Skapek et al. analyzed the outcome of patients with intermediate risk RMS treated on Children's Oncology Group Study D9803 in a uniform fashion and confirmed Williamson's findings, supporting utilization of fusion status in treatment allocation in the next generation of COG RMS studies (Skapek et al. 2013). The application of a five gene expres-

sion signature in fusion negative RMS is being investigated prospectively in future studies, as it has been shown separate children with COG intermediate risk RMS into separate prognostic groups (Hingorani et al. 2015; Missiaglia et al. 2012).

Over the past two decades, there has been a shift of risk stratification in the Children's Oncology Group, mainly involving reassigning of children under age 10 with stage 4 Group IV embryonal RMS from intermediate risk (D9803) to high risk (ARST0431) and back to intermediate risk again for the next generation of studies. This is because such patients did as well on D9803 when treated as intermediate risk patients as they did on the much more complex high risk study ARST0431 (Arndt et al. 2009; Weigel et al. 2016). As noted, in the next generation of studies, histologic classification will be replaced by presence or absence of FOXO1 fusion. Low-risk patients include FOXO1-negative histologic ERMS at favorable sites and group I/II ERMS at unfavorable sites. Intermediate risk patients include FOXO1 fusion positive stage 1–3, group I-III; FOXO1 fusion-negative stage 1 group III (non-orbit), stage 3 group I/II, stage 2/3 group III, and stage 4 group IV under age 10. Patients who have a histologic classification of ARMS but FOXO1 fusion negative, stage 1, group I/II, stage 1 group III orbit, and stage 2 group I/II will be included on the intermediate risk study but receive reduced chemotherapy compared to the remainder of the patients on the intermediate-risk study. High-risk patients will include FOXO1-positive stage 4 group IV patients.

RMS patients are stratified along similar lines in the European pediatric Soft Tissue Sarcoma Study Group (EpSSG) and in COG protocols. However, there are some differences, at least in the definition. With the identification of different prognostic factors, risk assessment has now become more complex and more precise in European protocols, increasing treatment intensity to improve outcomes in patients with less favorable disease and avoiding overtreatment and decreasing side effects without jeopardizing the outcome for patients with more favorable disease. In addition to the Clinical Grouping system shown in Table 3.1, risk stratification of EpSSG RMS 2005 protocols includes tumor histology (embryonal versus alveolar subtype); presence of regional lymph node involvement; favorable versus unfavorable primary tumor site; and combination of tumor size and age (favorable tumor size and age was the combination of both favorable size less than 5 cm and age less than 10 years) (Ferrari and Casanova 2005). Patients with metastatic disease are considered separately and are included in a different protocol for IRS group IV patients. The risk stratification based on these variables permits identification of different categories of patients with different outcomes. Nevertheless, it is clear that this group assignment is not perfect and is only partially based on the intrinsic aggressiveness of tumor. For example, these prognostic factors are often interdependent, i.e., alveolar subtype is more frequent in tumors of the extremities and in adolescents.

Using these prognostic variables, the EpSSG RMS 2005 protocol for patients with localized rhabdomyosarcoma identifies four risk groups (low, standard, high, and very high risk groups) and eight subgroups. The risk group definitions do not cover the same subsets of patients of the COG protocol: for example, the EpSSG High-Risk Group may grossly correspond to the COG intermediate-risk group, though the latter may also include some patients that in the EpSSG are classified as carrying a Standard and Very High Risk, as well as some metastatic cases with favorable prognostic findings. Table 3.3 shows the risk grouping and treatment assignment currently used in EpSSG 2005.

The EpSSG subgroup A is the cohort of patients at Low Risk. This is a highly selected subset of patients (mainly children less than 10 years with paratesticular tumor less than 5 cm, completely resected at diagnosis) that represents about 5–6% of all cases, with an estimated 5-year EFS and OS of around 90% and 95%, respectively.

The Standard Risk Group (subgroup B, C, and D) includes around 30% of cases, with estimated 5-year EFS and OS (based on the previous European experiences) in the range of 80% and 85–90%, respectively (Oberlin et al. 2012).

Table 3.3 Risk stratification in the EpSSG RMS 2005 protocol for localized rhabdomyosarcoma

Risk group	Subgroup	Histology	IRS	Nodal status	Site	Size and age
Low	A	Favorable	I	N0	Any	Favorable
Standard	B	Favorable	I	N0	Any	Unfavorable
	C	Favorable	II-III	N0	Favorable	Any
	D	Favorable	II-III	N0	Unfavorable	Favorable
High	E	Favorable	II-III	N0	Unfavorable	Unfavorable
	F	Favorable	II-III	N1	Any	Any
	G	Unfavorable	I-II-III	N0	Any	Any
Very high	H	Unfavorable	I-II-III	N1	Any	Any

Histology: favorable = embryonal RMS (and variants), not otherwise specified; unfavorable = alveolar RMS
IRS (Intergroup Rhabdomyosarcoma Study): Group I = complete resection; Group II = microscopic residual disease after initial surgery; Group III = macroscopic residual tumor after surgery (or biopsy)
N status: N0 = no nodal involvement; N1 = involvement of regional lymph nodes
Site: favorable = non-parameningeal head-neck (i.e., orbit), non-bladder/prostate genito-urinary (i.e., paratesticular, vagina); unfavorable = all the other sites
Size and age—favorable: tumor size less ≤5 cm *AND* <10 years

Historically, European groups adopted the response to initial chemotherapy as a variable to guide the use of local therapy or to shift the poor responder patients to second-line chemotherapy.

The High-Risk Group (subgroup E, F and G) includes more than half of the cases. The estimated 5-year EFS and OS are 50–55% and 60–65%, respectively. For this group, the EpSSG RMS 2005 protocol requires an intensified treatment.

The Very-High-Risk Group (subgroup H) includes patients with alveolar histology and regional lymph nodal involvement. This group represents 6–8% of cases, with an estimated 5-year EFS and OS in the range of 40–45% and 50%, respectively.

3.2 Staging of Nonrhabdomyosarcomatous Soft Tissue Tumors (NRSTS)

Nonrhabdomyosarcomatous tumors comprise a wide variety of histologies. Typically they can be grouped into three risk groups which take into account histologic grade of the tumor, presence of metastases, size, and degree of resection. For practical purposes of risk/stage assignment for treatment they are classified into low-, intermediate-, and high-risk tumors (Spunt et al. 1999, 2002; Pappo et al. 1999). Both the COG and the EpSSG have launched clinical trials specifically tailored to NRSTS, i.e., the COG ARST0332 study (conducted from February 2007 to February 2012) (Spunt et al. 2020) and the EpSSG NRSTS 2005 study (opened in August 2005 and still ongoing). Both studies used a stratification that was defined according to the extent of disease (non-metastatic vs. metastatic), histologic grade (low vs. high) size of the primary tumor (≤5 cm vs. >5 cm), and extent of surgical resection (resected vs. unresected). These prognostic factors are the same utilized for adult STS and have been demonstrated to also predict outcome in pediatric NRSTS (Ferrari et al. 2007). Low-risk tumors are those which are non-metastatic, grossly resected except those which are both high grade and >5 cm in maximal diameter. These patients typically have an excellent long-term outcome of >90% survival, and therapy is surgery alone. Intermediate-risk group of patients includes those with non-metastatic tumor that are both high grade and >5 cm in maximum diameter and those with non-metastatic unresectable tumors, irrespective of grade or size. High-risk patients include patients with metastases, both distant and regional lymph nodes. Both intermediate and high risk patients are usually treated with multimodality therapy including chemotherapy, surgery, and radiation.

In the EPSSG, synovial sarcoma is considered separately due to the high frequency in the pedi-

atric age and the possible higher sensitivity to chemotherapy. For synovial sarcoma, EpSSG stratification includes tumor site as one of the variables for risk stratification purposes. Tumors arising from axial sites, i.e., head-neck, trunk, lung-pleura, and retroperitoneum, have been shown to have worse prognosis than limb SS (Ferrari et al. 2008).

The next synovial sarcoma protocol in Europe is considering new biological tools for risk stratification, i.e., a 67-gene signature related to chromosome integrity and genome complexity named CINSARC (complexity index in sarcoma) and/or a genomic index (GI) analyzed using comparative genomic hybridization (CGH) on tumor cells. Those have recently been developed and shown a high prognostic value in adult STS and in SS in particular (in adult but also in pediatric SS) (Chibon et al. 2010; Chakiba et al. 2014).

3.3 Staging of Bone Sarcomas

Staging and risk assignment of bone tumors is much simpler than that of rhabdomyosarcoma and NRSTS. For Ewing sarcoma and osteosarcoma, patients are considered to be high risk or low risk according to whether they have evidence of distant metastases, with no separate stages assigned beyond metastatic or not. However the EuroEwing study 99 assigns patients with non-metastatic Ewing sarcoma who have a poor response to induction chemotherapy to different randomization and risk group (the same randomization as those who have lung metastases which includes randomization to continued chemotherapy or high dose myeloablative chemotherapy and stem cell rescue) than those who have a better response to induction treatment.

3.4 Imaging and Staging Procedures

Standard imaging and staging procedures for rhabdomyosarcoma, NRSTS, osteosarcoma, and Ewing sarcoma include computed tomography of the chest, magnetic resonance imaging or computed tomography of the primary tumor, technetium bone scan (or more recently fludeoxyglucose positron emission tomography (FDG-PET)), and, in the case of RMS and EWS, evaluation of the bone marrow. Evaluation of lymph nodes is indicated in certain types of NRSTS (epithelioid sarcoma and clear cell sarcoma) as well as RMS.

A recent review of over 1600 patients with RMS treated on Intergroup Rhabdomyosarcoma or Children's Oncology Group Studies from 1991 to 2004 have suggested that certain subgroups of patients with RMS can have more limited staging studies, resulting in less cost and radiation and procedure exposure to patients (Weiss et al. 2013). In patients without regional node involvement (N0) and without local tumor invasiveness (T1), metastatic workup can be omitted. Patients without regional lymph node involvement but locally invasive (T2) but favorable histology and molecular pathology with a negative chest CT scan can omit further evaluation with bone scan or bone marrow evaluations. Figure 3.1 shows the algorithm developed based on risk of metastasis.

Similarly, European pediatric groups recently published a critical reappraisal of the staging investigations used in synovial sarcoma, in relation to the rate of metastatic involvement at diagnosis, to see whether these diagnostic procedures were really necessary in all patients. The study (on 258 patients <21 years old) showed a 5.8% rate of distant metastases at diagnosis, 86% of which were pulmonary. The presence of metastases was associated with tumor size (the risk of metastases being 32 times higher for tumors >5 cm in size than for smaller lesions). On the basis of these findings, it was suggested that tumor diameter might be used as a variable for identifying patients at higher risk of metastases and warranting accurate radiological investigations (chest CT scan), while chest CT scanning might be omitted for patients with tumors ≤5 cm in size. Given the very low risk of bone metastases, bone scans could be recommended only in cases with evidence of lung metastases (Ferrari et al. 2012). Moreover, some investigations routinely performed in patients with rhabdomyosarcoma such as bone scan and bone marrow biopsy

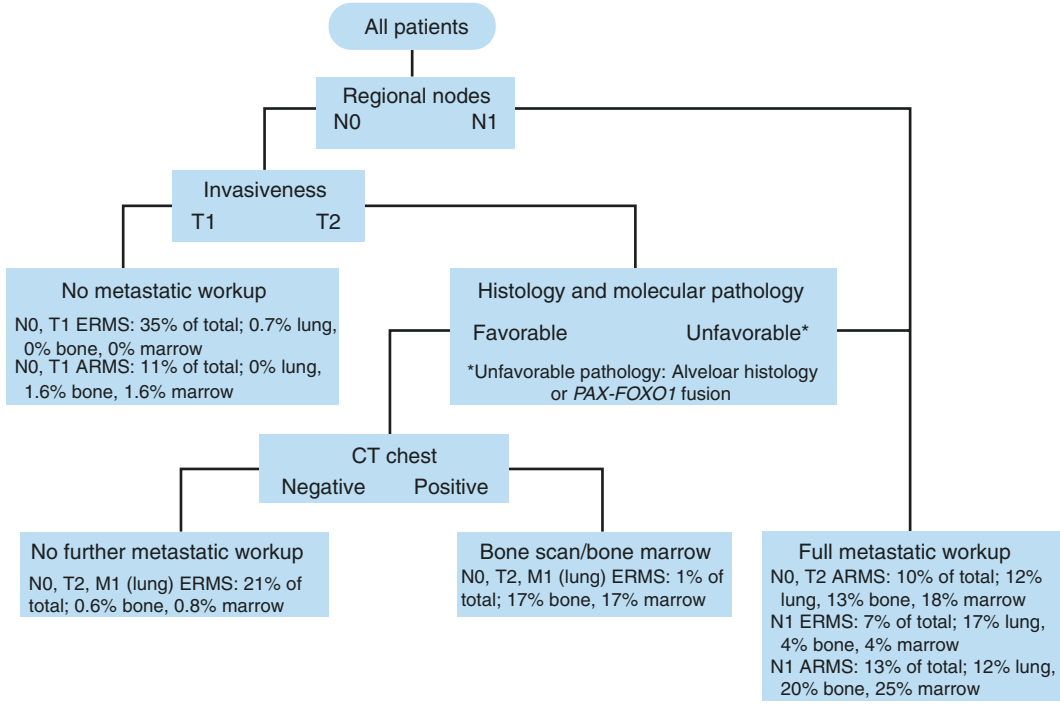

Fig. 3.1 Rhabdomyosarcoma initial staging algorithm using clinical and histologic characteristics. (Reprinted with permission. © (2013) American Society of Clinical Oncology. All rights reserved. Weiss et al.: Journal of Clinical Oncology 31:3226–3232)

may be omitted in patients with low grade NRSTS.

Lymph node positivity has been shown to affect outcome in patients with alveolar but not embryonal RMS (Rodeberg et al. 2011). Boys with paratesticular RMS who are older than 10 years have a higher rate of positive lymph nodes than those under 10 years. A population-based analysis based on the patient data reported by the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute demonstrated that lymph node dissection in boys >10 years of age significantly improved their outcome, whereas it had no effect on boys under 10. Therefore, on COG studies ipsilateral lymph node dissection is required for boys over 10 with paratestis primaries, and for boys under 10 with imaging findings concerning for nodal involvement. The European MMT group has taken a different approach, preferring more intensive therapy for paratestis primary tumors over node sampling (Stewart et al. 2003). The Italian and German study compared the clinical and surgical/pathologic stages in 95 children with paratesticular rhabdomyosarcoma: among 72 patients with negative radiologic findings (cN0), surgical/pathological assessment detected nodal involvement in only one case (pN1). This finding suggested that CT accurately evaluates the retroperitoneum. In analyses of only those patients older than 10 years, results did not seem different, even if the number of cases was substantially lower (13 of 13 cN0 patients were pN0 too) (Ferrari et al. 2002). On the basis of these results, the EpSSG protocol considered retroperitoneal lymphadenectomy or nodal sampling at diagnosis as not recommended unless there is uncertainty on imaging.

The planned COG intermediate risk study will require pathologic evaluation of nodes in all patients with extremity tumors and strongly

encourages it for patients with ARMS or clinically involved nodes regardless of histology. The preferred method of nodal evaluation is sentinel node sampling since this has been shown to be useful and feasible in pediatric patients with soft tissue sarcomas, even in a case reported with parameningeal RMS (De Corti et al. 2009; Weiss et al. 2011).

FDG-PET is being used more commonly in initial staging evaluation of sarcomas and is also being evaluated for response assessment. A small study of 13 children and adolescents with histologically proven RMS found FDG-PET scanning to be more sensitive than conventional imaging in detection of lymph nodes and bone metastases; however subcentimeter pulmonary nodules may not consistently be detected (Ricard et al. 2011). Moreover in one patient FDG-PET scan was positive in lymph nodes due to concurrent infection. In this series, FDG PET modified lymph node staging in four of 13 patients, bone involvement in two patients, and led to treatment alteration in two patients. A prospective study of 46 pediatric sarcoma patients, of whom 12 had RMS, found similar results in the superiority of FDG-PET scan in detection of lymph node and bone metastases, but not subcentimeter lung nodules (Volker et al. 2007). A limitation of both studies was that positive imaging was not consistently confirmed pathologically. Klem and colleagues also evaluated FDG-PET for initial staging in 24 patients with RMS and determined it to be 77% sensitive and 95% specific when compared with the final clinical determination of disease but still concluded that although "PET is a helpful adjunct… is not accurate enough to replace biopsy of suspicious nodes" (Klem et al. 2007). Federico and colleagues had similar conclusions in terms of high sensitivity for detection of nodal disease but low sensitivity in detection of lung disease (Federico et al. 2013). Mody et al. retrospectively evaluated 25 children with Ewing sarcoma family of tumors and rhabdomyosarcoma who had PET scans at various time points during therapy and found sensitivity of the PET scan was 86%, specificity was 80%, positive predictive value was 89%, and negative predictive value was 67% (Mody et al. 2010). Wegner and colleagues found PET scans to result in a change in management or be helpful in determining management of a wide variety of pediatric malignancies (Wegner et al. 2005). Gerth and colleagues found combining PET and CT to be significantly more accurate than PET alone in localization and detection of lesions in patients with Ewings sarcoma, and most PET imaging is now fused with CT imaging (Gerth et al. 2007). Eugene et al. evaluated 23 patients with RMS with PET/CT and conventional imaging and confirmed again that PET/CT provides important additional staging information as well as being prognostic for tumor response, but effect of PET response on outcome was not evaluated (Eugene et al. 2012). On the planned next intermediate risk Children's Oncology Group RMS study, data will be collected on results of FDG-PET scan and nodal biopsy to determine rates of false positivity and negativity for FDG-PET scans.

Surprisingly, tumor response by conventional anatomic imaging in patients with RMS does not correlate with outcome in two COG studies (Burke et al. 2007; Rosenberg et al. 2014). This is in contrast to the findings of two European groups, the Italian and German CWS group which found a correlation between the degree of shrinkage after induction chemotherapy (assessed radiologically after three courses of therapy) and final outcome, leading to subsequent European trials using radiologic response to tailor subsequent treatment (Koscielniak et al. 1999; Ferrari et al. 2010). Two recent studies from Memorial Sloan Kettering Cancer Center suggest that functional imaging with FDG-PET scan after local control with radiation as well as after up front chemo were predictive of EFS, overall survival, as well as local control (Casey et al. 2014; Dharmarajan et al. 2012). A small study of adults with high-grade sarcomas by Tateishi et al. suggested that metabolic reduction after neoadjuvant chemotherapy evaluated by FDG PET can be used for stratification of histopathologic response in patients with high-grade sarcoma and that percentage of SUV2 reduction as well as histopatho-

logic response were independent predictors of outcome (Tateishi et al. 2011). Correlation of PET response with outcome and histopathologic response in soft tissue sarcomas will require further evaluation and confirmation.

A number of investigators have investigated correlation of PET response with outcome and histopathologic response in Ewing sarcoma and osteosarcoma. Hawkins et al. evaluated 36 patients with Ewing sarcoma family of tumors and reported that not only did PET imaging corerlate with histologic response, but SUV2 less than 2.5 was predictive of progression-free survival (PFS) independent of initial stage (Hawkins et al. 2005). The same investigators found that PET response was only partially correlated with histopathologic response to chemotherapy in osteosarcoma, although an SUV2 <2.5 was associated with improved PFS (Hawkins et al. 2009). This is in contrast to the findings by Denecke et al. who found PET to be a useful tool in discriminating histologic response in osteosarcoma but not Ewings sarcoma (Denecke et al. 2010). Cheon et al. found that patients with osteosarcoma with an SUV2 of less than or equal to 2 showed a good histologic response whereas patients with an SUV2 of greater than 5 showed a poor histologic response, but did not attempt to correlate SUV2 with survival (Cheon et al. 2009). Benz et al. provides an excellent review of multiple studies of PET imaging in sarcoma (Benz et al. 2009). Fused whole body PET-MRI is also being investigated, especially in indications that require high soft tissue contrast for diagnosis and treatment planning (Buchbender et al. 2012).

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4 Multi-institutional Trials for Patients with Rhabdomyosarcoma: Lessons from North American Studies from 1967 Through 1997

R. Beverly Raney, Carola A. S. Arndt, and Harold M. Maurer

4.1 Introduction

The first large multi-institutional trial in children and adolescents less than 21 years of age with rhabdomyosarcoma (RMS) and undifferentiated soft-tissue sarcoma (UDS) was planned by members of the Children's Cancer Study Group A (CCSGA) in North America, under the leadership of Denman Hammond, MD, Group Chairman, and led by Ruth M. Heyn, MD. This study included pediatric oncologists in the United States and Canada and was composed of physicians specializing in surgery, pathology, radiation oncology, hematology/oncology, and statisticians. The rationale and results were published in two successive articles. The first part of the study, opened in 1967, was to answer this randomized question: would the addition of chemotherapy with dactinomycin (actinomycin D, denoted by A) and vincristine (VA) to surgery (S) and radiation therapy (RT) for patients with newly diagnosed RMS and UDS who had undergone complete removal of localized disease result in a more favorable outcome, compared to those who underwent only local therapy without VA chemotherapy? Finding a statistically significant improvement in disease-free survival with the addition of VA led to the conclusion that all young patients with RMS and UDS should receive chemotherapy for 1 year along with local treatments (Heyn et al. 1974). The second part of the study, later amended to include oral cyclophosphamide (C, collectively called VAC), opened in 1970 for patients with localized disease, grossly removed, with pathologically demonstrable microscopic residual tumor, patients with localized disease and grossly visible tumor after biopsy or subtotal resection, and those with distant metastases at diagnosis. The results were 3-year survival rates of 70.8%, 43.2%, and 27.2%, respectively (Heyn et al. 1977). The addition of C to VA did not result in a significantly improved survival rate compared to patients with the same amounts of residual disease in the first study (Heyn et al. 1974).

The first large, multi-institutional study of North American children with a solid tumor was the National Wilms' Tumor Study (NWTS 1969), which was funded by a grant from the National Cancer Institute (NCI) in Bethesda, Maryland. The collaboration of multiple insti-

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tutions was necessary to answer clinical and therapeutic questions with sufficient statistical power. The investigators found that a multidisciplinary approach with surgery, RT, and chemotherapy could treat those children successfully and improve prognosis. The Wilms' tumor model helped to pave the way for the first North American protocol for young patients with RMS and UDS, called the Intergroup Rhabdomyosarcoma Study (IRS).

4.2 The First 30 Years, 1970–2000

4.2.1 The IRS Era, 1972–1997

Members of the other two extant pediatric oncology groups in North America, the Acute Leukemia Group B (ALGB) and the Southwest Oncology Group (SWOG), joined the CCSGA and included members in the same specialties as those in Dr. Heyn's studies. Dr. Hammond led this effort, and Dr. Harold M. Maurer was chosen to chair the new study.

IRS-I, 1972–1978. The Intergroup Rhabdomyosarcoma Study (IRS, later called IRSG, or IRS Group) was sponsored by the National Cancer Institute and was opened for patient registration in 1972. Eligible for entry were children and adolescents aged 0–20 years with previously untreated RMS or UDS. The IRS-I protocol incorporated a system of categorizing patients based on location of the primary tumor's site of origin, extent of disease after initial surgical excision or biopsy, the presence of regional lymph node involvement by tumor, with or without distant metastases. Using this clinical postsurgical grouping system, Group I included patients with localized, pathologically proven completely removed tumor. Group II patients had microscopic residual disease at the margins of resection and/or the presence of grossly resected regional nodal metastases. Group III patients had gross residual disease after subtotal resection or biopsy only, and Group IV patients had distant metastases involving the lung, bone marrow, bone, liver, distant lymph nodes, or other sites (Table 4.1). Eligibility of the patients was established by the local pathologist and confirmed by the IRSG reference pathologists; tumor types were also specified as embryonal or alveolar rhabdomyosarcoma (ERMS or ARMS, respectively), and UDS. The overall plan was to use surgery, radiation therapy for residual local tumor, and chemotherapy for all patients. Based on data from single-institution studies, the major non-surgical therapies consisted of graded amounts of radiation and chemotherapy according to the likely risk of recurrence, least for Group I patients and more for patients with residual disease. Because Dr. Heyn and the CCSGA had found that chemotherapy with vincristine and dactinomycin (VA) improved disease-free survival in Group I patients treated with radiotherapy (Heyn et al. 1974); an unanswered question was whether local radiation therapy (RT) was necessary in these patients without proven local residual disease. The backbone chemotherapy for all patients in IRS-I also included cyclophosphamide, collectively called VAC (Heyn et al. 1977). A second question was whether the addition of doxorubicin in addition to VAC for those with Group III and IV disease would result in improved survival. Both of these questions were randomized. Central review by IRSG pathologists was required to confirm the diagnosis and to specify histologic subtypes of

Table 4.1 IRSG grouping system

Group	Description
I	Localized disease, completely resected (a) Confined to organ or muscle of origin (b) Infiltration outside organ or muscle of origin; regional nodes not involved
II	Compromised or regional resection of three types including: (a) Grossly resected tumors with microscopic residual (b) Regional disease, completely resected, in which nodes may be involved, and/or extension of tumor into an adjacent organ present (c) Regional disease with involved nodes grossly resected, but with evidence of microscopic residual
III	Incomplete resection of biopsy with gross residual disease
IV	Distant metastases, present at onset

RMS and UDS. Surgeons reviewed the pathologic and surgical data to confirm extent of disease at diagnosis. Radiation oncologists reviewed the RT data, and pediatric hematologists/oncologists reviewed the chemotherapy data.

Results. The final IRS-I report, comprising 686 previously untreated patients less than 21 years of age, was published in 1988 (Maurer et al. 1988). The major findings from this large cohort were the following. There was no significant therapeutic advantage to including RT for Group I patients, nor oral cyclophosphamide for Group II patients, nor doxorubicin for Group III and IV patients. Survival rates at 5 years were approximately 87% (Group I), 72% (Group II), 52% (Group III), and 20% (Group IV). The overall 5-year survival rate of the entire cohort of patients was 55%. Each Group's outcome results were significantly different from the others, validating the Clinical Grouping system as constituted. Patients with primary tumors of the orbit had the best prognosis and retroperitoneal tumors the worst, indicating that the site of tumor origin also had prognostic significance.

IRS-II, 1978–1984. Eligibility criteria for patients were the same as in IRS-I. They were stratified by Clinical Group, primary tumor site, and histologic subtype; non-osseous Ewing's Sarcoma patients were also included. Primary objectives were to determine whether cyclophosphamide could be eliminated for Group I patients (except for those with extremity ARMS) without decreasing disease-free survival (DFS) and overall survival (OS); whether intensified "pulse VAC" could improve DFS and OS rates for Group II patients compared to IRS-I, (excluding extremity ARMS); whether "pulse VAC" plus doxorubicin would improve outcomes over pulse VAC alone in Groups III and IV; whether intensive prophylactic meningeal radiation and intrathecal chemotherapy would eradicate cranial parameningeal sarcomas abutting or invading the central nervous system and improve survival; whether a primary chemotherapy approach for patients with bladder, prostate, vaginal, and uterine sarcomas (Special Pelvic sites) would result in less total cystectomies without compromising patient survival; and whether pulse VAC would improve DFS and OS rates for patients with Group I and II extremity ARMS.

Results. 999 eligible patients were enrolled. Five-year survival rates in Group I non-extremity ARMS patients were almost identical in the cyclophosphamide and non- cyclophosphamide-containing regimens, 84.5%. In Group II, non-extremity ARMS patients 5-year survival rates averaged 83.5%. For Group III patients (except those with special pelvic sites) 5-year survival rates were 59% with or without doxorubicin and significantly better than in Group III patients in IRS-I. Group IV patients were less fortunate: their 5-year survival rate was similar to those in IRS-I, 26.5% with or without doxorubicin. Five-year survival rates for Group III patients with cranial parameningeal sarcoma were increased significantly compared to IRS-I, 67% vs. 45%. The 5-year survival rate for all IRS-II patients was 63% compared to 55% in IRS-I, $P < 0.001$. Outcomes by primary site were the same as or better than those in IRS-I (Maurer et al. 1993).

IRS-III, 1984–1991. Patient eligibility criteria were identical to those of IRS-II The protocol was more elaborate than IRS-II, including two randomized groups of patients. Group II patients received VA and RT vs. VA + doxorubicin and RT, and Groups III and IV patients underwent a 3-way randomization among intensive VAC, VAC + doxorubicin + cisplatin, and VAC + doxorubicin + cisplatin + etoposide along with RT. Certain subgroups of patients with special pelvic sites, Groups I and II unfavorable-histology disease (non-embryonal RMS, UDS), and those with favorable sites (orbit, paratesticular, superficial head/neck) in Group II and III received modified versions of the above treatment schedules. Groups III and IV randomized patients who achieved a complete or partial response by week 20 were considered for second-look surgery to assess pathologic response. End-points were progression-free survival (PFS) and overall survival.

Results. 1062 eligible patients were entered. Group I, favorable histology (FH, including botryoid, embryonal, and spindle-cell tumors but excluding ARMS and UDS), had a 5-year OS rate of 93%, insignificantly different from those

in IRS-II. Group II FH patients had a 5-year OS rate 89%, insignificantly different from that in IRS-II, perhaps because of low numbers, and doxorubicin conferred no significant advantage. For Group III patients (excluding patients with orbit and superficial head and special pelvic sites), the intensified schedules of chemotherapy compared to that in IRS-II led to a significantly higher 5-year DFS rate of 62% vs. 52%, but the 5-year survival rate was 70%, insignificantly different from IRS-II. In Group IV, the 5-year survival rate averaged 29%, insignificantly better than those of IRS-II patients. The more intensive chemotherapy programs failed to show a significant difference compared to the standard VAC schedule, and thus VAC remained the "gold standard" of chemotherapy for those patients. For those with "special sites," results were as follows. Group III patients with cranial parameningeal sarcoma had outcomes similar to those in IRS-II after sequential deletion of intrathecal chemotherapy and total central nervous system RT in this and subsequent protocols, focusing instead on more localized RT to demonstrable sites of involvement. Primary sites in the bladder, prostate, vagina, and uterus had a 5-year survival rate of 83% and a bladder salvage rate of 60%, compared to 58% survival and 25% bladder salvage in the IRS-II patients. Patients with FH orbit and head tumors, Groups II and III, and paratesticular FH tumors, Group II, had outcomes similar with VA and RT without cyclophosphamide, which was administered to comparable patients in IRS-II. Patients with localized extremity tumors, including all histologies, had a 5-year overall survival of 74%. The overall clinical outcome of therapy on IRS-III was significantly better than on IRS-II, with a 5-year survival rate of 71% vs. 63%, respectively, $P < 0.001$. This result was due primarily to improved survival in the most frequent patients, those with Group III disease at diagnosis (Crist et al. 1995). Moreover, primary site of disease was shown to be of prognostic significance (Crist et al. 1995).

IRS-IV, 1991–1997. IRS-IV was similar to IRS-III in eligibility requirements but excluded patients with non-osseous Ewing's sarcoma, primary brain or spinal cord sarcoma, and embryonal sarcoma of the liver. Clinical Group was used to categorize patients as before. In addition, a new Presurgical Staging classification, based on the TNM system (tumor, lymph nodes, metastasis), was introduced. This was defined by primary tumor site and size, presence, or absence of tumor invasiveness of surrounding structures, involvement of regional lymph nodes, and metastases (Table 4.2). This was used to separate patients into prognostically differing groups, to clarify their likelihood of survival and to add another way to differentiate among good-risk, intermediate-risk, and poor-risk patients. Clinical Grouping was used to define RT doses, and the Presurgical Staging System was used to select chemotherapy alternatives according to likelihood of survival, greatest for Stage 1 patients and least for Stage 4 patients. The best-outlook patients (Stage 1) had localized disease with or without regional lymph node involvement, while others with localized disease had small primary sites with a less favorable outlook without regional lymph node disease (Stage 2) or with larger tumors ≥5 cm in diameter (Stage 3), with or without regional lymph node disease, either clinically or proven pathologically. Stage 4 patients had one or more distant metastases at diagnosis.

This protocol also had two randomized cohorts. The first was a comparison of conventionally delivered RT, 50.4 Gray (Gy) given in 1.8 Gy fractions vs. 59.4 Gy given twice daily, 6–8 h apart, to patients with Group III disease. This was

Table 4.2 Rhabdomyosarcoma staging system

Stage	Sites	T	Size	N	M
1	Favorable	T1 or T2	Any	N0 or N1 or Nx	M0
2	Unfavorable	T1 or T2	≤5 cm	N0 or Nx	M0
3	Unfavorable	T1 or T2	≤5 cm >5 cm	N1 No or N1 or Nx	M0
4	Unfavorable	T1 or T2	Any	N0 or N1	M1

T1 confined to anatomic site of origin, *T2* extension and/or fixed to surrounding tissue, *N0* regional nodes not clinically involved, *N1* regional nodes clinically involved by neoplasm, *Nx* clinical status of regional nodes unknown (especially sites that preclude lymph node evaluation)

preceded by an IRS-IV pilot study (Donaldson et al. 1995), which showed that it was feasible to deliver the hyperfractionated RT to similar patients along with chemotherapy. The second was a three-way randomization for eligible Group III patients for chemotherapy with VAC, VAI (I, ifosfamide), or VIE (E, etoposide). Granulocyte colony-stimulating factor (GCSF) was used to ameliorate dose-limiting neutropenia.

Results. 883 eligible patients were enrolled. There was no significant difference in outcome of 490 Group III patients who received conventional vs. hyperfractionated RT (Donaldson et al. 2001). VAC vs. VAI vs. VIE likewise led to 3-year failure-free survival (FFS) rates of 75–77% (Crist et al. 2001). Thus VAC + conventional RT remained the gold standard for patients with Group III, as less toxic and less expensive (Raney et al. 1994; Heyn et al. 1994). Overall, FFS at 3 years was 77% on IRS-IV and 76% on IRS-III, and no significant differences were seen in patients with ARMS or UDS; 3-year FFS rates were 64% in IRS-IV and 70% in IRS-III. However, FFS rates were improved for patients with ERMS compared to those of similar patients treated on IRS-III: 3-year FFS rates were 83% on IRS-IV vs. 74% on IRS-III. The improvement was restricted to patients with Stage 2 or Stage 2/3, Group I or II ERMS; 3-year FFS rates were 93% on IRS-IV vs. 76% on IRS-III (Crist et al. 2001; Baker et al. 2000). Patients with orbital and bladder/prostate tumors fared similarly as their counterparts on IRS-III. However, in IRS-IV patients with paratesticular RMS compared to IRS-III, there was a decrease in FFS at 3 years, 81% on IRS-IV vs. 95% on IRS-III, primarily due to lack of requirement for retroperitoneal lymph node sampling on IRS-IV, affecting boys aged ≥10 years. Fortunately, 3-year survival was not significantly compromised, 92% in IRS-IV vs. 96% in IRS-III. A requirement for retroperitoneal node sampling was reinstituted for subsequent patients on IRS-V.

Meanwhile, 152 eligible IRS-IV Pilot patients (1988–1991) with metastases at diagnosis (Group IV/Stage 4) received a Phase II "up-front window" combination of ifosfamide + doxorubicin (ID) for 6–12 weeks followed by VAC and RT, to assess their efficacy. The 5-year survival rate was 34% (Sandler et al. 2001). Subsequently, IRS-IV (1991–2005) entered 128 eligible patients on a randomized Phase II upfront window trial of vincristine + melphalan (VM) vs. ifosfamide + etoposide (IE), also given over 6–12 weeks, followed by VAC and RT (Breitfeld et al. 2001). Three-year survival rates were 27% for VM and 55% for IE. The inferior outcome for those receiving VM was attributed to excess marrow toxicity from the melphalan, which limited the ability to give adequate doses of subsequent chemotherapy. A subsequent multivariate analysis identified two prognostic factors associated with improved 3-year survival: ERMS and 2 or less metastatic sites. The patients with both these characteristics had a 3-year survival rate of 47% (Breneman et al. 2003).

Summary. The IRS Group's protocols were the first series of studies to set the stage for future studies in young patients with rhabdomyosarcoma. In the 1960s, cure of such patients was unusual. A review of 48 patients from the Memorial-Sloan-Kettering Institute found that only 9 of the patients (19%) were alive at the time of publication (Lawrence et al. 1964). In the 1990s, the 5-year survival rate was approximately 65–70%. The IRS clearly showed that VAC chemotherapy was the "gold standard," but other agents such as doxorubicin, etoposide, ifosfamide, irinotecan, and topotecan also had activity and were explored later. Sites of tumor origin and spread, extent of tumor removal, and histology were critical for assessing risk and designing proper chemotherapeutic approaches. Local tumor control with surgery and/or radiation therapy were required for long-term survival. A very important discovery was the lethality of Group III cranial parameningeal rhabdomyosarcoma. Once identified and treated, the majority of these patients could be cured (Raney Jr et al. 1987).

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Treatment of Rhabdomyosarcoma

5

Carola A. S. Arndt, Ewa Koscielniak, and Gianni Bisogno

5.1 Introduction

Soft tissue sarcoma comprises 7.4% of childhood soft tissue malignancies with rhabdomyosarcoma (RMS) being the most common soft tissue sarcoma in children. RMS can arise in virtually any site in the body. Presenting symptoms depend on the site of origin of the tumor and can range from urinary obstruction or constipation in patients with pelvic or bladder/prostate tumors to proptosis in patients with orbital RMS, sinusitis symptoms in patients with parameningeal/sinus tumors, or a painless mass in extremity tumors. In North America there are about 350 new cases of RMS in children annually, with a similar number of new cases in Europe. The staging system for rhabdomyosarcoma has already been discussed in Chap. 3 and will not be repeated here. Risk stratification has evolved over the past several decades. Meza and colleagues analyzed patient and disease characteristics of patients with nonmetastatic RMS treated on the third and fourth Intergroup Rhabdomyosarcoma Studies and identified the prognostic significance of histology (alveolar (ARMS) and embryonal RMS (ERMS)), stage, group, and primary site (Meza et al. 2006) and resulted in stratification of patients into two low risk, one intermediate risk, and one high risk group for North American studies from 1997 to 2004. In Europe ongoing protocols use IRS Group, histology, patient age, primary tumor site, and size and nodal involvement to assign patients into four groups: low risk, standard risk, high risk, and very high risk. A multivariate analysis of risk factors in 788 patients with metastatic RMS treated in nine studies in Europe and North America from 1984 to 2000 identified age under 1 year or older than 10, unfavorable site of primary tumor, presence of three or more sites of metastatic disease, and presence of bone or bone marrow involvement as being correlated with inferior event-free survival (EFS) (Oberlin et al. 2008).Risk group assignment for therapy differs between European and North American trials, so comparison of outcomes for clinical trials needs to take this into consideration. For example, in the European trials, positive lymph nodes in patients with unfavorable histology and Group III tumors result in assignment of patients to very high-risk therapy,

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whereas they are treated as intermediate risk on Children's Oncology Group (COG) trials. Many other COG "intermediate-risk" patients on the "D" series of studies were considered in European Pediatric Soft Tissue Sarcoma Group (EpSSG) or Cooperative Weichteilsarkom Studie (CWS) to be "high risk" (Sultan and Ferrari 2010).

Recently, study classification is moving away from histologic designation as embryonal RMS (ERMS) or alveolar RMS (ARMS) and more toward molecular classification using presence or absence of *PAX7-FOXO1 or PAX3-FOXO1* or variant fusion to risk assign patients to protocol therapy. Almost all ERMS lack this fusion (Parham and Barr 2013). Studies on the prognostic importance of the presence of this fusion have been conflicting. Williamson and colleagues found an inferior outcome in patients with presence of the fusion compared to those with absence of the fusion, whereas Stegmaier found no association between fusion status and outcome in patients with ARMS (Williamson et al. 2010; Stegmaier et al. 2011), although the definition of ARMS differed between the CWS and COG. However, a recent analysis of low and intermediate risk patients treated on a series of Children's Oncology Group trials confirmed the prognostic importance of fusion status (Arnold et al. 2016; Skapek et al. 2013). A subsequent analysis of 1727 patients treated on COG trials from 1997–2013 utilizing survival tree regression analysis for EFS demonstrated FOXO1 status to be the most important prognostic factor in patients with RMS and improved risk stratification of patients with localized RMS (Hibbitts et al. 2019). The study incorporated the following prognostic factors in an analysis: age at diagnosis (categorized based on previous studies into <1, 1–9, 10+ years), sex, primary site (favorable (orbit, head, and neck (excluding parameningeal), genitourinary (excluding bladder/prostate), and biliary tract/liver) versus unfavorable (bladder/prostrate, extremity, cranial parameningeal, others (includes trunk, retroperitoneum, pelvis, perineal/perianal, intrathoracic, gastrointestinal)), tumor size (tumor >5 cm), histology (ARMS, ERMS), FOXO1 fusion status (positive, negative), clinical group (I, II, III, IV), nodal status (N0, nodal involvement absent; N1, nodal involvement present), number of metastasis, and presence or absence of metastasis in certain specific sites. FOXO1 status and not histology was found to be the most important factor in patients with nonmetastatic disease. Other molecular risk factors such as amplification of CDK4, mycN, and myoD1 or presence of TP53 mutation are also being explored to be used in risk stratification (Agaram et al. 2019; Kohsaka et al. 2014; Rekhi et al. 2016) in future studies.

5.2 Initial Evaluation

Initial staging and evaluation of a child with rhabdomyosarcoma has been extensively discussed in Chap. 3 and will only be briefly discussed here. Following diagnosis, the patient should be evaluated for presence of regional as well as distant spread of disease, with MRI of primary site, chest computed tomography, PET scan, and bilateral bone marrow aspirate and biopsy. A recent analysis of patient data from North American Cooperative Groups (IRSG and COG) suggested that certain subgroups of patients can have limited staging evaluation. For example, in patients without regional node involvement (N0) and without local tumor invasiveness (T1), metastatic workup can be omitted. Patients without regional lymph node involvement but locally invasive (T2) but favorable histology and molecular pathology with a negative chest CT scan can omit further evaluation with bone scan or bone marrow evaluations (Weiss et al. 2013). Certain sites require lymph node sampling. For example, in boys 10 years and older with paratesticular RMS, the North American approach has been to evaluate retroperitoneal lymph nodes via node sampling, whereas the European approach has been to avoid routine sampling, opting instead for more intense chemotherapy. Tumor should be evaluated for presence or absence of FOXO1 fusion, since this is used by most groups in risk stratification. Other fusions are also being investigated for use in risk assignment.

5.3 Treatment Assignment

Once initial staging studies have been completed, patients are assigned a pretreatment group, stage, and risk group. The staging and grouping definitions have been described in Chap. 5.

The initial surgical approach should not attempt to resect tumor completely unless it is possible to obtain a resection with negative margins without mutilating surgery or surgery that would result in loss of organ function.

5.4 Treatment

Chemotherapy for RMS in North America has relied on variations of a backbone of vincristine, actinomycin, and cyclophosphamide for the past several decades, whereas in Europe, once ifosfamide was identified as an active agent in adult soft tissue sarcomas and initial data from CWS studies demonstrated a higher response rate compared to cyclophosphamide containing regimens, vincristine, actinomycin, and ifosfamide (IVA) was adopted as the standard backbone (Koscielniak et al. 1999).

5.4.1 North American Perspective

Chapter 4 has discussed the historical aspects of therapy from the First North American Intergroup Study through the Fourth Intergroup Rhabdomyosarcoma Study (IRS IV). The remainder of this chapter will focus on studies since then and contemporaneous European studies.

The "D" series of studies (1997–2004) in the Children's Oncology Group assigned patients to risk groups as determined by analysis of risk factors on IRS III and IV (Meza et al. 2006). The low risk protocol, D9602, treated patients with ERMS stage 1, Group I and IIA, and Group III orbit, as well as stage 2.

Group I (subgroup A) was treated with VA chemotherapy for 45 weeks, plus RT for patients with residual tumor; patients with ERMS with stage 1 Group III nonorbital tumors, Groups IIB/C, stage 2 Group II, and stage 3 Group I/II (subgroup B) received VAC for 45 weeks, plus XRT for patients with residual tumor; and selected groups of patients were given XRT doses 5–10 Gy lower than in IRS-III/IV. Estimated 5-year failure-free survival (FFS) rates were 89% for subgroup A and 85% for subgroup B. Five-year FFS and overall survival (OS) rates were similar to those observed in comparable IRS-III patients, including patients receiving reduced RT doses but were lower than in comparable IRS-IV patients receiving VAC, largely driven by subgroup A patients not receiving cyclophosphamide. Five-year FFS rates were similar among subgroups A and B patients (Raney et al. 2011).

Intermediate-risk patients were treated on D9803, which compared vincristine, actinomycin, and cyclophosphamide (VAC, cyclophosphamide dose 2.2 g.m^2) with VAC alternating with vincristine, topotecan, and cyclophosphamide (VTC, cyclophosphamide dose 1.25 g/m^2 when given with topotecan), and found no difference in outcome. 4-year FFS was 73% with VAC and 68% with VAC/VTC.

For high-risk patients, D9802 consisted of two consecutive phase 2 window studies with irinotecan alone or combined with vincristine for patients with metastatic rhabdomyosarcoma (except those under 10 years of age with ERMS who were treated on D9803), followed by VAC. Although the outcome at 2 years revealed a poor FFS of only 26%, the combination of vincristine and irinotecan was found to be highly active with a partial and complete response rate of 42%, leading to its inclusion in subsequent studies (Pappo et al. 2007).

ARST0331 (2004–2011) for low-risk patients successfully reduced the length of therapy for a subset of low-risk patients (stage 1/2 Group I/II ERMS, stage 1 Group III orbit). This regimen utilized four cycles of vincristine, actinomycin, and cyclophosphamide (VAc, dose of cyclophosphamide 1.2 g/m^2 per cycle) followed by eight cycles or VA along with radiation therapy in patients with Group II tumors, with a 3-year FFS of 89% and survival of 98% (Walterhouse et al. 2014). This approach can be considered effective therapy for this subgroup of low-risk patients.

Patients with stage 1 Group III nonorbit or stage 3 Group I/II ERMS were given an additional 12 cycles of VA on this study. Unfortunately, those patients had an outcome inferior to patients treated with much higher cumulative doses of cyclophosphamide on D9602 (3-year FFS of 70% on ARST0331 vs 83% on D9602). The decrement in outcome on ARST0331 in this subgroup of patients was partially due to the inferior local control rate in girls with vaginal rhabdomyosarcoma who had radiation eliminated and received significantly lower doses of cyclophosphamide (Walterhouse et al. 2011, 2017).

The intermediate-risk patients were treated on ARST0531 which compared VAc (cyclophosphamide dose 1.2 g/m2) with VAc alternating with vincristine/irinotecan (VI), based on the activity of VI noted in D9802. There was no difference in outcome in the two regimens, with 4-year FFS being 62% in both arms (Hawkins et al. 2018). However, local failure rates for patients with Group III ERMS on ARST0531 were higher than in similar patients on D9803 (27.9% vs 19.4%). Even after exclusion of patients under 2 years of age who did not receive radiation, local failure remained significantly increased on ARST0531 (Casey et al. 2019). This was particularly notable in patients who had tumors greater than 5 cm.

The high-risk patients with metastatic disease were treated with a complex intensive multiagent regimen including vincristine, doxorubicin, cyclophosphamide, ifosfamide, etoposide, actinomycin, and irinotecan (ARST0431) which failed to improve the historically poor outcome of these patients in all subgroups except those with one or no "Oberlin risk factors," where the 3-year event-free survival was 69% (Weigel et al. 2016).

The current open study in the Children's Oncology Group (COG), ARST1431, is evaluating the addition (in a randomized fashion) of m-TOR inhibition with temsirolimus to VAc/VI therapy, along with addition of 24 weeks of maintenance therapy with vinorelbine and oral low dose daily cyclophosphamide in all patients. The addition of maintenance chemotherapy was based on the results of EpSSG 2005 (discussed later). The study was modified from its original design (which did not include maintenance) based on the EpSSG results as well as increased local failure on ARST0531 compared to D9803, which may have been due in part to a lower cumulative dose of cyclophosphamide on ARST0531, although the reasons are not entirely clear (Casey et al. 2019; Bisogno et al. 2019). The selection of temsirolimus as an agent worthy of further investigation is based on evidence that mTOR inhibition plays a role in several tumor-promoting intracellular signaling pathways. Regulation of the mTOR pathway is highly complex and is mediated through a series of interactions linking growth factor receptor signaling and other cell stimuli as well as phosphatidylinositol 3-kinase and the Akt/protein kinase B pathway activation. This process leads to production of hypoxia inducible factor-1α that regulates transcription of genes that stimulate cell growth and angiogenesis including vascular endothelial growth factor (VEGF). Other effects of mTOR activation include stimulation of increased mRNA translation that encodes cell cycle regulators such as c-myc, cyclin D1, and ornithine decarboxylase resulting in increased cell division and survival (Rini 2008). Thus, targeting the mTOR pathway is a relevant strategy to inhibit cancer. Preclinical data in cell lines showed growth inhibition of RMS xenografts by temsirolimus, and the Pediatric Preclinical Testing Program (PPTP) reported on single agent activity of sirolimus against its models, in addition to synergy of mTOR inhibition in combination with cyclophosphamide, vincristine, or irinotecan (Wan et al. 2006; Houghton et al. 2008). ARST0921, which was a randomized phase 2 selection design study in relapsed RMS that compared bevacizumab (BEV) to temsirolimus, both administered in combination with cyclophosphamide and vinorelbine demonstrated that the temsirolimus arm had a superior 6-month EFS (65%) compared to the bevacizumab arm (50%). The complete and partial response rate on the temsirolimus arm was 46% compared with 28% on the bevacizumab arm ($p = 0.12$) (Mascarenhas et al. 2019). The results of this study, along with the preclinical data above, provided the rationale for including temsirolimus in the COG intermediate-

risk RMS study. In this study FOXO1 status is also used to assign patients to therapy in certain categories.

5.4.2 European Perspective

In Europe, three independent cooperative groups have coordinated pediatric soft tissue sarcoma studies, i.e., the International Society of Paediatric Oncology—Malignant Mesenchymal Tumor Committee (SIOPMMT), the Soft Tissue Sarcoma Cooperative Group (Cooperative Weichteilsarkom Studie (CWS) participating countries, Germany, Switzerland, Austria, Sweden, Poland, and Finland), and the Italian Soft Tissue Sarcoma Committee (STSC) affiliated to the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP). In 2004 the SIOP-MMT and the AIEOP STSC groups joined to form the European Paediatric Soft Tissue Sarcoma Study Group (EpSSG), initially also including the CWS. Although there are several similarities between North American and European strategies, different approaches were attempted in Europe in particular to minimize the aggressiveness of local treatment and therefore late sequelae and to explore the role of chemotherapy intensification in poor-risk patients. This review only focusses on the more recent studies conducted by these groups since the late 1990s. Prior studies are well summarized in a recent comprehensive review (Arndt et al. 2018).

The SIOP MMT promoted four consecutive multicenter multinational trials dedicated to patients with localized RMS. These studies were different from those conducted by other cooperative groups in the approach to local therapy. SIOP MMT systematically tried to avoid radiotherapy and mutilating surgery in patients with complete tumor regression after chemotherapy and limited surgery. This translated into a rate of local relapse generally higher in comparison with the results obtained by other cooperative groups, but the overall survival was in the end similar. This demonstrated that radiotherapy could be avoided in a selected group of patients and that children may have a second chance of cure if treated less aggressively in first line (Stevens 2005). The attempt to intensify treatment using most of the drugs active against RMS was investigated in the MMT95 trial where the IVA regimen was compared with the six drugs CEVAIE (carboplatin, epirubicin, vincristine, actinomycin, ifosfamide, and etoposide) regimen. No survival improvement was achieved, and IVA regimen remained the standard combination in Europe (Oberlin et al. 2012). This result was confirmed by the parallel CWS/STSC study that was not able to demonstrate that CEVAIE was superior to the VAIA regimen (Treuner et al. 2003).

CWS-2002P was conducted as a pilot study (P) as at that time EpSSG had planned a RMS 2005 study in which CWS (member of the EpSSG 2003–2004) had considered to participate. The trial was for localized RMS and further modified the stratification system to include RMS type, IRS group, site, size, age, and node status. Low-risk patients were treated with VA, standard-risk patients with IVA, and high-risk patients with VAIA. Surgery and/or radiation were utilized for local tumor control, depending on histology, response to therapy, and resectability. Optional maintenance chemotherapy (nonrandomized) with vinblastine and oral cyclophosphamide was included for high-risk patients who were in CR at the end of intensive therapy. With a median follow-up 4.7 years, the 5-year EFS (OS) rates were 100% (100%), 78% (92%), and 70% (79%) for low-, standard-, and high-risk patients, respectively (Koscielniak et al. 2013). The 5-year EFS for high-risk patients treated additionally with maintenance therapy was 70% and for patients who did not receive maintenance therapy 61%. (n.s) CWS 2007 HR study is also evaluating the addition of oral maintenance chemotherapy with trofosfamide, idarubicin, and etoposide to patients with RMS at high risk of recurrence https://clinicaltrials.gov/ct2/show/NCT00876031 and https://www.kinderkrebsinfo.de/health_professionals/clinical_trials/pohkinderkrebsinfotherapiestudien/cws_2007_hr/index_eng.html. The randomization is currently closed and patients are in follow-up.

In 2009, the CWS also created a registry for soft tissue sarcoma and other soft tissue tumors in children, adolescents, and young adults: CWS-SoTiSaR (Registry for **So**ft **Ti**ssue **Sar**coma and other soft tissue tumors in children, adolescents, and young adults). This registry has a number of aims, with the primary aim being to prospectively register all newly diagnosed patients (children, adolescents, and young adults) with soft tissue tumors. Information collected includes incidence of different types of soft tissue tumors, treatment, and outcome in order to determine whether a relationship exists between outcomes and specific interventions. Other goals include assessing the quality of treatment by the means of data collection, data check, and an advisory service provided by the registry and the CWS reference centers, creating a database for the reassessment of the present therapy stratification system and finding new risk factors by linkage of biological studies to long-term outcome, as well as identifying sarcoma specific surrogate endpoints, providing a basis for innovative clinical phase I, II, and III trials. This registry provides a clinical data basis for a sarcoma tumor and tissue repository and facilitates the conduct of other clinical and laboratory-based sarcoma research. SoTiSaR is a base for selecting patients with STS for the INFORM precision medicine study, a nationwide German program for children with high-risk (relapsed/refractory) malignancies which aims to identify therapeutic targets on the individual basis (Worst et al. 2016). 95% of new diagnosed children and adolescents <18 years of age with STS in Germany (according to the Deutsches Kinderkrebsregister (http://www.kinderkrebsregister.de/dkkr/ueber-uns/uebersicht.html)) have been registered in the CWS SoTiSaR.

CWS has also developed a "CWS guidance" document to provide therapy guidance for risk-adapted treatment of soft tissue sarcomas and soft tissue tumors in children, adolescents, and young adults (Cooperative Weichteilsarkom Studiengruppe (CWS) of the Gesellschaft für Pädiatrische Onkologie und Hämatologie (GPOH) 2014). The objective of the project is to improve the quality of care for children, adolescents, and young adults with STS and soft tissue tumors by providing standard recommendations for treatment including diagnostic procedures, pathological and biological investigations, chemotherapy, surgery, and radiotherapy, and for follow-up procedures (disease-free survival and late effects). Almost all patients registered in the CWS SoTiSaR are treated according to the recommendation in the CWS Guidance.

In Europe the IRS grouping system was used in the initial protocols, but since 1995 a more sophisticated stratification system was adopted taking into account also histology, patient age, primary tumor site, and size and nodal involvement. More recently the European Paediatric Soft Tissue Sarcoma Study Group (EpSSG) defined four risk groups: low risk, standard risk, high risk and very high risk to assign the treatment to patients with nonmetastatic RMS (Fig. 5.1). Therefore, although the COG intermediate-risk and the EpSSG high-risk population broadly overlap, the definition of each risk group has distinctly different meanings when used in North American and European studies.

The European Pediatric Soft Tissue Sarcoma Group trial EpSSG RMS2005 included prospective studies for patients with localized RMS included in the low-, standard-, and very high-risk groups and two randomized trials for high-risk patients. The randomized trials evaluated the addition of doxorubicin to IVA therapy as well as the role of maintenance chemotherapy. The addition of dose-intensified doxorubicin to standard IVA chemotherapy did not result in improved outcome in high-risk nonmetastatic patients and was associated with more toxicity than the standard IVA regimen alone. The 3-year event-free survival was 67.5% (95% CI 61.2–73.1) in the IVA plus doxorubicin group and 63.3% (56.8–69.0) in the IVA group (hazard ratio 0.87, 95% CI 0.65–1.16; $p = 0.33$) (Bisogno et al. 2018). In the same study, the addition of maintenance chemotherapy with intravenous vinorelbine and daily oral low-dose cyclophosphamide did improve the survival of nonmetastatic patients at high risk of relapse who were in complete remission at the end of standard chemotherapy (Bisogno et al. 2019). 5-year disease-free survival was 77.6% (95% CI 70.6–83.2) with maintenance

Risk Group	Subgroups	Pathology	Post surgical Stage (IRS Group)	Site	Node Stage	Size & Age
Low Risk	**A**	Favourable	I	Any	N0	Favourable
Standard Risk	***B***	Favourable	I	Any	N0	Unfavourable
	C	Favourable	II, III	Favourable	N0	Any
	D	Favourable	II, III	Unfavourable	N0	Favourable
High Risk	***E***	Favourable	II, III	Unfavourable	N0	Unfavourable
	F	Favourable	II, III	Any	N1	Any
	G	Unfavourable	I, II, III	Any	N0	Any
Very High Risk	***H***	Unfavourable	I, II, III	Any	N1	Any

- **Pathology:**

Favourable = all embryonal, spindle cells, botryoid RMS
Unfavourable = all alveolar RMS (including the solid-alveolar variant)

- **Post surgical stage** (according to the IRS grouping, see appendix A.2):

Group I = primary complete resection (R0);
Group II = microscopic residual (R1) or primary complete resection but N1;
Group III = macroscopic residual (R2);

- **Site:**

Favourable = orbit, GU non bladder prostate (i.e. paratesticular and vagina/uterus) and non PM head & neck
Unfavourable = all other sites (parameningeal, extremities, GU bladder-prostate and "other site")

- **Node stage** (According to the TNM classification, see appendix A1 and A.5):

N0 = no clinical or pathological node involvement
N1 = clinical or pathological nodal involvement

- **Size & Age:**

Favourable = Tumour size (maximum dimension) ≤5cm *and* Age <10 years
Unfavourable = all others (i.e. Size >5 cm *or* Age ≥10 years)

Fig. 5.1 EpSSG risk stratification for nonmetastatic rhabdomyosarcoma

chemotherapy versus 69.8% (62.2–76.2) without maintenance chemotherapy (hazard ratio [HR]0.68 [95% CI 0.45–1.02]; $p = 0.061$), and 5-year overall survival was 86.5% (95% CI 80.2–90.9) with maintenance chemotherapy versus 73.7% (65.8–80.1) without (HR 0.52 [95% CI 0.32–0.86]; $p = 0.0097$). It is important to again note that the risk group stratification differs between European and North American cooperative trials. Most patients considered high risk in Europe are considered intermediate risk in North America, so any comparison of studies needs to take that into consideration.

Recently EpSSG completed its first randomized study on relapsed patients demonstrating that the VIT (vincristine, irinotecan plus temozolomide) regimen achieved significantly better PFS (adjusted hazard ratio (HR) = 0.65, 95% CI,

0.43–0.97, $p = 0.036$) and OS (HR = 0.53, 95% CI, 0.33–0.83, $p = 0.005$) compared to vincristine and irinotecan alone (Defachelles et al. 2019).

In a pooled analysis of 788 patients with metastatic RMS from Europe and North America, Oberlin and colleagues identified four factors significantly and adversely associated with EFS: age younger than 1 year or at least 10 years, unfavorable site of primary tumor, bone or bone marrow involvement, and three or more metastatic sites. Multiple approaches have been taken to improve the outcome of patients with metastatic RMS, especially those with a high number of Oberlin risk factors. EpSSG investigated the use of bevacizumab in a randomized phase II study of multimodality chemotherapy in children with metastatic rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue sarcoma and did not find improved EFS with the use of bevacizumab (Chisholm et al. 2017). Unfortunately, neither high-dose chemotherapy with stem cell rescue nor aggressive-dose intense chemotherapy have been found to improve the outcome of patients with the highest-risk metastatic RMS, and their prognosis remains dismal (Chisholm et al. 2017; Carli et al. 2004; Bisogno et al. 2009; McDowell et al. 2010; Weigel et al. 2016). However, in a nonrandomized CWS-96 study, addition of oral maintenance therapy with trofosfamide, idarubicin, and etoposide in patients with metastatic rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue sarcoma, but without bone or marrow metastases, seemed to hold promise for this subgroup of patients (Klingebiel et al. 2008). The use of maintenance chemotherapy in patients with metastatic RMS is also being investigated in a prospective EpSSG study.

EpSSG is also planning a new overarching study for children and adults with frontline and relapsed rhabdomyosarcoma (FarRMS). It is a multi-arm, multi-stage study with three principal aims. These are to evaluate (1) systemic therapy through the introduction of new agent regimens in the most advanced disease states (very high risk (VHR), high risk (HR), and relapse), (2) the duration of maintenance therapy, and (3) radiotherapy to improve local control in VHR, HR, and standard risk (SR) patients and to treat metastatic disease. In addition the study will evaluate risk stratification through the use of *PAX-FOXO1* fusion gene status instead of histological subtyping and the use of FDG PET-CT response assessment as a prognostic biomarker for outcome following induction chemotherapy.

5.5 Future Directions

As already noted, risk stratification for RMS differs between North American and European trials. However the ultimate goal is to provide comprehensive molecularly based risk stratification that goes beyond FOXO1 fusion status. Whittle and colleagues have recently described a very favorable outcome in congenital spindle cell RMS associated with VGLL2 or NCOA2 fusion (Whittle et al. 2019). This is in contrast to the very poor outcome of spindle cell RMS associated with MYOD-1 mutations in older children and young adults with head and neck RMS (Agaram et al. 2014, 2019; Owosho et al. 2016; Rekhi et al. 2016; Szuhai et al. 2014). MYCN and CDK4 amplification have also been found to be unfavorable prognostic markers in fusion positive RMS (Shern et al. 2018). Recurrent alterations in genes including NRAS, KRAS, HRAS, FGFR4, PIK3CA, CTNNB1, FBXW7, and BCOR as well as alterations in the receptor tyrosine kinase/RAS/PIK3CA axis have been found and may provide opportunities for molecularly guided interventions and risk stratifications (Shern et al. 2014).

Recently, a series of workshops have pooled data, and an ongoing effort to develop a Pediatric Cancer Data Commons through creation of an International Soft Tissue Sarcoma Consortium https://commons.cri.uchicago.edu/instruct/ is underway. Through collaboration across continents, it is hoped that more can be learned about this rare group of tumors.

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6 Current Approaches to Therapy: Soft Tissue Sarcomas Other than Rhabdomyosarcoma in Children and Adolescents

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6.1 Introduction

Sarcomas in children and adolescents under 18 years of age are rare diseases and include various different histiotypes that include soft tissue sarcomas of "pediatric-type" (i.e., rhabdomyosarcoma), "adult-type" (i.e., liposarcoma and leiomyosarcoma tumor), or special entities (infantile fibrosarcoma, desmoid tumor, etc.). The group of "non-rhabdomyosarcoma soft tissue sarcomas" (NRSTS) includes all soft tissue sarcomas, except rhabdomyosarcoma and Ewing sarcoma, occurring during childhood and adolescence (Ferrari and Casanova 2005). The incidence varies with age, but NRSTS are more frequent during adolescence and in early adulthood (Fig. 6.1). Because treatment decisions are complex, management of these sarcomas in patients under 18 years is best overseen by a multidisciplinary team, preferably starting before any biopsy. The onset of these sarcomas in growing patients makes the local treatment and subsequent reconstruction more complex, requiring broad expertise in pediatrics, oncology, surgery, radiotherapy, and psychosocial disciplines. Sensitivity to medical therapies depends on the type of disease but must also be adapted to the age of the patient, the tumor extent, and the potential resectability of the primary tumor. The evolution of the treatment philosophy has historically differed between pediatric oncology where primary chemotherapy is frequently used prior to surgery and medical oncology where local treatment is often the first step in the therapeutic strategy. However, collaboration between pediatric and medical oncology is increasingly frequent, particularly in sarcomas occurring in adolescents and young adults who may be treated by either pediatric or medical oncologists (van der Graaf et al. 2017). International collaboration in studying pediatric soft tissue sarcomas, as well as engagement of both pediatric and medical oncologists, has facilitated our understanding of the clinical and therapeutic issues and optimized the care of these patients. The survival of most of these sarcomas is good, although lower in adolescents than in younger patients and in certain histologies and in metastatic presentations that are difficult to cure with current treatments. In these cases, patients should be included in biology-driven protocols as much as possible.

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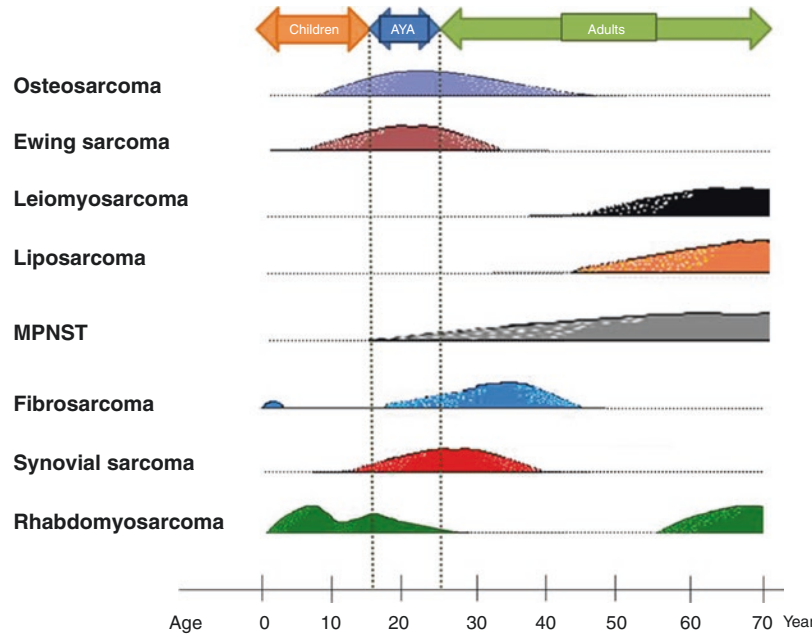

Fig. 6.1 Age distribution of the most frequent soft part sarcomas. *AYA* adolescents and young adults, *MPNST* malignant peripheral nerve shealth tumor

6.2 Local Therapy

6.2.1 Surgery

Surgery is the mainstay of therapy in NRSTS. The quality of the surgical operation is so important that children and adolescents with deep, large (>3–5 cm) soft tissue lesions suspected of being sarcomas should be referred to centers of excellence, preferably before undergoing biopsy. Surgery aims to obtain negative margins with no or minimal long-term sequelae. A resection with negative margin is defined as complete tumor removal with a surrounding rim of normal tissue, with no macroscopic tumor visible and no microscopic tumor cells present at the edge of the resected specimen. The crucial issue is the quality and quantity of the resected tissue surrounding the tumor mass or in other words the definition of the "safety distance" between the tumor and the resection margins. As for adult STS, the surgical guidelines for NRSTS should be based on the concept of compartmentalization in the extremities as popularized by Enneking (Enneking et al. 2003). Local management of extremity STS historically necessitated entire "compartmental resection" (en bloc removal of the tumor with the entire anatomical compartment, covered by intact deep fascia). With better local imaging which improves the definition of local tumor extent and with the introduction of combined multimodal treatment, more conservative and function-preserving surgery has been utilized. Compartmentalization is based on fascial boundaries, which generally act as barriers to tumor growth: sarcoma originating within a muscular compartment may grow longitudinally along fascial planes but typically cannot grow in a radial pattern beyond the fascia which acts as a barrier to local growth. Though fascial compartments do not exist in some anatomic sites, the concept of fascia and compartments has been traditionally utilized to define different types of resection. The so-called "wide" resection according to Enneking is a resection with either an intact fascial boundary (even within a few millimeters of the tumor) or an adequate surrounding layer of tissues indicating that the tumor was removed with an intact pseudocapsule (the first surrounding tissues compressed by the tumor), reactive zone (both the pseudocapsule and the reactive zone may contain microscopic disease),

and some additional normal tissue. A metric definition of this "additional normal tissue" is somewhat arbitrary and challenging: 1–2 cm of healthy tissue (when the tissue is a muscle) around the tumor has been selected as a cut-off in some studies, but it is important to realize that the margin can be minimal (>1 mm) when the tissue is a resistant anatomical barrier, such as muscular fasciae, periosteum, and perineurium (O'Donnell et al. 2014). Since 1–2 cm margins may be difficult to achieve in small patients, some studies have utilized a 0.5 cm margin based on studies in adults suggesting that this smaller margin may be adequate. However, inadequate surgical margins adversely affect local outcome and consequently also overall survival (Gronchi et al. 2010).

In children IRSG staging (Intergroup Rhabdomyosarcoma Staging (IRS) grouping system) is frequently used, and in adults the UICC "R system" is used to define surgical margins. Surgical resection margins are currently defined as:

- R0, which corresponds to IRS I (negative margin), includes both the "compartmental" and the "wide" resection as defined by Enneking but potentially also resection with close but negative margins (associated therefore with a higher risk of local relapse).
- R1/IRS II (microscopically positive margin), the so called "marginal resection," the dissection extends into or through the reactive zone that surrounds the tumor, with microscopic tumor extension at the margin of resection (without evidence of macroscopic disease residue).
- R2/IRS III (grossly positive margin), the Enneking intralesional resection that occurs if the tumor is entered at any point during surgery or if macroscopic tumor residue is left in situ. R2 also includes patients with only a biopsy at diagnosis.

6.2.2 Radiotherapy

Radiotherapy is an important component of local tumor control for pediatric patients with NRSTS, where it has a role in facilitating tumor resection in those with unresectable tumors and preventing local tumor recurrence in those who have undergone resection. Radiotherapy produces considerable long-term toxicity including growth impairment, organ dysfunction, and secondary neoplasia and must be used cautiously in children. Conformal techniques and proton radiotherapy may spare normal tissues, although these approaches have undergone limited study in pediatric NRSTS (Krasin et al. 2010; Merchant 2009). Nevertheless, brachytherapy and proton radiotherapy may be beneficial in selected anatomic sites where sparing of radiotherapy dose to normal structures can be achieved. In considering whether or not radiotherapy should be administered, the likelihood of local tumor control must be assessed. The factors that most influence local tumor control include tumor size, tumor histologic grade, and extent of surgical resection (Spunt et al. 1999; Ferrari et al. 2005a). Patients with high-grade tumors >5 cm and those with unresectable disease are at the greatest risk for local disease progression or recurrence. Aggressive surgical resection and radiotherapy may not be warranted in the patient with widely disseminated disease who has a short life expectancy.

Rendering an unresectable tumor amenable to gross resection is among the clearest indications for radiotherapy in pediatric NRSTS since unresected disease is rarely curable. Retrospective studies suggest that neoadjuvant radiotherapy combined with chemotherapy produces a better tumor response than either modality alone, but there has been no prospective evaluation of this hypothesis (Spunt et al. 2002). A recently completed clinical trial in NRSTS patients under 30 years of age showed that combined neoadjuvant chemotherapy and radiotherapy was feasible and produced a 15% estimated 5-year cumulative incidence of local failure when combined with delayed surgery when feasible (Tinkle et al. 2017). Neoadjuvant radiotherapy alone is used infrequently in pediatric patients, although this approach has been used successfully in adults for whom chemotherapy is not warranted (Tsagozis et al. 2018).

A second indication for radiotherapy is adjuvant use for microscopic residual disease following maximal resection of high-grade NRSTS. In this setting, local tumor control can be achieved in about 85% of cases (Tinkle et al. 2017). When microscopic residual disease is anticipated after resection of a high-grade tumor, preoperative radiotherapy should be considered. Preoperative irradiation may allow a 10–15% reduction in dose, and the volume irradiated may be smaller, thereby potentially producing fewer long-term effects on normal tissues.

Recent studies suggest that radiotherapy may not be required for all widely excised high-grade NRSTS (Ferrari et al. 2017). In a retrospective series of 100 IRS group I patients for a high-grade tumor, the estimated cumulative incidence of local failure was 14% at 5 years (Ferrari et al. 2005b). It is unknown whether widely resected >5 cm, high-grade tumors require radiotherapy, although there is some retrospective evidence in adults that these patients may also be safely treated with surgery alone even though a small pediatric retrospective analysis showed a better outcome with adjuvant radiotherapy in IRS group I > 5 cm adult-type sarcomas (overall survival 90% vs. 54%; $p = 0.02$) (Ferrari et al. 2005b; Baldini et al. 1999).

Radiotherapy is rarely indicated for treatment of resected low-grade NRSTS in pediatric patients. Those with widely excised tumors have excellent local tumor control rates exceeding 95% without radiotherapy (Spunt et al. 2020). Historically, patients with marginally resected low-grade NRSTS received radiotherapy. Although a lower local failure rate might be expected with radiotherapy, it appears that about 85% of patients can be cured without radiotherapy. Given the potentially serious toxicities of radiation, it seems prudent to restrict radiotherapy for low-grade tumors to settings where a local recurrence would be highly problematic or where the tumor has recurred after surgery alone. In a recent published large COG study, patients with nonmetastatic low-grade tumors or high-grade tumors up to 5 cm who have undergone negative (R0) or microscopically positive (R1) tumor resection were treated with conservative approach of omitting adjuvant radiotherapy (Spunt et al. 2020). Despite limited retrospective data showed that omission of adjuvant radiotherapy for R1 low-grade tumors produced a local recurrence rate of only 25% without any effect on disease-specific survival (Brennan 1997), authors adopted a surgery-only strategy for patients with low-grade tumors even with R1 margins following maximal surgery, aiming to avoid the majority of these patients receiving radiotherapy while recognizing that a small subset would require further treatment for local recurrence. Overall, 205 low-risk patients were treated with exclusive surgery and reached a 5-year event-free survival of 88.9% (95% CI 84.0–93.8) and an overall survival of 96.2% (93.2–99.2). Main tumor events were isolated local recurrence or progression (15/26 cases, 58%).

Radiotherapy has a palliative role in patients with NRSTS whose disease cannot be cured. Treatment of unresectable metastases must be individualized based on the number of sites, their locations, and whether disease control at each site is likely to produce meaningful clinical benefit. Radiotherapy may be helpful in relieving focal bone pain or pain from nerve compression since small changes in tumor volume may have a significant impact on symptoms. Conformal techniques such as stereotactic radiosurgery are an ideal way to limit normal tissue damage when delivering high radiotherapy doses over a short timeframe, as in a palliative setting. Whole lung, liver, or abdominal-pelvic radiotherapy are not recommended for diffuse metastases.

6.3 Conventional Chemotherapy

The role of chemotherapy in NRSTS is still controversial. Like their adult counterparts, NRSTS are generally considered minimally sensitive to chemotherapy. However, more than 50 different subtypes of soft tissue sarcomas are indeed heterogeneous not only in their biology and clinical behavior but also in their therapeutic sensitivity, e.g., synovial sarcoma is far more sensitive to standard chemotherapy compared to more resistant subtypes like alveolar soft part sarcoma or clear cell sarcoma (Orbach et al. 2013; Reichardt et al. 2003). In addition, treatment strategies for

these tumors have changed to some degree in recent years, and multiple-modality treatments that also include chemotherapy have increasingly been attempted. In fact, though approximately 70% of patients with localized NRSTS can currently be cured, the outcome depends on various prognostic factors; prognosis may be unsatisfactory for patients with high-grade, large, invasive tumors if the treatment is limited to local therapies alone, because these neoplasms have a marked tendency to spread. It is therefore essential to identify patients who are at high risk of metastatic failure and consequently in need of systemic treatment to try and improve their outcome.

In addition, certain histological characteristics make NRSTS more likely to respond to chemotherapy, e.g., in general, high-grade sarcomas may have greater benefit from chemotherapy than low-grade tumors (Spunt et al. 2019). The chemosensitivity of synovial sarcoma is intermediate between that of typical adult sarcomas (with fewer than 40% of tumors responding to chemotherapy) and that of pediatric small round cell tumors, such as rhabdomyosarcoma (with up to 80% of responders). The intensive ifosfamide (9 g/m^2 cycle) and doxorubicin (75 mg/m^2 cycle) regimen is currently considered to be the best front-line systemic therapy for most NRSTS (Ferrari et al. 2005b).

Apart from patients with metastatic disease (for whom chemotherapy, although rarely curative, may lengthen survival and possibly quality of life), the best indication for chemotherapy may be in NRSTS patients with unresectable advanced disease (Spunt et al. 2019; Ferrari et al. 2011). Chemotherapy may achieve tumor shrinkage and facilitate complete resection, as well as helping to treat any micrometastases promptly, since these patients have a high risk of distant dissemination. A study pooling series from various international research groups on initially unresected NRSTS showed a 41% response rate (in terms of major response), but the figure rose to 57% when minor responses were included (Ferrari et al. 2011). The study reported an overall survival of 51% at 10 years, with better outcome for patients whose tumors responded to chemotherapy.

The role of adjuvant chemotherapy in preventing distant recurrences after initial surgery in NRSTS remains uncertain (Ferrari et al. 2005a). This has long been a point of controversy in clinical studies on adult soft tissue sarcomas, where trials have suffered from the heterogeneity of patients with different histiotypes and clinical factors, the relatively small sample size, and the use of different chemotherapy regimens. The historic Sarcoma Meta-analysis Collaboration (pooling together data from 14 trials of anthracycline-based adjuvant chemotherapy conducted between 1973 and 1990 showed only a small benefit of chemotherapy (Sarcoma Meta-analysis Collaboration 1997). A significant benefit for adjuvant chemotherapy was documented in the Italian randomized trial that strictly selected high-risk patients (with high-grade, large, deep-seated tumors) and delivered a regimen of full-dose ifosfamide plus anthracyclines (Frustaci et al. 2001). The contribution of pediatric oncologists to this debate has been limited. The only randomized trial of adjuvant chemotherapy in pediatric patients was conducted by the Pediatric Oncology Group (POG) (1986–1992) and failed to adequately assess the role of adjuvant chemotherapy because the majority of patients refused randomization (Pratt et al. 1999). This study demonstrated how difficult it is to conduct prospective randomized studies in pediatric patients with such rare tumors, for which no standard therapy has been established. A potential role for chemotherapy in high-risk NRSTS has been suggested by pediatric retrospective analyses (Spunt et al. 1999; Ferrari et al. 2005a, 2011).

An accurate risk-adapted stratification is essential to identify patients who are more likely to benefit from chemotherapy. In the Children's Oncology Group (COG) ARST0332 and the European Pediatric Soft tissue Sarcoma Group (EpSSG) NRSTS 2005 protocols, adjuvant ifosfamide-doxorubicin was only given to patients with initially resected high-grade and large (>5 cm) tumors.

In the recently completed Children's Oncology Group study ARST0332, patients were assigned to four treatment groups: A (surgery only), grossly excised low-grade and ≤5 cm widely

excised high-grade tumor; B (55.8 Gy radiotherapy [RT]), ≤5 cm marginally resected high-grade tumor; C (ifosfamide-doxorubicin chemotherapy + 55.8 Gy RT), >5 cm grossly resected tumor ± metastases; and D (neoadjuvant ifosfamide-doxorubicin chemotherapy and 45 Gy RT, then surgery and an RT boost based on margins), >5 cm unresected tumor ± metastases. The three risk groups defined were low (nonmetastatic R0 or R1 low-grade or ≤5 cm R1 high-grade tumor); intermediate (nonmetastatic R0 or R1 >5 cm high-grade or unresected tumor of any size or grade); or high (metastatic tumor). Risk group predicted event-free survival and overall survival ($p < 0.0001$) (Spunt et al. 2020). The most common subtype was synovial sarcoma followed by malignant peripheral nerve sheath tumor and undifferentiated sarcoma. Chemotherapy included six cycles of ifosfamide 3 g/m^2 per dose intravenously on days 1–3 and five cycles of doxorubicin 37.5 mg/m^2 per dose intravenously on days 1–2 every 3 weeks (Spunt et al. 2020). At a median follow-up of 6.5 years, 5-year event-free survival and overall survival were 88.9% (95% CI 84.0–93.8) and 96.2% (93.2–99.2) in the low-risk group. Patients in the intermediate-risk group (227 cases) had a 5-year EFS of 65.0% (95% CI 58.2–71.8) and OS of 79.2% (73.4–85.0). Metastatic recurrence or progression with or without local failure was the main tumor event (52/84 cases, 62%). In the high-risk group (80 cases), estimated 5-year EFS was 21.2% (95% CI 11.4–31.1) and OS 35.5% (23.6–47.4). Similarly, in this latter group, metastatic tumor relapse or progression was the main event (57/63, 90%). For this group, authors admitted that this treatment strategy was modestly efficacious.

6.4 Specific Therapy for Diseases

The recent development of new approaches targeted to specific molecular targets may overcome the limitations of systemic therapies in the near future, possibly identifying specific agents tailored to each histotype: imatinib for GIST and dermatofibrosarcoma and sunitinib for alveolar soft part sarcomas, for instance. While awaiting these developments, however, a more precise use of standard chemotherapy may prove important in improving the cure rate for these patients.

6.4.1 Synovial Sarcoma

Synovial sarcoma (SS) is a malignant mesenchymal tumor that occurs in both pediatric and adult patients and accounts for 8–10% of all soft tissue sarcomas (STS) in children (Fig. 6.1). SS tends to be locally invasive and has a propensity to metastasize. At diagnosis, fewer than 10% of patients present with metastases (mainly to the lung), but the disease subsequently spreads in 25–50% of cases. Lymph node metastases are rare. The biological hallmark of SS is the t(X;18)(p11.2;q11.2) chromosomal translocation which produces the SYT-SSX transcript. A 67-gene signature related to chromosome integrity and genome complexity named CINSARC (*complexity index in sarcoma*) and a genomic index (GI) analyzed using comparative genomic hybridization (CGH) have recently been developed and shown a high prognostic value in STS and in SS (Lagarde et al. 2013; Orbach et al. 2018). The molecular signatures identified (CINSARC and GI) may discriminate patients likely to benefit from chemotherapy from those for whom chemotherapy is not beneficial. The prognosis for SS patients depends on several variables and particularly on the tumor extension, the feasibility of the surgical resection, tumor size (±5 cm), and tumor site (worse prognosis for axial tumors vs. limbs).

The EpSSG NRSTS 2005 protocol included a prospective non-randomized trial of SS assessing the role of ifosfamide-doxorubicin chemotherapy in improving the response rates for patients with unresectable disease and elimination of adjuvant chemotherapy in low-risk cases. Patients were stratified by surgical stage, tumor size, nodal involvement, and tumor site as follows: (a) "low-risk," IRS group I and tumor size ≤5 cm; (b) "intermediate-risk," IRS group I, >5 cm in size, and all IRS group II; and (c) "high-risk," IRS group III tumors, nodal involvement (N1), or axial disease. "Low-risk" patients were treated

with surgery alone. "Intermediate-risk" patients received 3–6 courses of adjuvant ifosfamide-doxorubicin chemotherapy to prevent distant recurrences after initial local treatment, along with radiotherapy. "High-risk" patients had six courses of chemotherapy, delayed surgery (when feasible), and radiotherapy (local treatment was planned after three cycles of neoadjuvant chemotherapy). Neoadjuvant chemotherapy was considered the treatment of choice not only for patients with unresectable advanced disease but also whenever the surgeon was unsure whether a complete resection could be achieved at the first attempt. Ifosfamide + doxorubicin was given for a maximum of four cycles (maximum cumulative dose of doxorubicin: 300 mg/m^2). Two cycles of ifosfamide were given concomitantly with radiotherapy to patients in IRS group II, with tumors >5 cm in size, and to IRS group III patients. This trial (involving 138 patients <21 years old treated between 2005 and 2012) resulted in 5-year EFS and OS rates of 80.7% and 90.7%, respectively (Ferrari et al. 2014). In this series, 24 patients were classified as "low-risk" and were therefore treated with surgery alone; only two local relapses were observed in this cohort, with no metastatic relapses. Though the number of cases was relatively small and caution is needed, this finding suggests that adjuvant chemotherapy might be safely omitted for low-risk patients without jeopardizing their outcome (Ferrari et al. 2017).

6.4.2 Other "Adult-Type" Sarcomas (Fibrosarcoma, Liposarcoma, Mesenchymal Chondrosarcoma, PECOMA, Leiomyosarcoma, Epithelioid Sarcoma, Clear Cell Sarcoma, Angiosarcoma, Undifferentiated Sarcoma)

A formal definition of "adult-type NRSTS"—i.e., definitely malignant mesenchymal tumors, typical of adulthood, with morphological features resembling differentiated/mature tissues—was developed some years ago and has been utilized by the EpSSG to identify a more homogeneous subset of histiotypes within the large heterogeneous group of NRSTS (thus excluding from a common analysis, for instance, borderline tumors, infantile histiotypes, and small round cell tumors, which are biologically and clinically different entities that were sometimes studied together, giving rise to misleading results). However, this group still includes different entities whose biology and clinical history may be very different. For example, epithelioid sarcomas present typical features such as superficial distal location (i.e., hand, fingers), indolent growth along tendon sheaths, and a tendency for lymph node involvement (Spunt et al. 2019). Clear cell sarcoma mimics melanoma and is characterized by a prolonged clinical course with multiple local recurrences, late metastases, and a high rate of tumor deaths; chemotherapy is generally considered ineffective. Leiomyosarcomas may involve skin, superficial and deep soft tissues, and viscera and may arise as a second malignancy in patients treated with radiotherapy. Liposarcomas in children mainly occur in lower extremities, with the conventional myxoid liposarcoma being the most frequent histiotype. Undifferentiated high-grade pleomorphic sarcomas (in the past called malignant fibrous histiocytoma) are highly aggressive tumors and may be associated with a family history of cancer. The PEComa family of tumors (perivascular epithelioid cell tumors, including the classic benign angiomyolipomas, epithelioid angiomyolipomas of the kidney that may have malignant behavior, and clear cell myomelanocytic tumor that may be an aggressive disease) are generally treated with surgery alone, and neither radiotherapy nor chemotherapy is efficacious. Mesenchymal chondrosarcomas are high-grade sarcomas suggested to be closely related to Ewing sarcomas, for which multimodal regimens (as for Ewing sarcoma protocols) may be indicated. Acknowledging this heterogeneity, it is evident that the definition of "adult-type" sarcoma has been helpful for descriptive purposes, but for the future, these tumors should no longer be studied as a whole group. Rather, by concentrating separately on each histiotype, the hope is to have new targeted molecular therapies for each histology.

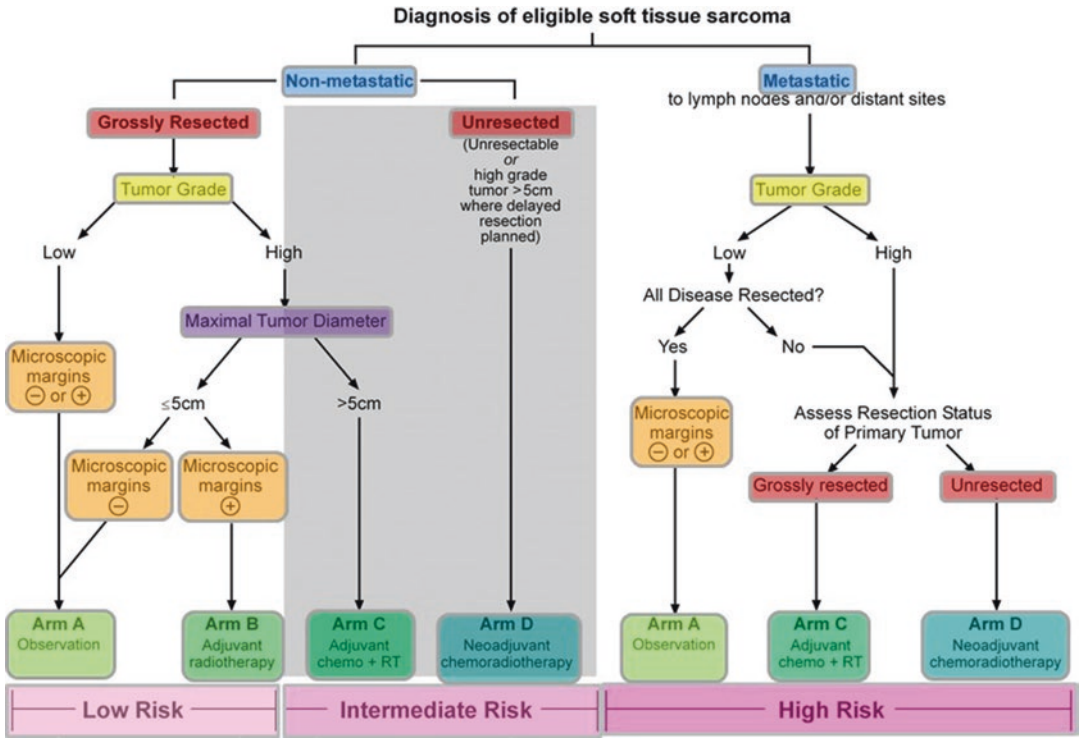

Fig. 6.2 Children's Oncology Group (ARST0332) treatment schema for adult-type soft tissue sarcomas

In addition, each of these tumors is very rare in the pediatric age group, and data on their natural history in children (and therefore on the best possible treatment) are limited. Extrapolating data from adult populations (where these tumors are more frequently observed) may be useful but must be performed cautiously, not only because the distribution of histologic subtypes differs considerably in adults and children but because certain histiotypes may behave differently in different age groups (e.g., in general, a less aggressively clinical course in children than in adults).

The COG ARST0332 study and the EpSSG NRSTS 2005 study stratified these patients according to histologic grade, size of primary tumor, extent of initial surgical resection, and presence or absence of metastatic disease. These variables have been shown to predict outcome in the few prospective and retrospective pediatric series, as well as in the large adult series (Spunt et al. 1999; Ferrari et al. 2011; Italiano et al. 2014). Surgery is the mainstay of treatment for these tumors. Radiotherapy and chemotherapy have been used in both protocols according to the risk stratification schema (Ferrari and Casanova 2005) (Fig. 6.2).

The successor study for NRSTS, ARST1321, Pazopanib Neoadjuvant Trial in Non-Rhabdomyosarcoma Soft Tissue Sarcomas (PAZNTIS), A Phase II/III Randomized Trial of Preoperative Chemoradiation or Preoperative Radiation Plus or Minus Pazopanib, was a combined effort of COG and NRG. The rationale for investigation of tyrosine kinase inhibitors (TKI) in NRSTS is based on the fact that tyrosine kinases are critical regulators of cellular growth, proliferation, and survival and TK dysregulation is felt to be a major contributor to tumorigenesis in a variety of cancer types (Krause and Van Etten 2005). Certain TKs have been found to be expressed in a range of NRSTS subtypes. Among these, vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), c-Kit, and epidermal growth factor receptor (EGFR) have been the most prevalent and dysregulated across histologic subtypes

(Tamborini et al. 2004). Tumor growth and metastatic spread are critically dependent on tumor angiogenesis. VEGF and PDGFR are two of the main receptor proteins involved in this process (Potti et al. 2004; Holtkamp et al. 2006; Park et al. 2010). Further, their elevated expression has correlated with higher malignancy grade and worse outcome (Chao et al. 2001; Yudoh et al. 2001) Preclinical research demonstrated that the effect of simultaneous inhibition of VEGF and PDGFR on tumor angiogenesis and growth is additive suggesting that concurrent targeting of multiple signaling pathways may be more effective than targeting either pathway alone (Bergers et al. 2003; Erber et al. 2004) . The multi-targeted TKI pazopanib (GW786034) is a potent inhibitor of VEGFR, PDGFR, and c-Kit (Le Tourneau et al. 2008) . While VEGFR and PDGFR are critical regulators of tumor angiogenesis, c-Kit is associated with tumor progression (Masson and Ronnstrand 2009). A number of phase I and II pediatric and adult studies demonstrated activity of pazopanib in advanced soft tissue sarcomas, leading to its selection for study in ARST1321 (Hurwitz et al. 2009; Sleijfer et al. 2009; van der Graaf et al. 2012; Glade Bender et al. 2013). Patients in the chemotherapy cohort received the standard ifosfamide-doxorubicin backbone and were randomized to receive pazopanib. Patients in the non-chemotherapy cohort underwent radiotherapy and were randomized to receive pazopanib. The primary goal, after identifying the dose of pazopanib that was feasible when given in combination with radiation or chemoradiation, was to compare the rates of near complete pathologic response (> 90% necrosis) with the addition of pazopanib to preoperative chemoradiation (or radiation alone) versus preoperative chemoradiation (or radiation alone) alone for potentially resectable >5 cm, Grade 2 or 3 intermediate to high-risk NRSTS in the phase II portion of the study. The rate of near complete pathologic response was significantly greater with the addition of pazopanib to preoperative chemoradiation in children and adults with intermediate/high-risk NRSTS (Aaron et al. 2019) (58% vs. 22% >90% necrosis). The rate of wound complications was similar to that in current and historical literature. However, a longer follow-up seems necessary to analyze if these early results translate to an improvement on patients' outcome (Aaron et al. 2019).

6.4.3 Malignant Peripheral Nerve Sheath Tumor

Malignant peripheral nerve sheath tumor (MPNST) is a spindle cell sarcoma arising from or differentiating toward peripheral nerve sheath cells. The term *MPNST* is preferred to the "synonyms" *malignant schwannoma* or *neurofibrosarcoma*. There are no histologic markers predictive of clinical behavior and tumor grade does not appear to have prognostic significance (intermediate vs. high grade), but low-grade MPNST does have a less aggressive behavior. MPNST are rare (expected incidence being around 0.1/100,000 a year) and occur mainly in adults: only 10–20% are diagnosed in the first two decades. Nevertheless, they represent one of the most frequent subtypes among pediatric NRSTS (Fig. 6.1) (Ferrari and Casanova 2005; Carli et al. 2005). The clinical behavior of MPNST is generally that of an aggressive highly malignant tumor, often arising in axial sites (trunk or, less frequently, head and neck region). Association with a sizable nerve can be identified in more than 70% of cases. In approximately 40% of patients, MPNST develops in a preexisting neurofibroma, particularly in patients with neurofibromatosis type 1 (NF-1). In about 21–67% of cases, in fact, MPNST arises in patients affected by NF-1. The lifetime risk of developing MPNST in NF-1 patients has been estimated to be 8–13%, as compared to 0.001% in the general population. The molecular mechanisms responsible for malignant transformation of neurofibromas are still unclear. NF-1 is caused by mutation in the NF-1 suppressor gene, on chromosome band 17q11. This gene encodes neurofibromin, a protein inhibiting p21-RAS. NF-1 inactivation is not sufficient for malignant transformation, and further genetic alterations are needed. Several alterations in tumor suppressor genes playing a pivotal role in

cell cycle, such as mutations of TP53 and CDKN2A (p16INK4), have been reported in neurofibromas as they transform into MPNST.

MPNST is generally characterized by uncertain prognosis, in both adults and children. A large series on pediatric MPNST from the Italian and German cooperative groups included 167 cases treated over a 25-year period using a multimodality therapeutic approach (Carli et al. 2005). That series confirmed the aggressiveness of MPNST, for which complete surgical resection is the mainstay of successful treatment. Unfortunately, achieving a complete resection at the time of initial diagnosis is rarely feasible (Carli et al. 2005; Ferrari et al. 2007). In this experience, most of the patients had a large, invasive, and unresectable tumor at diagnosis. Progression-free and overall survival at 5 years were 37% and 51%, respectively, significantly lower than that generally reported for other pediatric soft tissue sarcoma subtypes. Local progression or relapse after therapy represented the main cause of failure. Outcome was only satisfactory for the small group of resected and small tumors. Survival rates look especially poor in patients with NF-1: 5-year PFS and OS were 19% and 32% in NF-1, versus 42% and 55% in non-NF-1 cases, but this is controversial (Kolberg et al. 2013).

Among NRSTS, MPNST is generally regarded as among the least chemosensitive (Ferrari et al. 2007). The Italian and German study reported a chemotherapy response rate of 28% in terms of major responses that rose to 45% when minor responses were considered too. However, response to chemotherapy was higher when considering patients who had received regimens containing ifosfamide (65%) and when analyzing the group of non-NF-1 patients (55%, versus 18% in NF1). In both the ongoing EpSSG NRSTS 2005 and the recently completed COG ARST0332 protocols dedicated to NRSTS, localized MPNST was treated according to risk stratification based on histologic tumor grade, tumor size, initial resectability, and extent of disease (Spunt et al. 2020; van Noesel et al. 2019). Surgery was the keystone of treatment; given its local aggressiveness, for MPNST the surgical approach may be more aggressive than for other pediatric NRSTS. The need for adjuvant therapies should be decided according to the risk of local and distant recurrence. Radiotherapy may improve local control after initial marginal resection, after wide resection of large tumors, and in large and invasive unresectable tumors. In NF-1 cases, the risk of second malignancies must always be borne in mind when considering the use of radiotherapy. The role of chemotherapy remains uncertain. Ifosfamide and doxorubicin may be considered as neoadjuvant therapy in locally advanced and metastatic disease. This approach may be debatable in completely resected MPNST (regarded as one of the NRSTS least likely to benefit from chemotherapy), particularly considering the serious toxicity of chemotherapy. For the future, there is a strong need for a new therapy specific for this histiotype. In the EpSSG risk-adapted strategy, 51 patients were stratified in four groups according these risk factors (van Noesel et al. 2019). Outcome for patients with resectable MPNST was excellent, but even if response rate to ifosfamide-doxorubicin regimen was 46%, outcome for patients with initially unresectable tumor was dismal (5 year EFS 29–42%). In this experience, the presence of NF1 was confirmed to be an independent poor prognosis factor for OS and EFS.

6.4.4 Desmoid Tumor

Desmoid fibromatosis, also known as *desmoid tumor* (DT), *deep fibromatosis*, or *aggressive fibromatosis*, is a locally aggressive soft tissue lesion arising from deep fascial or soft tissue structures (musculo-aponeurotic structures) (Kasper et al. 2017). Although surgery is the traditional treatment, it now appears that repeated stimulation of connective tissue by surgery is a risk factor for tumor recurrence (Orbach et al. 2017). Furthermore, complete resection (IRS I) in DT is rare even in small tumors (≈13%) (Oudot et al. 2012; Meazza et al. 2010). Moreover, DT can stabilize or even spontaneously resolve. This observation has led to a recommendation for a period of observation for tumors that are mini-

mally symptomatic and to restrict therapy to documented progressions. For unresectable or recurrent tumors, some nonsurgical approaches have been developed with conventional chemotherapy drugs, antiestrogens, nonsteroidal anti-inflammatory drugs, and targeted therapy (Orbach et al. 2017; Meazza et al. 2010; Sparber-Sauer et al. 2018a; Ferrari et al. 2019). The respective roles of these strategies remain to be specified in adults as well as in children. Furthermore, if the tumor appears to be unresectable, surveillance first is also recommended with complementary medical treatment only in the case of progression. There is no indication for partial resection in desmoid tumors. If the tumor has nevertheless been resected, simple surveillance is recommended in all cases (after complete or incomplete resection) with delayed treatments proposed only in the case of local progression. The duration of chemotherapy is arbitrary, but it is proposed to continue treatment for at least 6–9 months after stabilization of the tumor (often 12–18 months). The choice of treatment must take into account the "benign" nature of the lesion, the child's age and gender, the potential long-term effects of different therapies, the expected benefit of these treatments (overall expected partial response of about 30–40%, stabilization in 30%), and the expected local risks in the case of tumor progression (failure 20%) (Skapek et al. 2007). The goal of systemic therapy in AF should not be only tumor shrinkage to permit a subsequent resection but rather the induction of growth arrest and tumor stabilization. Due to the absence of long-term effects, one of the first-line treatment commonly used is methotrexate-vinblastine (Orbach et al. 2017). The efficacy of treatment is assessed on surveillance examinations, which must not be performed too frequently (every 3 months). Treatment should be continued in the case of stabilization of tumor volume. Only frank progression of tumor volume (>30%) should be considered to reflect treatment failure, and another second-line treatment should be considered (Fig. 6.3). Second-line treatments that may be considered include VAC/IVA alternating with VA (vincristine, actinomycin-D, cyclophosphamide or ifosfamide), tamoxifen with a nonsteroidal anti-inflammatory (NSAID) drug such as Sulindac-Arthrocine® or Celecoxib-Celebrex® [but the overall response rate to this last association (tamoxifen + NSAIDs) is estimated at only 8% in children] (Skapek et al. 2013), or a targeted therapy (imatinib, sorafenib®) (Kasper et al. 2017; Gounder et al. 2011). Furthermore, some recent limited data showed the efficacy of hydroxyurea in DT (Ferrari et al. 2019). In the rare case of emergency (huge mesenteric primary, rapidly growing tumor in a threatening site), liposomal doxorubicin can be considered (Constantinidou et al. 2009). As DT may have a hormonal sensitivity, oral contraceptive with estrogen treatments in adolescents may be avoided.

Finally, due to the benign condition, as far as possible radiotherapy should be avoided in children due to the expected long-term effects (cosmetic, functional morbidity, second malignancy), even if this therapy is efficient in DT. The current overall EpSSG strategy in DT is summarized in Fig. 6.3. Some new drugs showed promising effect in adults with DT and are under experimentation in children (Messersmith et al. 2015).

6.4.5 Rhabdoid Tumor

Extracranial rhabdoid tumors (RT) are rare and often present in infants or children at any anatomical site as a rapidly growing mass. The vast majority contain biallelic inactivating mutation of the *SMARCB1* gene, which is part of the chromatin remodeling complex SWI/SNF, which is important in cell cycle control and functions as a classic tumor suppressor gene. The primary tumor can be found in a variety of locations including the soft tissues of the trunk, extremities, head and neck, abdomen, pelvis, and retroperitoneum, as well as in a variety of organs such as the liver, heart, kidney, and bladder. Multifocal or metastatic disease is not uncommon and should be carefully checked at diagnosis. Early progressions are common in RT even during induction therapy. In the Bourdeaut et al. series of extrarenal non-cranial RT, the median time to progression was 5 months (0–44) (Bourdeaut

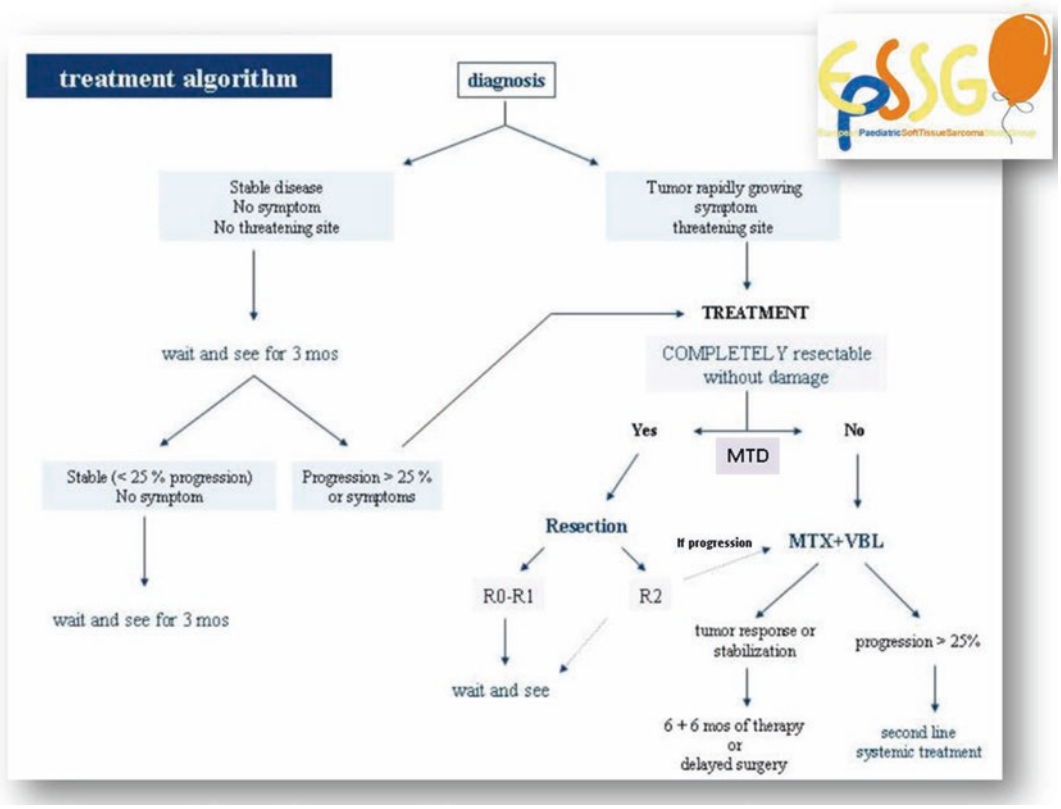

Fig. 6.3 E*pSSG* strategy for patients with desmoid tumors (protocol NRSTS 05). *R0* complete resection, *R1* marginal resection, *R2* gross residual disease, *MTX + VLB* methotrexate with vinblastine, *MTD* multidisciplinary team discussion

et al. 2008). Given the rarity of RT, there is no standard therapeutic pathway, and there have been no randomized trials examining the role of chemotherapy combinations or addition of new agents. The best therapeutic strategy in this tumor remains to be defined, but all decisions, especially radiotherapy, should take into account the young age of the patients with RT (median age: 28 months) and the aggressive nature of the disease (Fig. 6.4). RT are often described as rapidly lethal, with little evidence of improvement in survival in recent years (1-year survival <30%) (Bourdeaut et al. 2008; Brennan et al. 2016).

The general surgical principles apply to RT, i.e., complete surgical resection as early as possible, due to potential early progression. As soon as workup is completed, the tumor has been completely excised or biopsied, and the diagnosis of RT has been made; neoadjuvant or adjuvant chemotherapy should be given. Patients with initially unresectable or incompletely resected tumors should receive chemotherapy and undergo reassessment earlier in order to plan a delayed surgical resection to remove the primary tumor and any residual resectable metastases (Brennan et al. 2016).

Evidence for the role of chemotherapy, in particular ifosfamide, initially comes from a single historical institutional series from St Jude Children's Research Hospital. The inclusion of doxorubicin in chemotherapy combinations is suggested as important for survival in extracranial RT. Due to the absence of effective standard chemotherapy protocols, patients should be enrolled in prospective studies that contain sequential multidrug regimens for 6–12 months with mainly alkylating agents, anthracycline and platinum compounds. The current EpSSG strategy is interval-compressed chemotherapy with VDCy (vincristine, doxorubicin, cyclo-

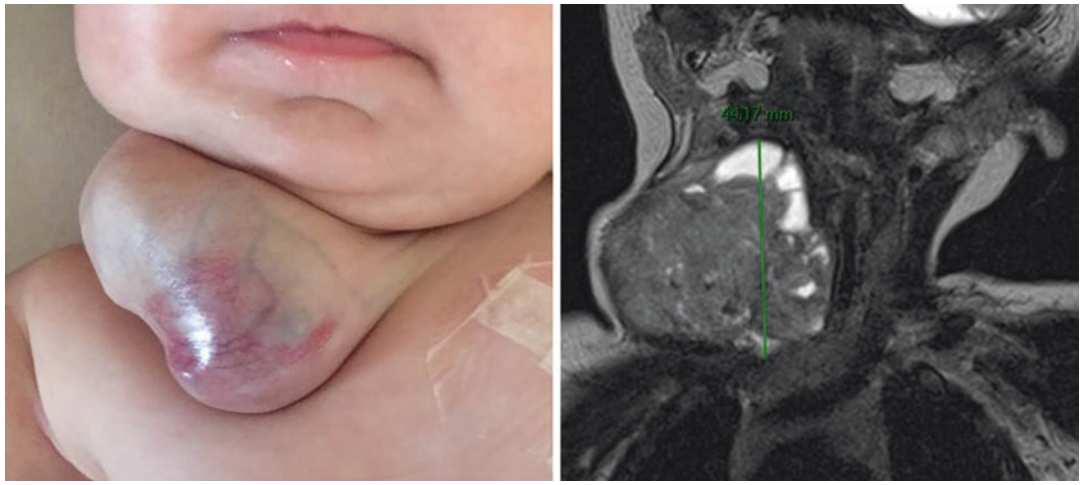

Fig. 6.4 Clinical and MRI presentation of localized cervical rhabdoid tumor (*SMARCB1* somatic mutation) in a 4-week-old female. The young age of the patient and the extent of local tumor invasion argue against the use of external beam radiotherapy

phosphamide) alternating with IE (ifosfamide, etoposide) in order to increase the dose intensity (Waldron et al. 1999). The value of intensification with high-dose chemotherapy or maintenance therapy after induction treatment is not known and should be explored. For the future, improved understanding of the biology and role of SMARCB1 in RT has enabled identification of new targets for small molecule inhibitors (EZH2 inhibitors, for instance) to combine with chemotherapy backbones that might be tested in future EpSSG and COG studies (Brennan et al. 2004).

Due to the aggressiveness of the tumor, local radiation should be considered early in all cases. The role of radiotherapy in local control of extracranial RT is suggested from small series (Bourdeaut et al. 2008). The real benefit of radiation is difficult to analyze in the literature because radiation tended to be given to those with a higher stage, without early progressive disease, and in older age groups. Furthermore, older patients were more likely to receive a higher radiation dose. Radiation dosages and fields are frequently limited by the tumor extent at diagnosis and the young age of the patients (Brennan et al. 2016) (Fig. 6.4).

6.4.6 Infantile Fibrosarcoma

Infantile fibrosarcoma (IFS) is a rare tumor, but it is the commonest soft tissue sarcoma in children less than 1 year of age (median age 1.43 months) and mainly arising in the extremity (54.0%) (Atallah et al. 2016; Sparber-Sauer et al. 2019). IFS is currently classified as a soft tissue tumor of intermediate malignancy characterized by a quite specific t(12;15)(p13;q25) translocation coding for an *ETV6-NTRK3* gene fusion. It often presents with initial rapid growth, sometimes with indolent evolution, and metastatic spread is uncommon (1–13%). Recent studies confirm the very good overall survival of children with IFS with a 5 year-OS >90% and emphasize the challenge of tumor resection without anatomic or functional damage (Atallah et al. 2016; Sparber-Sauer et al. 2019). Due to the very young age of patients, special attention should be paid to minimizing therapeutic late effects. Primary surgery should only be considered in small localized tumors that can easily be completely resected without any functional consequences. In case of complete surgery or microscopic residue (IRS I or II group) of a localized tumor, no further adjuvant treatment is needed. Since IFS is a chemo-

sensitive tumor, chemotherapy may play a role in the treatment strategy (Parida et al. 2013; Orbach et al. 2009; Surico et al. 2003). Recently, the VA regimen (vincristine-actinomycin-D) has been confirmed to be efficacious and may produce a response that facilitates surgery (Atallah et al. 2016; Orbach et al. 2009). The aim of neoadjuvant chemotherapy is to reduce the tumor size in order to allow delayed non-mutilating tumor resection. Response to chemotherapy can be slow (several months), and nonresponse to chemotherapy should only be considered in cases of tumor growth (>25% volume increase) or absence of tumor reduction after at least 3 months of therapy. If the tumor responds to VA and surgery could become feasible without anthracyclines and alkylating agents, VA is to be continued up to the surgery, and chemotherapy is discontinued after surgery. Other effective regimens include VAC (vincristine-actinomycin-D-cyclophophamide) and VAdriaC (vincristine-doxorubicin-cyclophophamide) but should be reserved for nonresponse to VA chemotherapy (Sparber-Sauer et al. 2019). The tumor shrinkage achieved in the majority of cases with initially unresected tumor will allow a conservative surgical approach in most cases (>95%). Radiotherapy should not be used due to its toxic consequences in infants. The EpSSG current treatment strategy is summarized in Fig. 6.5. Retrospective data of 66 infants (median age 1.7 months; range, 0–21.5) with IFS treated in

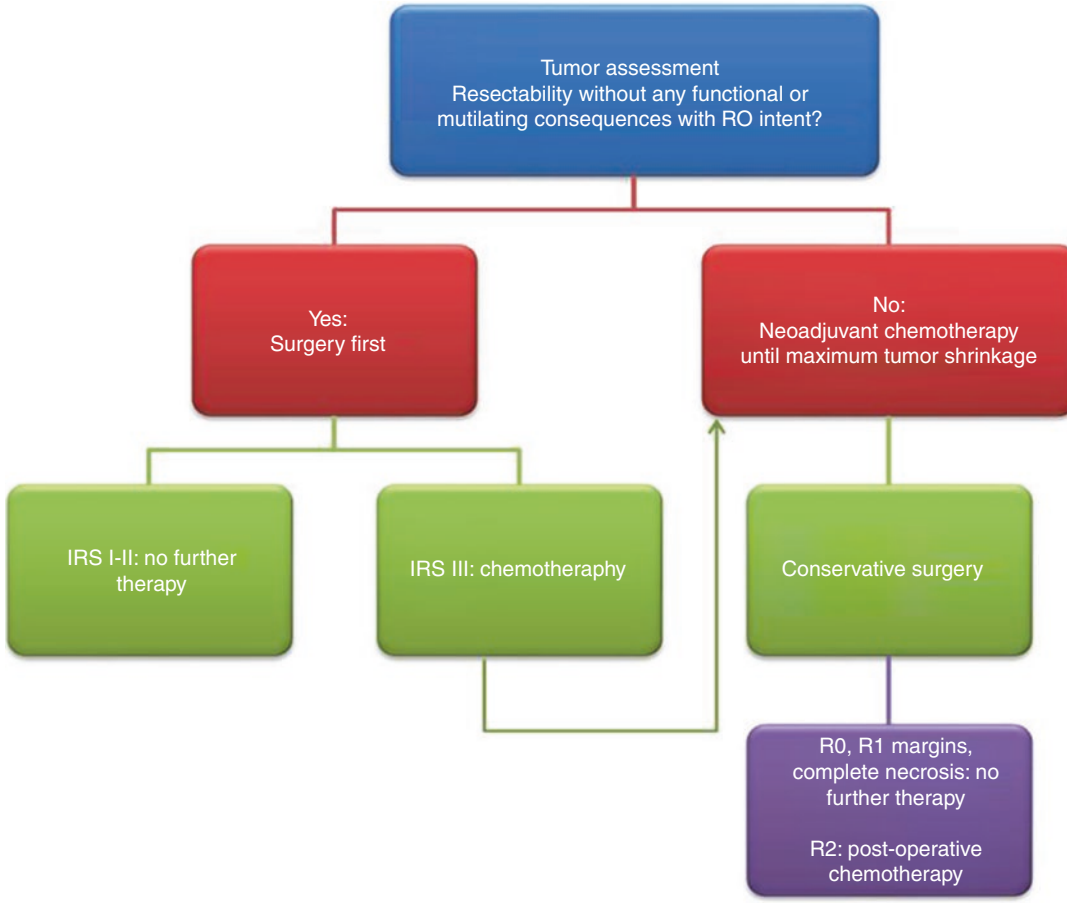

Fig. 6.5 Therapy summary for patients with infantile fibrosarcoma (EpSSG protocol). *IRS I* complete resection, *IRS II* microscopic residual disease, *IRS III* macroscopic residual disease, *R0* wide resection, *R1* marginal resection, *R2* gross residual disease after maximal surgery

the *Cooperative Weichteilsarkom Studiengruppe* (CWS) studies have been recently published (Sparber-Sauer et al. 2019). Main regimens used were vincristine, actinomycin-D, cyclophosphamide, Adriamycin (VACA) in CWS-81 and -86 and vincristine, actinomycin-D, cyclophosphamide (VAC) since the CWS-91 study (Ferrari et al. 2007). Since the CWS-96 study, a "wait and see" strategy was recommended after microscopically complete (R0, IRS group I) or microscopically incomplete (R1, IRS group II) resection. VAC was recommended for patients after macroscopically incomplete resection (R2 or biopsy, IRS group III) and in case of progressive disease (PD) (Sparber-Sauer et al. 2019). These regimens were followed by delayed surgery.

Since several decades, malignant tumors harboring a NTRK fusion transcript (Neurotrophic receptor tyrosine kinase) have been described. Initially, this fusion was highly associated to ETV6 (NTRK –ETV6) and mainly described in infantile fibrosarcoma (IFS). Progressively, this "specific" fusion transcript has also been discovered in other tumors as hypercellular mesoblastic nephroma, salivary gland carcinoma (Mammary analogue secretory carcinoma of salivary glands: MASC), or secretory breast carcinoma (Pavlick et al. 2017). In these tumors, the presence of this transcript is considered being frequent and remains an important tool for diagnosis (by FISH, RT-PCR, or RNA seq). Moreover, other partners (as, for instance, EML4-NTRK3 TMP3-NTRK1, LMNA-NTRK1, SCYL3-NTRK1) have been less frequently founded to be associated to NTRK gene in other malignant tumors (as other infantile mesenchymal tumors, colic carcinoma, lung carcinoma, inflammatory myofibroblastic tumor, brain tumor (low and high grade), or thyroid carcinoma, for instance) leading to consider that nowadays approximately the prevalence of the NTRK fusion transcript could be present in some more common tumors up to 1–2% (Wong et al. 2016). The recent clinical developments of a new class of compounds blocking the NTRK molecular pathway, which are still currently under early clinical investigation, give an important hope to find a specific new way to treat patients with these tumors. First results showed a real efficacy of these new drugs (larotrectinib, crizotinib, entrectinib) (DuBois et al. 2018). As an example, recently, Drilon et al. have tested a highly selective TRK inhibitor (larotrectinib) in adults and children who had tumors with these fusions and have found an overall response of 75% with a median time to response of 1.8 months (Drilon et al. 2018). Larotrectinib (LOXO-101) is the first highly selective pan-TRK inhibitor to enter clinical development with IC50 values in the low nanomolar range of inhibition for all the three TRK family members. Very encouraging efficacy results have been obtained in a phase I clinical trial (NCT02637687) (Laetsch et al. 2018). Larotrectinib is Food and Drug Administration (FDA)- and (European Medicines Agency) EMA-approved for the treatment of pediatric and adult patients with all solid tumors harboring *NTRK* gene fusions, and entrectinib is FDA and Japan approved.

6.4.7 Desmoplastic Small Round Cell Tumor

Desmoplastic small round cell tumor (DSRCT), seen mainly in adolescent and young adult males, usually presents as an abdominal or pelvic primary tumor with serosal dissemination and frequent invasion into other organs such as the liver, pancreas, and spleen. The tumor may also involve regional lymph nodes and frequently metastasize to the liver, kidney, lung, bone, and bone marrow (Philippe-Chomette et al. 2012).

Aggressive multimodality treatment with intensive chemotherapy may lengthen survival but usually is not curative. Although DSRCT is often responsive to sarcoma-directed chemotherapy, patients with this disease have an overall survival of only about 15% at 5 years. Tumor resection is critical for cure, as survival is vanishingly rare when the tumor is not grossly excised. Whole abdominal-pelvic radiotherapy has been used to treat serosal tumor dissemination. However, the dose that is feasible to deliver is usually insufficient for durable tumor control

even for microscopic residual disease. Hyperthermic peritoneal infusion with cisplatin chemotherapy has been shown to lengthen median survival in patients who have undergone a total or near total tumor resection but remains controversial in the literature, and some authors advise external beam radiotherapy instead (Atallah et al. 2016; Hayes-Jordan et al. 2014; Honore et al. 2017). Myeloablative chemotherapy with autologous stem cell transplant does not appear to significantly improve outcome and has fallen out of favor.

6.4.8 Dermatofibrosarcoma Protuberans

Dermatofibrosarcoma protuberans (DFSP) is a locally aggressive and rarely metastasizing neoplasm that is among the most common childhood soft tissue sarcomas. It usually arises in the trunk or extremities in superficial locations and is characterized by a t(17;22) translocation that produces the *COL1A1-PDGFB* fusion gene.

Wide local excision is curative in most cases, so primary re-excision should be considered after inadequate surgery. Mohs micrographic surgery may produce durable local tumor control while allowing smaller surgical margins than wide excision and should be considered in the appropriate clinical setting (Loghdey et al. 2014). In patients with unresectable, metastatic, or recurrent disease not amenable to surgery, systemic therapy is indicated. Imatinib, an inhibitor of the platelet-derived growth factor receptor tyrosine kinase, produces tumor responses in about half of patients with DFSP and can be curative in some cases. However, it is most often used in conjunction with surgical resection. Imatinib may also be considered in the adjuvant setting for patients with microscopic residual disease after maximal surgery. In adults whose tumor is resistant to imatinib, sunitinib also produces a high rate of disease control (Fu et al. 2015; Ugurel et al. 2014; Kerob et al. 2010). However, sunitinib has not been evaluated in pediatric patients for this indication. Although dermatofibrosarcoma protuberans is radiosensitive, radiotherapy is rarely used in pediatric patients since its toxicities are thought to exceed those of imatinib.

6.4.9 Gastrointestinal Stromal Tumor (GIST)

Although the most common adult soft tissue sarcoma of the gastrointestinal tract, gastrointestinal stromal tumor (GIST) is very rare in pediatric patients, where it disproportionally affects females. Unlike adult GISTs that are characterized by mutations causing constitutive activation of *KIT* or *PDGFRA*, most pediatric GISTs lack these mutations and are referred to as "wild-type." A substantial proportion of wild-type GIST have loss of function of the succinate dehydrogenase complex, which in some cases may be due to germline mutations that predispose to other types of cancer including paraganglioma (Miettinen et al. 2011).

Surgery is the mainstay of therapy for all GISTs. Those with tumors that can be removed with adequate margins and without significant functional consequences should undergo wide resection. Lymph node sampling should also be considered since nodal involvement is more common in young patients. Adjuvant therapy has not been studied in pediatric patients and is generally not recommended. The approach to treatment of pediatric GIST depends on the underlying tumor biology. If *KIT* or *PDGFRA* activating mutations are identified, treatment guidelines established for adults, such as those of the National Comprehensive Cancer Network, may be used. Because pediatric wild-type GIST may behave in an indolent fashion and there are no known effective treatments, patients with unresectable or metastatic disease who are asymptomatic may not require treatment. Symptomatic patients may be given tyrosine kinase inhibitors such as imatinib or rather sunitinib, although there are no published data yet comparing the efficacy of these two agents in pediatric GIST patients (Benesch et al. 2011).

6.4.10 Inflammatory Myofibroblastic Tumor

Inflammatory myofibroblastic tumor (IMT) is a rare soft tissue sarcoma that has a variable biologic behavior ranging from a benign course to aggressive disease dissemination. More than half have rearrangement of the *ALK* gene on chromosome 2p23; those without *ALK* rearrangement often have *ROS1*, *NTRK*, or *PDGFRB* fusions (Lovly et al. 2014).

The variable clinical behavior of IMT makes treatment selection challenging. When feasible, wide tumor resection is the treatment of choice. Cytotoxic chemotherapy has been used for unresectable or metastatic disease, but efficacy data are limited. Nonsteroidal anti-inflammatory drugs have also been used with some success; high VEGF and COX-2 expression in IMTs suggest that this may be due to disruption of angiogenic signaling. Steroids have also been used. *ALK* inhibitors such as crizotinib and *ROS1* or *NTRK* inhibitors (entrectinib, larotrectinib) may produce rapid and sustained tumor responses in *ALK/ROS/NTRK*-rearranged IMT, but acquired drug resistance can develop (Mosse et al. 2013; Rolfo et al. 2015).

6.4.11 Alveolar Soft Part Sarcoma

Alveolar soft part sarcoma (ASPS) is a very rare sarcoma that can occur at any age but mainly affects adolescents and young adults, with a peak of incidence in the third decade. About one third of ASPS occur in children and adolescents (Orbach et al. 2013; Sparber-Sauer et al. 2018b). The critical role of surgery in localized disease is emphasized by all published studies. In case of incomplete initial resection, a second surgery before any other treatment (primary re-excision) is recommended to achieve clear margins and avoid the need for further treatment (Casanova et al. 2000). The possibility to obtain a complete tumor resection seems strictly related to the tumor dimensions and the absence of extension to nearby organs. This is little modified by the addition of chemotherapy and/or radiotherapy due to the unsatisfactory response of ASPS to neoadjuvant treatment.

Radiotherapy with doses ranging from 45 to 60 Gy has been administered to children with unresectable tumors. Although this seems a rational approach, there are no convincing data that irradiation may significantly change the natural history of the disease (Orbach et al. 2013; Casanova et al. 2000). Patients with complete tumor resection (IRS group I) likely do not need systematic adjuvant radiotherapy, unlike those who undergo marginal resection (IRS II) even if these latter case data are scarce (Orbach et al. 2013). In the literature, most patients with gross residual tumor (IRS group III) had delayed surgery after neoadjuvant medical therapy. Almost all received adjuvant radiotherapy even after complete secondary resection, preventing analysis of the precise benefit of radiotherapy in this situation.

ASPS is one of the less chemosensitive NRSTS. In different series of adult ASPS, no objective response to conventional chemotherapy has been reported. Recently, the European Cooperative Groups published a joint analysis on a large series of pediatric and adolescent patients with ASPS and reported a 17% response rate to conventional chemotherapy (Orbach et al. 2013). The better characterization of the biological features of ASPS has allowed testing of new molecules that can target the genetic alteration. In particular, gene expression profiling of ASPS specimens demonstrates an array of potentially therapeutically targetable, angiogenesis-related molecules. Reports about clinical use and efficacy of targeted therapies focus on sunitinib, cediranib, pazopanib, or bevacizumab or immunotherapy as immune checkpoint inhibitors (Bisogno et al. 2014). The systematic adoption of targeted agents as first-line chemotherapy for unresectable or metastatic ASPS should be considered given the low likelihood of response to standard chemotherapy. The response to tyrosine kinase inhibitors supports the inclusion of ASPS patients in specific trials testing the activity of these targeted therapies (Fig. 6.6). However, a recent publication showed that cediranib in a phase II study did not reach the targeted response

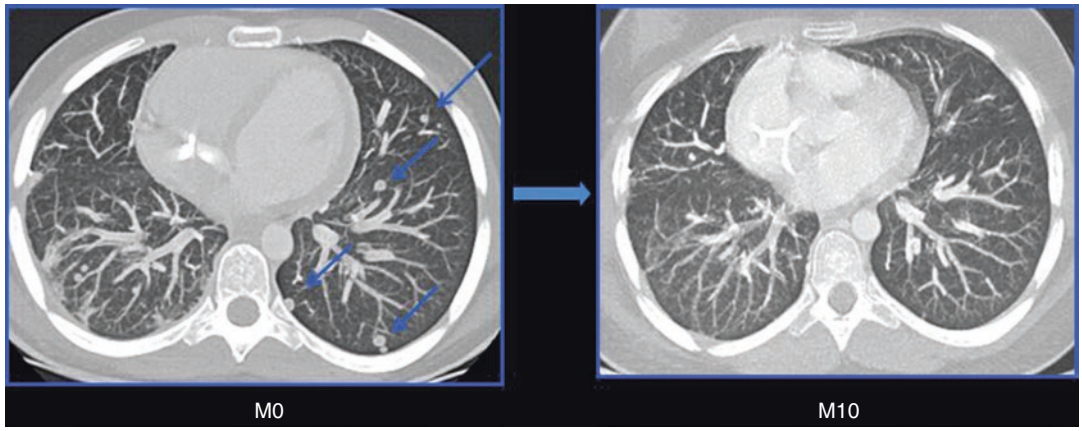

Fig. 6.6 Example of partial response of lung metastases to sunitinib in an 8-year-old female with stage IV thoracic wall alveolar soft part sarcoma (with ASPSTR1/TFE3 transcript). *M0* prior to initiation of therapy, *M10* after 10 months of therapy (sunitinib 25 mg/day, 4 weeks/6)

rate with no tumor response among seven children with ASPS and nevertheless prolonged stable disease (Cohen et al. 2019).

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Osteosarcoma: History of Therapy

7

Paul Meyers

Successful treatment for osteosarcoma (OS) requires the combination of effective systemic chemotherapy and surgical resection of all sites of clinically detectable disease. Prior to the introduction of systemic chemotherapy, patients who presented with OS of the extremity without clinically detectable metastatic disease underwent immediate surgical resection of the primary tumor. Five-year survival rates using this approach ranged from 11 to 25% (Anninga et al. 2011). In the early 1970s, trials evaluated OS response to single agents including high-dose methotrexate (HDMTX) with leucovorin rescue, cisplatin (CDDP), and doxorubicin (DOX) (Table 7.1) (Anninga et al. 2011; Pratt and Shanks 1974). Several studies reported that single agent or combination chemotherapy administered to patients with OS after primary tumor resection resulted in improved survival compared to historical controls (Anninga et al. 2011). Other reports suggested that the apparent improvement in outcome was related to improvement in diagnosis and surgery rather than a benefit from adjuvant chemotherapy (Carter 1984; Taylor et al. 1985). Two randomized prospective trials subsequently confirmed the benefit of adjuvant chemotherapy following resection of primary OS (Eilber et al. 1987; Link et al. 1986). Trials evaluating regimens including doxorubicin and high-dose methotrexate or doxorubicin and cisplatin following resection of the primary tumor reported 3- to 5-year event-free survival ranging from 50 to 60% or more in patients who presented without clinically detectable metastases (Anninga et al. 2011; Ettinger et al. 1981; Goorin et al. 1987; Pratt et al. 1990). In the 1980s, several investigations established the activity of ifosfamide or ifosfamide and etoposide in recurrent and metastatic OS (Harris et al. 1995; Miser et al. 1987).

Investigators at Memorial Sloan-Kettering Cancer Center (MSKCC) pioneered initial administration of chemotherapy followed by definitive surgical resection in OS (Rosen et al. 1976, 1979). A randomized study comparing this strategy to that of immediate definitive surgery followed by adjuvant therapy did not detect any survival difference (Goorin et al. 2003). Initial chemotherapy permits evaluation of primary tumor necrosis at the time of definitive surgical resection, which correlates with event-free and overall survival. Clinical trials from 1990 to the present day, using combinations of the agents with demonstrated activity (doxorubicin, cisplatin, high-dose methotrexate, ifosfamide (IFOS) [with or without etoposide]) reported EFS for localized OS ranging from 60 to 70% with no best combination (Bielack et al. 2009; Meyers et al. 2005; Petrilli et al. 2006; Picci et al. 2010). Anninga reported a

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C. A. S. Arndt (ed.), *Sarcomas of Bone and Soft Tissues in Children and Adolescents*, Pediatric Oncology, https://doi.org/10.1007/978-3-030-51160-9_7

Table 7.1 Objective response of patients with osteosarcoma to single-agent chemotherapy (Anninga et al. 2011)

Drug	Number of patients	Complete response	Partial response	Response rate (%)
Doxorubicin	108	14	32	43
Ifosfamide	246	30	50	33
Methotrexate	164	26	26	32
Cisplatin	174	18	28	26
Etoposide	27	0	1	4

meta-analysis of all published trials for the treatment of osteosarcoma (Anninga et al. 2011). He observed that any combination of three of the known active agents achieved results which were superior to any combination of two of the agents, and treatment with four agents was not superior to treatment with any three of the active agents. The Children's Oncology Group (COG) performed a randomized trial that compared CDDP, HDMTX, and DOX to the same combination with the addition of IFOS. EFS and OS were the same for both regimens (Meyers et al. 2005, 2008). The randomized prospective trial performed by the COG also investigated the addition of liposomal muramyl tripeptide (L-MTP) to standard combination chemotherapy. It demonstrated a trend toward improved EFS and statistically significantly improved overall survival for patients with localized and OS who received L-MTP (Meyers et al. 2005, 2008). It showed improved event-free and overall survival for patients with metastatic OS who received L-MTP, although the improvement was not statistically significant (Chou et al. 2009). The analysis, however, was complicated by what appeared to be an interaction between one intervention, the addition of ifosfamide to CDDP, HDMTX, and doxorubicin, and the other intervention, the addition of L-MTP (Bielack 2011). The putative interaction was observed in the results for EFS but not in the results for overall survival. The putative interaction was seen only in the cohort of patients with localized disease and was not observed in the cohort of patients with primarily metastatic disease. On the basis of this randomized trial, the US Food and Drug Administration did not approve L-MTP, but the European Medicines Agency did. L-MTP is licensed for the front-line treatment of osteosarcoma in conjunction with multi-agent chemotherapy in many countries, but remains investigational in the United States.

The degree of necrosis in the primary tumor after an initial period of chemotherapy correlates strongly with event-free and overall survival and is one of the strongest predictors of outcome. An international consortium, the European American Osteosarcoma study group (EURAMOS), was formed to conduct a prospective randomized trial of the strategy of modifying treatment based on histological necrosis, sometimes referred to as tailored therapy (Whelan et al. 2015). All patients received initial therapy with cisplatin, doxorubicin, and high-dose methotrexate and had definitive surgical resection of the primary tumor and evaluation of necrosis. Patients with a higher degree of necrosis were randomly assigned to continue the same chemotherapy agents following surgery or to receive the same agents followed by treatment with recombinant alpha-interferon. The addition of interferon to the postoperative treatment did not result in improved outcome (Bielack et al. 2015). Patients with a lower degree of necrosis were randomly assigned to continue the same chemotherapy agents following surgery or to receive the same agents with the addition of high-dose ifosfamide and etoposide. The addition of high-dose ifosfamide and etoposide to the postoperative regimen did not improve outcome and was associated with increased toxicity and risk of secondary leukemia (Marina et al. 2016).

The standard of care for the treatment of OS includes an initial period of multi-agent chemotherapy, followed by definitive surgical resection of clinically detectable disease, followed by additional adjuvant chemotherapy. This standard was established in the 1980s and modifications have not changed the EFS or overall survival for patients.

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8 Osteosarcoma-Approach to Therapy

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8.1 Introduction

Osteosarcoma demonstrates that treatment success will only be achieved through close interdisciplinary collaboration. The most common subtype, high-grade central osteosarcoma, carries a very high risk of systemic dissemination, so that surgery alone will rarely lead to cure (Casali et al. 2018; Fletcher et al. 2013; Jaffe 1972; Link et al. 1986; Marcove et al. 1970). Chemotherapy without sufficient local therapy will also result in failure (Bielack et al. 2002; Jaffe et al. 2002). Combined uses of both approaches together, however, will often result in cure (Bielack et al. 2004, 2008; Ferrari and Serra 2015).

Rare low-grade central, parosteal, and periosteal osteosarcoma variants are of lower malignant potential and treated by surgery alone (Casali et al. 2018; Cesari et al. 2011; Fletcher et al. 2013; Grimer et al. 2005; Laitinen et al. 2015). Craniofacial osteosarcomas also carry a lower risk of metastatic spread, but a high risk of local failure, and recent guidelines favor a multidisciplinary approach (Casali et al. 2018). This chapter will focus on treatment of young patients with high-grade osteosarcoma.

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C. A. S. Arndt (ed.), *Sarcomas of Bone and Soft Tissues in Children and Adolescents*, Pediatric Oncology, https://doi.org/10.1007/978-3-030-51160-9_8

8.2 Clinical Presentation

The typical presentation of osteosarcoma is in a young mid-teenage male complaining of pain around the knee, gradually worsening over 2–3 months, and swelling, both impacting to an increasing degree on normal activities including broken sleep and reduced mobility. Systemic symptoms are unusual even if metastases are present. The male/female ratio of osteosarcoma patients aged <24 is 1.28, and median age at diagnosis is 15 years. Approximately 90% of primaries are located in the limbs (Mirabello et al. 2009; Whelan et al. 2012). Primary lung metastases are detected in 10–15% and metastases to other sites, most often bones, in a further 5% (Kager et al. 2003; Marko et al. 2016; Meyers et al. 1993). Osteosarcomas arising in non-extremity sites are uncommon in young persons. Both nonspecific symptoms and low levels of awareness of cancer among both professionals and public result in diagnostic delays (Brasme et al. 2012).

8.3 General Outline of Multidisciplinary Treatment

High-grade osteosarcoma is one of few pediatric tumors in which the value of systemic chemotherapy has been rigorously proven through a randomized clinical trial (Link et al. 1986). Even prior to this study, it was known that few patients with radiographically localized high-grade osteosarcoma would survive with surgery alone, suggesting microscopic metastatic disease was frequently present (Jaffe 1972; Marcove et al. 1970).

Local control of the primary tumor, typically by surgery, remains as critical as systemic chemotherapy. Until only a few decades ago, surgery usually meant amputation. This was subsequently replaced by limb-sparing resections followed by reconstruction, usually with an endoprosthesis. Initiating chemotherapy some months prior to surgery offers the advantages of allowing time for surgical planning, and the degree of necrosis of the resection specimen is prognostic (Bielack et al. 2002; Rosen et al. 1981), even if clinically exploiting that information has been challenging (Bielack et al. 2015; Marina et al. 2016; Whelan et al. 2015). The principles of managing metastatic high-grade osteosarcoma generally mirror those also used for localized disease with the addition of surgical clearance of all metastatic deposits.

8.4 Imaging at Diagnosis and During Systemic Treatment: Essentials

A plain radiograph is the first imaging study for a patient with suspected osteosarcoma. In cases of malignancy, the radiographic characteristics suggest an aggressive lesion (Meyer et al. 2008). Radiographs are followed by magnetic resonance imaging (MRI) to delineate the locoregional extent of disease as well as the tumor's relationship to neurovascular structures.

Computed tomography (CT) of the chest is the preferred method to detect metastatic lung disease (Bielack et al. 2002). It is now common to identify subcentimeter pulmonary lesions on chest CT (Ginsberg and Panicek 2000; Meyer et al. 2008). There is evidence that lesions >5 mm are more likely to be malignant (Ginsberg and Panicek 2000; Picci et al. 2001). The presence of at least one lung lesion >1 cm or more than three lesions at least 5 mm in diameter is often accepted as evidence of metastatic disease. The presence of lesions <5 mm raises the possibility of metastases, but in these instances tissue confirmation is recommended.

Extrapulmonary metastases are searched for by either technetium bone scintigraphy, whole body MRI, or positron emission tomography/computed tomography (PET/CT). Either modality provides information as long as the primary tumor is avid. The role of functional imaging remains controversial. In one study, PET/CT could not be consistently used to determine histological response of the tumor (Hawkins et al. 2009).

Imaging evaluation during systemic treatment should occur approximately every 3 months, its

purpose being to confirm the absence of local or distant progression. Patients are followed with chest X-ray and/or CT and MRI.

8.5 Biopsy: Procedure

Nearly all masses that require surgical resection will have biopsies performed prior to excision. It is essential that representative tissue be delivered to the pathologist. Heterogeneous sarcomas may require biopsy from multiple locations to ensure a representative sample. The biopsy tract should be placed as to be incorporated and excised en bloc with the definitive resection. Longitudinal incisions are used, and for extremity lesions, a direct approach with minimal extension into adjacent tissue planes is usually possible.

MRI can prove invaluable in deciding where to biopsy. Tumor margins can be distinguished from surrounding muscle, fat, and neurovascular bundles. Areas of necrosis, liquefaction, myxoid degeneration, hemorrhage, and fibrosis are typically avoided. Abrupt signal changes within a mass could indicate dedifferentiation and should be sampled. For many bone sarcomas the most viable portions are near the periphery or within a soft tissue component. Specimens are typically sent fresh to pathology, which allows use of specific fixatives for immunohistochemistry and molecular genetic studies. Newer sequencing technologies may also allow use of formalin-fixed paraffin-embedded samples in molecular testing (van de Rijn et al. 2014). Nearly all suspected osteosarcoma specimens require decalcification.

Core needle biopsies have been shown to be safe and less invasive than incisional biopsies and are accurate if adequate cores are obtained even in sclerotic bony lesions (Mitsuyoshi et al. 2006; Taupin et al. 2016). Core needle biopsies may not be as effective in telangiectatic osteosarcoma, misdiagnosing over one third of cases as aneurysmal bone cysts in one series (Gao et al. 2013). Frozen sections are often used to assess intramedullary marrow margins during definitive resection and should be interpreted in tandem with the gross specimen (Anderson et al. 2014).

8.6 What Can Be Learned from the Biopsy Specimen?

Osteosarcoma is defined as a malignant spindle cell tumor which produces osteoid (Fletcher et al. 2013). Despite being a genetically complex tumor, its diagnosis is not based on any molecular testing at present (Gorlick 2009), but on routine hematoxylin and eosin staining and conventional light microscopy. Beyond the histologic classification, all osteosarcomas can be categorized as low, intermediate, or high grade. Although most osteosarcomas in young patients are high grade, this distinction is of critical importance as it defines the need for systemic chemotherapy. Osteosarcoma can similarly be broken into histologic subtypes such as osteoblastic, chondroblastic, fibroblastic, and telangiectatic dependent on the predominant pattern of differentiation (Fletcher et al. 2013). Although these subtypes may be associated with characteristic radiographic appearances, they do not impact on treatment and in most studies have not been shown to influence prognosis.

Hampered by osteosarcoma's genetic complexity, defining biological risk factors and molecular alterations which can be targeted remains elusive. That said an explosion in the available knowledge with regard to osteosarcomas biology has occurred with efforts such as whole tumor genome sequencing (Bishop et al. 2016). One could be nihilistic and suggest that in the absence of clinical relevance of osteosarcoma biology studies, the only purpose of a biopsy is making the diagnosis. On the other hand, many remain hopeful that additional clinical progress can be made through enhanced biological understanding, provided that additional osteosarcoma specimens are available for analyses. In both North America and Europe, coordinated research efforts exist putting forward biology and banking studies for the collection of biomaterials from patients with osteosarcoma (Glover et al. 2015). Success of these efforts will require obtaining adequate samples to permit biological analyses from osteosarcoma patients, with the consent of the patients/guardians. These efforts are strongly supported by the authors of this chapter.

8.7 Systemic Treatment

8.7.1 Choice of Drugs

For over 30 years, systemic treatment of osteosarcoma has relied on the same few cytotoxic agents. High-dose methotrexate with folinic acid rescue (HD-MTX) (Jaffe et al. 1973, 1974), doxorubicin (DOX, adriamycin) (Cortes et al. 1974), and cisplatin (cis-diamminedichloridoplatinum(II), CDDP) thereafter (Freeman et al. 1979; Ochs et al. 1978) were introduced in the 1970s. Soon, combination regimens were employed (Pagani et al. 1975; Rosen et al. 1974, 1975; Winkler et al. 1977). Starting in the early 1980s, several protocols also included ifosfamide (IFOS) (Bielack et al. 2013; Ferrari and Serra 2015). A recent meta-analysis concluded that combining any three of those four drugs led to better results than using only two, but that adding the fourth was not associated with further improvements (Anninga et al. 2011). A combination of HD-MTX, DOX, and CDDP (MAP) (Meyers et al. 2005, 2008; Whelan et al. 2015) is considered a standard (Table 8.1), but other regimens which include several of the mentioned drugs may achieve similar results (Daw et al. 2011; Fuchs et al. 1998; Le Deley et al. 2007; Smeland et al. 2011). Outside of specific trials, patients with primary metastases generally receive the same systemic treatment as those with localized disease (Carrle and Bielack 2009; Kager et al. 2003; Meyers et al. 1993).

Table 8.1 Standard MAP regimen for osteosarcoma

Adriamycin (doxorubicin)	37.5 mg/m^2	i.v.	24 h infusion	Days 1, 2
Cisplatin	40 mg/m^2	i.v.	24 h infusion[a]	Days 1–3
Hyperhydration and forced mannitol diuresis required to reduce otherwise severe cisplatin nephrotoxicity				
Weeks 1; 6; 12; 17				
Adriamycin (doxorubicin)	37.5 mg/m^2	i.v.	24 h infusion[a]	Days 1, 2
Weeks 22; 26				
Methotrexate	12,000 mg/m^2	i.v.	4 h infusion	Day 1
Folinic acid (leucovorin)	15 mg/m^2	p.o./i.v.	Every 6 h, beginning 24 h from MTX Total of 12 doses	
Meticulous supportive care including hyperhydration, urinary alkalinization, repeated measurement of MTX serum levels, and folinic acid rescue obligatory to prevent life-threatening toxicity				
Weeks 4 + 5; 9 + 10; 15 + 16; 20 + 21; 24 + 25; 28 + 29				
Local therapy			Surgery of the primary tumor[b]	
Always strive for wide resection margins. Pathology must assess margins and grade histologic response to preoperative chemotherapy				
Week 11				

The MAP regimen as used for osteosarcoma (Ahmed et al. 2015; Marina 1997; Meyers et al. 1993, 2008; Wasilewski-Masker et al. 2009)

[a]Short infusion with dexrazoxane can be an alternative

[b]Primary metastases, if present, must also be resected. This is usually done during the months which follow surgery of the primary tumor

8.7.2 High-Dose Methotrexate

HD-MTX, commonly at 12 g/m^2, in combination with vigorous hydration and urinary alkalinization along with pharmacokinetically guided folinic acid "rescue" (FAR), is an essential component of osteosarcoma treatment (Jaffe et al. 1974). Methotrexate (MTX) is an analogue of folic acid which penetrates into cells via a specific membrane transport system used by physiological folates. Carrier-mediated transport limits the entry of MTX into cells until the extracellular concentration is as high as 20 μmol/L and passive diffusion occurs. Inside the cell MTX rapidly binds to and inhibits its target enzyme dihydrofolate reductase (DHFR), leading to an inhibition of purine and pyrimidine synthesis. In addition to direct inhibition of DHFR, the intracellular formation of MTX polyglutamyl metabolites is also thought to (a) increase intracellular drug accumulation, (b) increase intracellular drug retention, and (c) inhibit folate-dependent nucleotide synthesis, by effects at loci other than DHFR (Adamson et al. 2011).

High-dose methotrexate regimens are designed to circumvent MTX resistance.

Achieving and sustaining high plasma levels of the drug promotes passive diffusion of MTX, thus overcoming defective transmembrane transport systems (Guo et al. 1999). The doses of MTX required to achieve such high plasma concentrations must be followed by the antidote, folinic acid, to prevent excessive toxicity to normal tissues. Folinic acid replenishes the intracellular source of reduced active folates. Although this decreases the degree of MTX toxicity, patients will remain at risk as long as elevated MTX levels persist in their circulation. Moreover, if the extracellular MTX concentration is very high, folinic acid alone may prove inadequate.

Despite supportive measures, MTX-induced toxicity (myelosuppression, mucositis, hepatic and renal toxicity) still occurs and results in morbidity, patient discomfort, costs, and potentially reduced treatment efficacy, due to suboptimal chemotherapy doses and/or delays in chemotherapy administration (Widemann and Adamson 2006). Elevation of serum creatinine points out renal injury, which can result in delayed excretion of MTX, so close monitoring during administration is critical. In case of life-threatening MTX intoxication, administration of high-dose leucovorin or in selected cases glucarpidase may become necessary (Flombaum et al. 2018; Ramsey et al. 2018).

8.7.3 Doxorubicin

Doxorubicin in an anthracycline antibiotic isolated from cultures of *Streptomyces peucetius*. Doxorubicin intercalates to nucleotide base pairs and binds to the lipid membrane. Intercalation interferes with nucleotide replication and the action of DNA and RNA polymerases. Its interaction with topoisomerase II appears important for cytotoxic activity. Approximately 40% of the drug is excreted in the bile in 5 days. Only up to 12% of the drug is excreted in the urine (Adamson et al. 2011; Bedford Laboratories 2012).

The most common acute toxicities include nausea, vomiting, myelosuppression, and a pink or red color to the patient's secretions. It can also produce mucositis and liver toxicity. The most serious complication of doxorubicin administration is cardiotoxicity [Krischer et al. 1997; Lipshultz 2006; Nysom et al. 1998, see below]. Pediatric patients are usually treated with doses ranging from 25 to 90 mg/m^2 per dose. Cumulative doses >300 mg/m^2 are associated with higher long-term toxicity. As doxorubicin is one of the most effective agents against osteosarcoma, affected patients often receive such high cumulative doses (Smith et al. 1991). Strategies to decrease doxorubicin cardiotoxicity include the use of continuous infusions (Hortobagyi et al. 1989; Legha et al. 1982) and of cardioprotective agents (Huh et al. 2010; Sanchez-Medina et al. 2010; Wexler et al. 1996). Continuous infusion has successfully reduced cardiotoxicity in adults but is associated with increased mucositis (Bielack et al. 1996; Hortobagyi et al. 1989; Legha et al. 1982) and has been reported as unsuccessful in children (Lipshultz et al. 2013). Dexrazoxane has been successfully used in pediatric patients (Asselin et al. 2016; Huh et al. 2010; Lipshultz et al. 2013; Sanchez-Medina et al. 2010; Wexler et al. 1996). While it has been claimed that it may increase the risk of second malignancies (Tebbi et al. 2007), recent reports suggest this is not the case (Chow et al. 2015; Seif et al. 2015).

8.7.4 Cisplatin

Cisplatin is a platinum-containing DNA-damaging agent, the intracellular presence of which leads to DNA cross-linking and consequent apoptosis. Initially reported active as a single agent at a dose of 100 mg/m^2, it quickly came to be used in combination with doxorubicin (Gasparini et al. 1985; Pratt et al. 1985).

CDDP is highly emetogenic, a side effect which if uncontrolled can exacerbate renal impairment, the most challenging problem arising from its administration (Arany and Safirstein 2003). Acute glomerular toxicity can be ameliorated by hyperhydration, forced diuresis, and prolonged intravenous administration over 48–72 h rather than short infusions. Renal tubular damage may also occur, leading to electrolyte

imbalance and the need for replacement particularly of magnesium, calcium, and potassium. Ototoxicity and peripheral neuropathy are also limiting factors. CDDP causes tinnitus, often reversible, and irreversible hearing loss especially affecting high tones. Avoidance of other nephro- or neurotoxic agents such as aminoglycoside antibiotics and furosemide is advised. CDDP is also significantly myelosuppressive.

8.7.5 Ifosfamide

IFOS is a cyclophosphamide analogue which requires hepatic activation to the reactive 4-hydroxyifosfamide, which exists in equilibrium with aldoifosfamide. Aldoifosfamide is then converted to acrolein and ifosfamide mustard, the active bifunctional alkylating agent. Acrolein is presumed to be the cause of hemorrhagic cystitis, a side effect of both cyclophosphamide and ifosfamide. The metabolism of IFOS is autoinducible resulting in increased clearance and decreased toxicity over time (Kerbusch et al. 2001). The amount of IFOS excreted in the urine is directly proportional to the dose administered. There is more oxidation of chloroethyl groups by IFOS, which produces more chloroacetaldehyde (thought to be responsible for neurotoxicity and renal toxicity).

Evaluation of this drug in an upfront window approach revealed clinical responses in patients with recurrent and metastatic osteosarcoma (Goorin et al. 2002; Harris et al. 1995; Harris et al. 1998). The drug is usually administered by short infusions lasting 1–4 h depending on the total doses used, which range from 6–9 g/m^2 over 2–5 days (Fuchs et al. 1998; Meyers et al. 2005, 2008) to 14 g/m^2 over 5 days (Schwartz et al. 2016; Whelan et al. 2015). Fractionation reduces urotoxicity (Kerbusch et al. 2001), as does the use of mesna, which helps to prevent hemorrhagic cystitis. Patients receiving ifosfamide are monitored with urinalysis to make certain they do not develop hemorrhagic cystitis (Kerbusch et al. 2001) as well as with electrolyte measurements to evaluate for renal tubular dysfunction (Buttemer et al. 2011). Neurotoxicity can be managed by stopping the infusion and administration of methylene blue (Kerbusch et al. 2001). Other acute toxicities associated with IFOS include nausea, vomiting, hair loss, myelosuppression, and liver toxicity.

8.7.6 Other Agents

Addition of more cytotoxic chemotherapy to the standard treatment backbones has failed to further improve survival outcomes (Gatta et al. 2014; Mirabello et al. 2009; Stiller et al. 2018). This was most recently evident in the prospective, randomized European and American Osteosarcoma Study (EURAMOS)-1 which tested the addition of high-dose ifosfamide (14 g/m^2) plus etoposide to preoperative MAP in poor responders to preoperative MAP (Marina et al. 2016; Whelan et al. 2015). Early protocols had included the BCD combination of bleomycin with cyclophosphamide and actinomycin D (Mosende et al. 1977; Rosen et al. 1981; Winkler et al. 1977, 1988); however, this was largely abandoned when a phase 2 study failed to demonstrate activity (Pratt et al. 1987).

The macrophage activator mifamurtide (liposomal muramyl-tripeptide-diphosphatidyl-ethanolamine, MTP) was investigated in a randomized trial, INT0133, which also tested ifosfamide in a randomized 2 × 2 factorial design (Meyers et al. 2005, 2008). A first report concluded that interaction between the two randomizations precluded definitive statements regarding MTP (Meyers et al. 2005). With three additional years of follow-up, the authors performed a second analysis of event-free and overall survival in the localized disease cohort of the trial. They reported that the addition of MTP to chemotherapy resulted in a statistically significant improvement in overall survival and a trend toward better event-free survival (Meyers et al. 2008). Persisting statistical and interaction concerns, however, led others to caution that these results did not meet generally accepted standards for practice-changing conclusions and that confirmatory trials would be required before MTP should be considered for routine use (Bielack et al. 2008;

Hunsberger et al. 2008). The US-FDA denied a license stating that the applicants had failed to demonstrate substantial evidence of efficacy (U.S. Food and Drug Administration 2007). The European Medicines Agency approved mifamurtide for use in the treatment of newly diagnosed osteosarcoma, and the agent is now licensed for that indication in many countries.

Interferon alpha-2b (IFN-α-2b), which can act as an immune modulator as well as exerting anti-angiogenetic and direct antitumor effects (Whelan et al. 2010), was investigated in the good responder cohort of EURAMOS-1, where patients in the experimental arm received the agent as maintenance after concluding MAP chemotherapy (Bielack et al. 2015; Marina et al. 2009; Whelan et al. 2015). Compared to MAP alone, MAP plus IFN-α-2b was not statistically different. The interpretation of this finding is hampered by the fact that a considerable proportion of patients never started IFN-α-2b or stopped prematurely (Bielack et al. 2015).

In order to move things forward, effective drugs with novel mechanisms of action need to be identified. In the Children's Oncology Group, the decision has been made to await identification of a novel agent with efficacy in osteosarcoma prior to embarking upon a phase 3 trial testing the value of its addition to standard chemotherapy (Gorlick et al. 2013). Instead, the group is focused on performing a series of phase 2 trials in patients with recurrent osteosarcoma attempting to identify novel agents with efficacy. The rationale for each of these studies is varied but includes encouraging data obtained from the Pediatric Preclinical Testing Consortium, transgenic mouse models, and tumor profiling. A phase 2 trial of eribulin in unresectable recurrent osteosarcoma was rapidly completed but unfortunately did not demonstrate activity (Isakoff et al. 2018). Trials of denosumab in resectable and unresectable recurrent osteosarcoma, ch14.18 antibody in resectable recurrent osteosarcoma, and glembatumumab vedotin in unresectable recurrent osteosarcoma are ongoing. Other agents are being incorporated into additional phase 2 trials in development (Bishop et al. 2016).

Numerous other novel agents are being tested by different groups. The anti-PD-1 antibody pembrolizumab failed to show encouraging activity against osteosarcoma in the SARC028 phase 2 trial (Tawbi et al. 2017). Current European activities were most recently discussed in 2015 and 2017 during workshops of European Bone Sarcoma Research Networks (Kager et al. 2016; Strauss et al. 2018). Several trials are worth mentioning. While addition of zoledronate to standard osteosarcoma therapy was feasible, a French multicenter randomized trial failed to demonstrate it provided a survival advantage (Piperno-Neumann et al. 2016). The Italian Sarcoma Group has performed phase 2 trials of sorafenib and of sorafenib with everolimus (Grignani et al. 2012, 2015). Signals of activity were observed, as has been the case for regorafenib in a randomized French phase 2 trial (Duffaud et al. 2018). A group of investigators from Baylor have published results of CAR-T cells directed to HER-2 for the treatment of osteosarcoma (Ahmed et al. 2015). The US National Cancer Institute is investigating CAR-T cells directed to GD2 as a treatment for osteosarcoma (Bishop et al. 2016). It is hoped that some of the agents will prove to be effective driving phase 3 trials of these agents in the future.

8.8 Local Treatment of the Primary Tumor

Chemotherapy alone is insufficient to cure osteosarcoma (Bielack et al. 2002; Jaffe et al. 2002) and local therapy therefore remains an integral component of curative treatment. A successful osteosarcoma resection should have a wide surgical margin (DeLaney et al. 2005; Enneking 1986) while providing optimal functional status. Mutilating procedures are still indicated if this goal cannot be reached otherwise, but the role for amputation is dwindling (Fig. 8.1), and it does not confer significant survival benefit over limb salvage (Reddy et al. 2015; Schrager et al. 2011). Radiotherapy is reserved for situations where appropriate surgery cannot be performed (Schwarz et al. 2009).

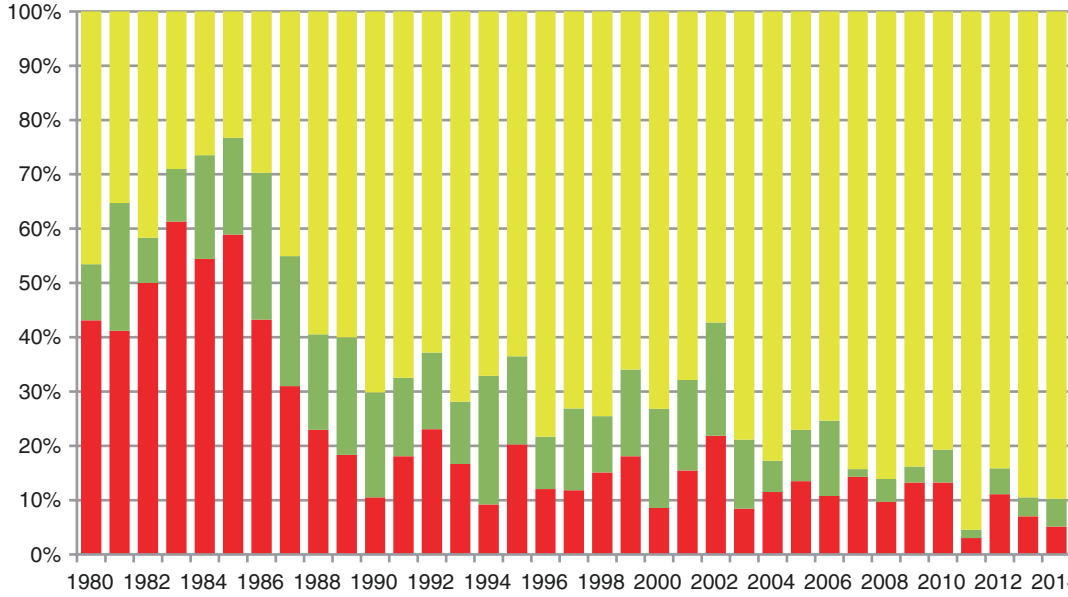

Fig. 8.1 Choice of definitive surgical procedures by year of osteosarcoma diagnosis. Data are from 2585 operated Cooperative Osteosarcoma Study Group COSS patients with previously untreated, localized high-grade central extremity osteosarcoma. Red, amputation; green, rotationplasty; yellow, limb salvage

Common limb salvage options include endoprosthetic reconstruction, allograft prosthetic composite (APC), massive bone allograft with soft tissue attachments, and rotationplasty. Reconstruction durability must be considered in children and adolescents, whose lifestyle expectations can vary tremendously.

In the pediatric or adolescent patient, endoprosthetic fixation is usually via a press-fit stem or a compress device. Screw and plate fixation is used with rotationplasty and massive bone allograft. Cement, while commonly used for prosthetic fixation in older patients, is used with caution in younger patients due to concerns of long-term aseptic loosening (Jeys et al. 2008).

General oncologic surgical principles should be adhered to during tumor resection, namely, meticulous hemostasis, careful identification and dissection of neurovascular structures, and maintenance of a soft tissue envelope around the osteosarcoma. Preoperative MRI is used for determining bony resection length (2 cm is recommended), and marrow margins should be sent as frozen sections after bone cuts are made and the canal plugged with bone wax (Loh et al. 2015). Once the tumor has been resected, gloves should be changed, fresh drapes placed over soiled portions of the surgical field, and separate instruments used during the reconstruction to avoid contamination. The tumor specimen should be oriented with a surgical pathologist, with mention of certain areas such as synovium or epineurium that may contain potentially close margins.

Proximal humerus tumors often necessitate resection of rotator cuff insertion(s). Unresected portions of anterior deltoid, supraspinatus, and subscapularis should be tagged as they are encountered to assist with reconstruction, and Gore-Tex or Alloderm can be used to reconstruct the joint capsule. In larger resections an APC with rotator cuff attachments may be favored over a traditional endoprosthesis. In younger children the biological alternative of clavicula pro humero should be considered (Calvert et al. 2015) as well as a vascularized free fibular transfer. In the proximal tibia, extensor mechanism involvement often dictates implant choice. The tibial insertion should be preserved when possible to promote bony ingrowth to the reconstruction. If the insertion is sacrificed with the articular

surface, then an APC with patellar tendon can be used. If the joint and epiphysis are spared, then massive intercalary allograft can be considered. The gastrocnemius may be included with the resection as a soft tissue margin or used as a rotational flap to cover the reconstructed extensor mechanism. If the tumor involves the tibial tubercle with extensive soft tissue involvement of the calf musculature, then rotationplasty may be the only alternative to through-knee amputation. In fact, rotationplasty, because of its predictable durability, may actually be preferred. Distal femur resections usually incorporate the vastus intermedius as a margin, and the reconstruction is covered with remaining musculature and a robust quadriceps tendon repair. In the proximal femur, although rare in children, critical soft tissue reconstruction involves reattachment of the abductors (preferably with a portion of the greater trochanter) and purse-string reapproximation of the joint capsule when possible.

The most common locations for osteosarcoma in the growing child are the distal femur, proximal tibia, and proximal humerus (Mirabello et al. 2009). Coincidentally this is where the most proliferative physes reside. If the physis can be spared, an intercalary reconstruction with allograft or fibula autograft may be considered, with graft incorporation and hypertrophy possible in skeletally immature patients (Aponte-Tinao et al. 2015). Remaining limb growth will aid with reconstruction choice if the physis is resected. A rotationplasty will continue to lengthen from the distal tibial physis (albeit half the rate of the distal femoral physis), and modular implant design can facilitate future lengthening procedures without sacrificing a well-incorporated base. Reconstruction can be intentionally long to accommodate for remaining growth or contralateral epiphysiodesis performed. Growing prostheses are still associated with poor implant survival rates and continued need for eventual surgical revision to adult-sized implants (Cipriano et al. 2015; Schinhan et al. 2015; Staals et al. 2015).

While surgery with wide margins remains the gold standard for local therapy, not all osteosarcomas are suitable candidates. This is particularly true for primaries located in the axial skeleton. It was demonstrated decades ago that radiotherapy can achieve temporary local remissions and that doses in excess of 60 Gy will lead to better control than lower doses (Cade 1952). The rate of permanent local control may increase if radiotherapy is administered within a multimodal context which also includes chemotherapy (DeLaney et al. 2005; Machak et al. 2003; Schwarz et al. 2009), when employed as part of first-line treatment rather than used against recurrences (DeLaney et al. 2005; Schwarz et al. 2009), and when it is used against osteosarcomas which show an imaging response to chemotherapy (Machak et al. 2003). Unless very high doses in excess of 70 Gy are used, it seems advisable to combine radiotherapy with subtotal resection (Ciernik et al. 2011; Schwarz et al. 2009). Innovative techniques such as proton (Ciernik et al. 2011) or carbon ion (Combs et al. 2012; Matsunobu et al. 2012; Sugahara et al. 2012; Zhang et al. 2016) radiotherapy have led to encouraging local control rates in some settings, but data on long-term effectiveness and side-effects are lacking (Leroy et al. 2016). A prospective trial of carbon ion radiotherapy in skeletally immature patients with unresectable osteosarcoma is currently ongoing (Blattmann et al. 2010).

8.9 Primary Metastases

Unless treated on specific trials, chemotherapy for patients with primary metastases usually reflects that used for those with localized disease. A good histological response to such therapy again confers a more favorable prognosis (Kager et al. 2003). All primary metastases should be resected if treatment is to be curative (Carrle and Bielack 2009; Kager et al. 2003). Owing to their matrix content, osteosarcoma metastases are often quite hard, and palpation may therefore detect more lesions than does CT (Kayton et al. 2006; Picci et al. 2001). Surgery for lung metastases is therefore generally performed by open thoracotomy (Carrle and Bielack 2009; Casali et al. 2018). Nonsurgical approaches such as per-

cutaneous computed tomography-guided thermal ablation (Yevich et al. 2016), radiofrequency ablation (Saumet et al. 2015), or stereotactic radiosurgery (Yu et al. 2014) may offer alternatives for lung metastases not eligible for surgery, their non-inferiority remaining to be established. Unresolved issues include the merit of contralateral exploration in seemingly unilateral pulmonary disease and how to proceed with small, nonspecific pulmonary nodules (Bhattasali et al. 2015; Carrle and Bielack 2009). Given the dismal outcome of patients in whom definitive metastases remain after surgery (Kager et al. 2003) and the inability to reliably distinguish small benign lung lesions from small lung metastases (Picci et al. 2001), many advocate an aggressive approach even for such "possible" metastases, but the benefit of this approach remains to be proven.

8.10 Prognosis After Multimodal Treatment

Despite chemotherapy and surgical resection, 30–40% of patients who initially present with localized disease will develop a recurrence. Recurrences most often affect the lungs but can also involve the former primary tumor site, distant bones or, less frequently, other sites including the brain, skin, and intraabdominal organs (Ferrari et al. 2003; Kempf-Bielack et al. 2005). At present the strongest prognostic factors for newly diagnosed osteosarcoma patients are the presence or absence of radiographically detectable metastatic disease and whether or not the primary tumor is resectable (Bielack et al. 2002). A large size of the primary tumor, a tumor site in the axial skeleton, or the proximal extremities and increased serum levels of alkaline phosphatase or lactate dehydrogenase have all been linked to inferior outcomes (Anderson 2016; Bielack et al. 2002). The presence of primary metastases is associated with an unfavorable prognosis particularly if these are multiple, involve both lungs, or involve several organ systems (Kager et al. 2003). Metastases which involve the pleura, chest wall, pericardium, or diaphragm are associated with very poor long-term survival expectancies. The prognosis of osteosarcoma patients with bone metastases, more than three lung nodules, or bilateral lung nodules is generally less than a 20% 5-year disease-free survival (Bielack et al. 2002; Kager et al. 2003).

Predictors of local recurrence include poor histologic response and narrow resection margins (Picci et al. 1994). Poor histologic response of the primary tumor is also a powerful predictive factor for distant recurrence and reduced overall-survival expectancies (Bielack et al. 2002) (Fig. 8.2).

8.11 Physical Rehabilitation and Surveillance for Late Effects

Complete surgical tumor resection is required for long-term survival (Bielack et al. 2002) but associated with functional impairments even years after treatment. Compared to other survivors bone tumor survivors are at higher risk of physical limitations (Ness et al. 2008, 2009) and chronic health conditions (Oeffinger et al. 2006). The degree of impairment appears related to the surgical procedure performed with greater functional impairment and activity limitations in patients treated with amputations (Marina et al. 2013).

Postoperative rehabilitation and physiotherapy can involve immediate passive range of motion, with partial weight-bearing recommended over the first 12 weeks for non-cemented implants to allow for bony ingrowth. Individual restrictions are made depending on the robustness of soft tissue reconstruction. Progressive weight-bearing as tolerated is possible with cemented components. It must be emphasized that function will likely not achieve preoperative levels, and activities of daily living are addressed and met first. Ensuring adequate time for rehabilitation before returning to sports and recreational activities can prove difficult with this age group.

Full-length standing films are taken for lower extremity resections to monitor for limb length

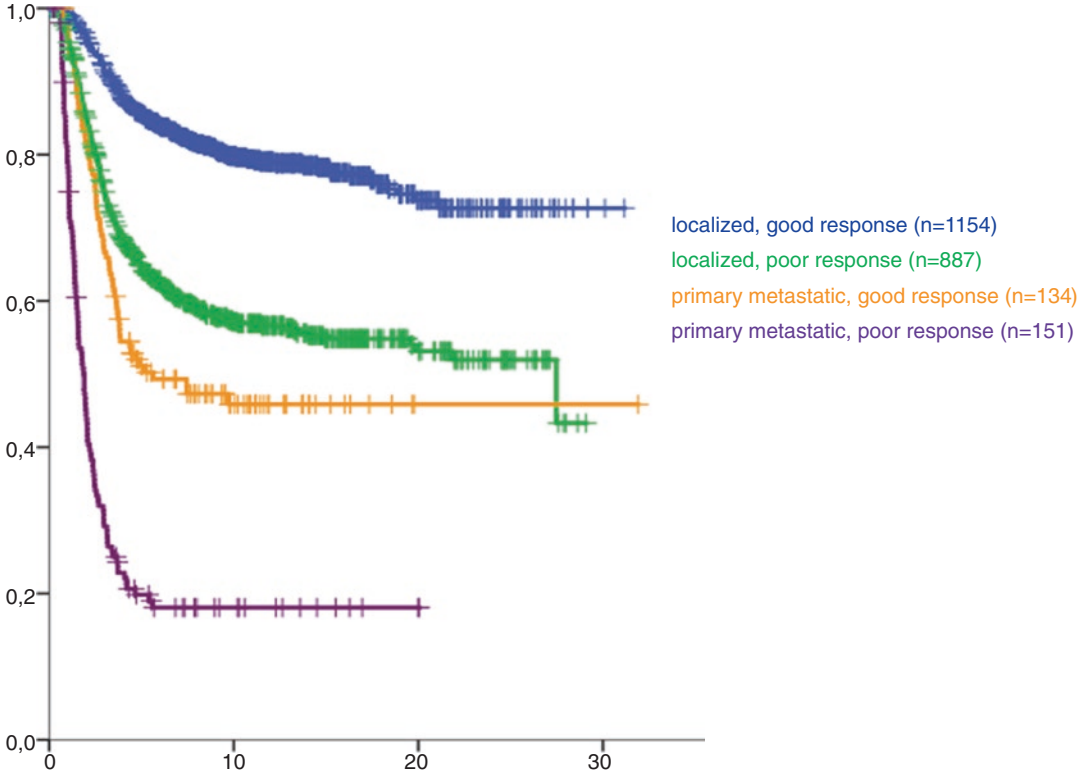

Fig. 8.2 Overall survival probability in correlation to primary metastatic status and response to preoperative chemotherapy. Data are from 2326 Cooperative Osteosarcoma Study Group COSS patients with previously untreated, high-grade central osteosarcomas of the extremities or axial skeleton. Good response: <10% viable tumor cells in the resection specimen

inequality. The need for eventual revision should be emphasized given the young age of these patients, with common transgressors including infection, aseptic loosening, limb length discrepancy, soft tissue instability, implant failure, and allograft nonunion (Jeys and Grimer 2009; Schinhan et al. 2015).

Treatment with multi-agent chemotherapy and surgery improves long-term survival expectancies (Link et al. 1991) but can also result in medical and psychosocial complications (Nagarajan et al. 2011). Medical consequences include the possibility of anthracycline-related cardiac dysfunction (Krischer et al. 1997; Lipshultz 2006; Nysom et al. 1998), gonadal dysfunction related to ifosfamide (Longhi et al. 2003; Williams et al. 2008), nephrotoxicity related to the use of cisplatin and/or ifosfamide (Buttemer et al. 2011; Rossi et al. 1999; Skinner 2003; Skinner et al. 1989), hearing loss related to treatment with cisplatin (Brock et al. 1991), and second malignant neoplasms most frequently associated with alkylating agents (Marina 1997; Smith et al. 2003; Tucker et al. 1987), etoposide (Le Deley et al. 2003; Stine et al. 1997), and anthracyclines (Le Deley et al. 2003).

Anthracycline cardiotoxicity ranges from subclinical to full-blown cardiomyopathy requiring chronic medical treatment (Lipshultz et al. 2008, 2013; Raj et al. 2014) and in some instances heart transplantation (Grande et al. 2003; Jenney and Jones 1992). The extent of cardiotoxicity is related to the total dose, cumulative dose, and the age at administration (Von Hoff et al. 1979). Since patients with osteosarcoma often receive high anthracycline doses, regular monitoring of

cardiac function by echocardiography is recommended (Armenian et al. 2015; Lipshultz et al. 2008, 2013).

Late toxicities can include gonadal dysfunction, particularly if treatment included the alkylator ifosfamide (Longhi et al. 2003; Williams et al. 2008). Patients should be offered the option of pretreatment fertility preservation so that they become knowledgeable about their options (Hug et al. 2012; Loren et al. 2013).

Patients treated with either cisplatin and/or ifosfamide should be monitored for renal dysfunction with electrolytes and creatinine once treatment is completed. Though renal failure is rare, tubular dysfunction leading to renal tubular acidosis and hypophosphatemic rickets can happen and continue for many years (Marina et al. 1995; Rossi et al. 1999; Skinner 2003; Skinner et al. 1989). Peripheral CDDP-associated neuropathy is especially problematic when treating older patients and, in contrast to vincristine-related neuropathy, most often presents after completion of CDDP and is irreversible (Avan et al. 2015).

Second malignant neoplasms are among the most feared complications (Nagarajan et al. 2011), and evaluation with complete blood count and radiograph for persistent pain and swelling are what is currently recommended.

8.12 Surveillance for Recurrence

Surveillance for recurrences focuses on the areas most likely to be affected, namely, the lungs and the former primary tumor site. Clinical surveillance usually involves at least 3-month intervals for 2 years, 3- to 6-month intervals for years 3–5, and semi-annual to annual visits thereafter (Meyer et al. 2008). Radiographs of the surgical site and particularly the chest are often performed at each visit. Rare recurrences may only arise during the second decade of follow-up (Wasilewski-Masker et al. 2009) or even later (Halldorsson et al. 2009), so that there is no unanimously agreed time point at which surveillance should end.

While recommended by some guidelines (Meyer et al. 2008), the use of chest CT rather than chest X-ray during routine follow-up has been challenged because of its high radiation burden (Dauer et al. 2008; McHugh and Roebuck 2014). Results from a recent randomized Indian study of sarcomas in general suggest that imaging during the first years should be every 3 rather than every 6 months, but that chest CT adds no survival benefit over chest X-ray (Puri et al. 2014).

8.13 Treatment Options in Case of Recurrence

Most patients with recurrent osteosarcoma will ultimately then die of their disease, but around 20–25% can be cured (Ferrari et al. 2003; Kempf-Bielack et al. 2005), and some may even survive multiple recurrences (Bielack et al. 2009). Factors favoring this are resectable disease and a first remission of 18 months or more. The mainstay of treatment is surgical resection (Daw et al. 2015). Repeated thoracotomies may be indicated. Surgical remission of disease at other sites can also be effective though repeated resection is associated with ever-shortening remission (Bacci et al. 2005; Bielack et al. 2009; Ferrari et al. 2003; Gelderblom et al. 2011; Kempf-Bielack et al. 2005).

The role of second-line chemotherapy is poorly defined by prospective evaluation. Its use correlates with limited prolongation of survival when used against unresectable recurrences but is more controversial in the setting of resectable recurrences (Ferrari et al. 2003; Kempf-Bielack et al. 2005). Responses are certainly seen to the most commonly applied regimen, ifosfamide and etoposide. These responses may reduce the risk of further recurrence when combined with surgical resection of all disease or provide palliative benefit. Responses have been increasingly reported to the combination of gemcitabine and docetaxel (Palmerini et al. 2016), but other effective new agents await to be identified. Radiotherapy can also improve symptoms and provide temporary disease control (Schwarz et al. 2009).

8.14 Conclusion

Successful treatment of osteosarcoma requires close collaboration between many diagnostic and therapeutic specialties. Multidisciplinary therapy consisting of surgery and chemotherapy leads to long-term, disease-free survival in 60–70% of patients. While the past decades have witnessed a major shift from amputation to limb-salvage surgery, chemotherapy still relies on the same few drugs as ever. Accordingly, despite dedicated multi-institutional and multinational efforts, survival expectancies stagnate. Efforts to better understand tumor biology are ongoing, and it is hoped that these will lead to the identification of suitable therapeutic targets for prospective trials and ultimately higher cure rates.

Acknowledgments The authors thank Matthias Kevric and Benjamin Sorg for their invaluable assistance with Figs. 8.1 and 8.2.

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9 Contemporary Approach to Therapy for Ewing Sarcoma

Steven G. DuBois and Uta Dirksen

9.1 Introduction

The management of patients with EWS requires a multidisciplinary team approach that includes pediatric/medical oncologists, orthopedic/general surgeons, radiation oncologists, and nurses. With this team approach and as a result of decades of cooperative group clinical trials, the majority of patients with newly diagnosed localized EWS will survive their disease. This chapter begins with a review of current therapies for this group of patients, along with the evidence base supporting current practice. The chapter then turns to the management of patients with more advanced disease (patients with newly diagnosed metastatic disease and patients with recurrent disease) who have not enjoyed the improvements in outcomes seen in patients with newly diagnosed localized tumors. The limited data on management of so-called Ewing-like sarcomas are then reviewed. The chapter will then conclude with a summary of late effects of the intensive therapies used to treat EWS.

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9.2 Treatment of the Patient with Newly Diagnosed Localized EWS

Stage at initial diagnosis (localized vs. metastatic) is a critical determinant of prognosis in EWS (Cotterill et al. 2000; Karski et al. 2016). Therefore, most cooperative groups have developed separate clinical trials or separate trial arms for patients with localized vs. metastatic disease. In North American cooperative group trials, additional variables have not been used to risk-stratify patients. In European cooperative group trials, tumor size and response to neoadjuvant chemotherapy have been used to assign patients to risk-adapted specific treatment regimens.

The successful management of patients with localized EWS requires systemic therapy as well as local measures (surgery, radiation, or combined modality approach) directed at the primary tumor. This portion of the chapter begins with an overview of contemporary clinical trials of systemic therapy approaches (summarized in Table 9.1) and concludes with a review of the studies evaluating approach to the primary tumor.

9.2.1 Systemic Therapy for Localized EWS

Trial INT-0091 established the combination of vincristine/doxorubicin/cyclophosphamide

C. A. S. Arndt (ed.), *Sarcomas of Bone and Soft Tissues in Children and Adolescents*, Pediatric Oncology, https://doi.org/10.1007/978-3-030-51160-9_9

Table 9.1 Contemporary clinical trials evaluating systemic therapies for patients with localized EWS

Trial	Group	Randomization (if applicable)	Event-free survival (EFS)	Reference
INT-0154	COG	VDC/IE VDC/IE aug	5-year EFS 72.1% 70.1%	Granowetter et al. (2009)
EICESS-92	EICESS (SR) EICESS (HR)	VDIA VDCA VDIA EVDIA	3-year EFS 74% 73% 47% 52%	Paulussen et al. (2008)
EW93	French SR IR HR	N.A.	5-year EFS 70% 54% 48%	Gaspar et al. (2012)
SSG IX	SSG	N.A.	5-year MFS 58%	Elomaa et al. (2000)
AEWS0031	COG	VDC/IE VDC/IE comp	5-year 65% 73%	Womer et al. (2012)
Euro-E.W.I.N.G 99	Euro-Ewing	Standard-risk localized VAI VAC	3-year EFS 78.2% 75.4%	Le Deley et al. (2014)
Euro-E.W.I.N.G 99	Euro-Ewing	High-risk localized Ongoing chemotherapy Busulfan/melphalan	3-year EFS 56.7% 69.0%	Whelan et al. (2018)
Protocol III	ISG/SSG	N.A.	5-year EFS 69%	(Ferrari et al. (2011)
Ewing1	BCSG	N.A.	5-year EFS 67.9%	Brunetto et al. (2015)

COG Children's Oncology Group, *EICESS* European Intergroup Cooperative Ewing's Sarcoma Study, *SSG* Scandinavian Sarcoma Group, *ISG* Italian Sarcoma Group, *BCSG* Brazilian Collaborative Study Group, *SR* standard risk, *IR* intermediate risk, *HR* high risk, *N.A.* not applicable, *V* vincristine, *C* cyclophosphamide, *D* doxorubicin, *E* etoposide, *I* ifosfamide, *A* actinomycin, *D* aug augmented dose regimen, *comp* interval-compressed regimen, *MFS* metastasis-free survival

alternating every 3 weeks with ifosfamide/etoposide (VDC/IE regimen) as a standard chemotherapy approach in North America for patients with localized EWS (Grier et al. 2003). Building upon the success of INT-0091, the subsequent North American Intergroup trial, INT-0154, sought to augment the doses of the alkylating agents used in the VDC/IE regimen (Granowetter et al. 2009). Specifically, the cyclophosphamide dose was increased from 1200 to 4200 mg/m^2/cycle (total planned cumulative dose of 10.8 vs. 12 g/m^2), and the ifosfamide dose was increased from 9 to 12 g/m^2/cycle (total planned cumulative dose of 72 g/m^2 in both arms). A total of 478 eligible patients with localized EWS were randomized to either the standard arm or the intensified arm. Survival outcomes were not significantly different between randomized arms, with a 5-year event-free survival (EFS) 72.1% vs. 70.1% for standard vs. intensified arms, respectively.

Following the results of INT-0154, the Children's Oncology Group (COG) attempted to intensify the VDC/IE regimen in trial AEWS0031 by studying administration of chemotherapy cycles every 2 weeks (interval compression) (Womer et al. 2012). In this randomized phase III trial, 568 eligible patients with localized disease were randomized to receive 14 cycles of VDC/IE administered every 3 weeks (standard timing) or every 2 weeks (interval-compressed timing). Interval compression was feasible in this context, with a median cycle interval of 15 days for patients randomized to that arm of the trial. Interval compression was associated with a statistically significant improvement in a 5-year EFS compared to standard timing (73% vs. 65%).

Rates of grade 3 and 4 toxicity and rates of second malignancy appeared to be similar between randomized arms. With these results, interval-compressed VDC/IE has become a standard regimen for the care of patients with localized disease in North America.

Based upon activity of topotecan/cyclophosphamide (TC) in the care of patients with relapsed EWS (reviewed below), the COG conducted a pilot study that established the feasibility of incorporating blocks of vincristine/TC in the context of interval-compressed chemotherapy (Mascarenhas et al. 2016). This pilot trial provided the data needed to launch a now completed COG trial for patients with localized disease (AEWS1031). This randomized phase III trial compared standard interval-compressed VDC/IE to VDC/IE with the addition of blocks of vincristine/TC, with results pending at this time.

In 1992, two multi-institutional European groups, the German Pediatric Hematology and Oncology with Austria, Switzerland, and the Netherlands and the UK Intergroup Children's Cancer Study group developed a randomized clinical trial, the Cooperative Ewing's Sarcoma Study (EICESS)-92 trial. This trial sought to compare the efficacy of ifosfamide and cyclophosphamide in treatment of patients with small (<100 mL tumor volume) localized EWS and to assess the role of etoposide in the care of patients with large (≥100 mL) localized EWS (Paulussen et al. 2008). One hundred fifty-five patients with small tumors were treated with induction therapy with vincristine/doxorubicin/ifosfamide/dactinomycin (VAIA regimen) and then randomized to post-induction therapy with either ongoing VAIA or with cyclophosphamide substituted for ifosfamide (VACA regimen). Three hundred twenty-nine patients with large tumors were randomized at study entry to receive VAIA or VAIA with the addition of etoposide (EVAIA regimen). The 3-year EFS rates were similar for patients with smaller tumors randomized to VAIA or VACA (74% vs. 73%), though there was a higher rate of hematologic toxicity with the latter regimen. Among patients with larger localized tumors, there was a trend to suggest a benefit of the EVAIA regimen over the VAIA regimen (hazard ratio 0.80 in favor of EVAIA).

The French EW93 trial adopted a risk-adapted, non-randomized approach for these patients (Gaspar et al. 2012). All 214 patients received identical initial chemotherapy with doxorubicin and cyclophosphamide for 3 cycles. Patients with at least 50% tumor regression received two additional cycles prior to planned local control. Patients with less than 50% tumor regression received two cycles of IE prior to local control. At the time of local control, patients were further risk-stratified based upon histologic response (for resected tumors) or initial tumor volume (for unresected tumors). Patients with <5% viable tumor or small unresected tumor were considered standard-risk and received consolidation therapy with VACA. Patients with 5–29% viable tumor or large unresected tumor were considered intermediate-risk and received VA and IE during consolidation. Patients with ≥30% viable tumor or an unresected tumor with <50% radiographic regression after 5 cycles of induction were considered high-risk and received IE followed by consolidation with high-dose busulfan/melphalan with autologous stem cell rescue. With this strategy, the overall 5-year EFS was 60%, with an estimate of 70% for standard-risk patients, 54% for intermediate-risk patients, and 48% for high-risk patients. Compared to the prior experience of the EW88 trial, the study team concluded that the intermediate-risk strategy did not improve outcomes, while the high-risk strategy may have improved outcomes.

The EURO-E.W.I.N.G. (EE) 99 trial, as a successor of the EICESS-92 and EW-93 trials, was a large European collaboration. The trial employed an intensive neoadjuvant chemotherapy regimen that includes cycles containing vincristine, ifosfamide, doxorubicin, and etoposide (VIDE). This regimen has been shown to be feasible, with nearly 90% of patients able to receive cycles at 21–28 day intervals (Juergens et al. 2006). This regimen has been applied as neoadjuvant chemotherapy in Euro-Ewing 99 for patients with localized disease and either a favorable response to neoadjuvant chemotherapy or a small initial tumor volume (Le Deley et al. 2014). Following neoadjuvant VIDE, patients in this "R1" standard-risk arm of the study were randomized to receive adjuvant therapy with

either vincristine, dactinomycin, and cyclophosphamide (VAC) or vincristine, dactinomycin, and ifosfamide (VAI). A total of 856 patients were randomized in this non-inferiority trial. The VAC arm was determined to be statistically non-inferior to the VAI arm, with 75.4 vs. 78.2% 3-year event-free survival rates, respectively. These results indicate that either regimen provides acceptable disease control in this group of patients. Late toxicity data are pending from this trial and will help to inform optimal approach for future patients. Patients, who were classified as "high-risk" localized disease, were those with a poor histologic response (≥10% viable cells after six courses of VAI therapy or had a tumor volume of ≥200 mL at diagnosis. High-risk localized patients were randomized to either high-dose chemotherapy (HDC) with busulfan/melphalan or to ongoing conventional chemotherapy. For these patients, HDC conferred a survival advantage (Whelan et al. 2018).

The successor trial of the EE99 trial, the international EWING 2008 trial, is a collaboration of 11 nations. All patients receive the VIDE induction regimen. For patients with standard-risk disease (tumors <200 mL and/or favorable histological response to induction chemotherapy), a gender-adapted maintenance treatment with VAC in female and VAI in male patients is used, and all patients are randomized for an add-on of zoledronic acid. Second, in the high-risk group (tumors ≥200 mL and/or unfavorable histological response to induction chemotherapy), a randomization between standard chemotherapy vs. high-dose chemotherapy with autologous stem cell rescue was continued from the EE99 trial, and the advantage from high dose for a subgroup of patients has been published (Whelan et al. 2018).

In the EE 2012 protocol, conducted by the UK, France, Spain, and the EORTC, the type of neoadjuvant chemotherapy is randomized between the VIDE regimen and an interval-compressed VDC/IE regimen. The randomization has been stopped prior to end of the trial as an advantage for VDC/IE has been observed in a 2-year event-free survival (personal communication, Dr. Brennan). Patients are also randomized in a second randomization for an add-on with zoledronic acid or no add-on.

Outside of the COG and EICESS/Euro-Ewing cooperative studies, a number of other groups have conducted prospective trials in patients with localized disease. The Italian and Scandinavian sarcoma groups employed a non-randomized approach for patients with localized disease and poor response to initial therapy (Ferrari et al. 2011). Patients were defined as having a poor response following neoadjuvant multiagent chemotherapy if they had macroscopic foci of residual tumor seen after resection or if they did not undergo resection and had imaging evidence of residual soft tissue disease prior to radiotherapy. Poor responders constituted half of the 300 patients and received busulfan/melphalan HDC in a non-randomized manner. The overall 5-year EFS for the entire study population was 69%, with good responders having a 5-year EFS of 75% compared to 63% for poor responders. Outcomes were superior for poor responder patients in this trial compared to historical comparisons.

A number of trials have investigated the use of platinum-containing regimens in the treatment of newly diagnosed patients with localized disease. The Scandinavian IX protocol utilized cycles of vincristine/doxorubicin/ifosfamide and cisplatin/doxorubicin/ifosfamide administered at 3-week intervals (Elomaa et al. 2000). The 5-year metastasis-free survival estimate was 58%. This outcome was improved compared to the group's prior regimen, though does not compare favorably with other contemporary regimens.

The Brazilian Ewing1 trial also evaluated a platinum-containing regimen (Brunetto et al. 2015). The trial included 107 patients with localized disease treated with VDC alternating with ifosfamide, carboplatin, and etoposide (ICE) during induction. After local control, patients with low-risk clinical features (resectable and normal LDH) then received consolidation therapy with VDC/IE. All other patients received consolidation therapy with VDC/IE and two additional cycles of ICE. The 5-year EFS rate was 67.9%, similar to that reported in other contemporary trials.

9.2.2 Local Control Strategies for Localized EWS

In contradistinction to osteosarcoma, EWS is a radiosensitive tumor. Therefore, local control of the primary tumor can consist of definitive surgical resection, definitive radiotherapy, or the combination of surgery with radiation. The optimal mode of local control in EWS has been an area of controversy in the field. While multiagent chemotherapy is the standard of care and has been structurally evaluated in prospective clinical trials, the best approach for local control remains a matter of discussion and may be dependent on site and size of the tumor. Consequently, local treatment requires a personalized approach. The controversy arises from the inherent barriers to conducting a randomized trial comparing modes of local control. Therefore, the evidence base in this area is comprised of observational studies, with varying attempts to control for confounding variables that impact the choice of local control for an individual patient. For example, two large pooled studies derived from serial cooperative group trials have demonstrated that patients selected for definitive radiotherapy have inferior outcomes compared to patients selected for definitive surgery (DuBois et al. 2015; Schuck et al. 2003). However, these studies have also typically shown the group of patients selected for definitive radiation is enriched for adverse prognostic factors, such as older age, pelvic primary site, and larger tumor size (DuBois et al. 2015; Schuck et al. 2003).

Studies that have controlled for confounders have helped to clarify the impact of choice of local control modality on clinical outcomes. For example, the COG performed a propensity score analysis of 465 patients with localized EWS all treated with VDC/IE (DuBois et al. 2015). After controlling for a range of potential confounders, including age, tumor site, and tumor size, there was not a difference in EFS, overall survival, or distant failure between patients selected for definitive radiotherapy and patients selected for definitive surgery. However, patients selected for definitive radiotherapy had approximately double the risk of local failure compared to patients selected for definitive surgery. Based upon findings such as these and the risk of second malignancy associated with radiation (see Late Effects subsection below), definitive surgery is the preferred approach whenever feasible, with definitive radiation reserved for the care of patients with unresectable tumors.

A number of studies have focused on local therapy for EWS arising at specific sites. For example, analyses of patients with chest wall EWS (ribs, scapula, clavicle, sternum, and soft tissue) have demonstrated several important points. First, the addition of radiation therapy following complete surgical resection does not appear to improve outcomes (Bedetti et al. 2015). Second, surgical outcomes are superior following delayed resection after neoadjuvant chemotherapy (Shamberger et al. 2003). Third, outcomes appear to be similar with resection of the entire involved rib compared to partial rib resection (Bedetti et al. 2015). In a retrospective study, 198 patients with nonmetastatic Ewing sarcoma of the chest wall treated in the EE 99 trial have been analyzed in detail (Bedetti et al. 2015). Local treatment included surgery alone (85 patients, 43%), surgery followed by radiotherapy (106 patients, 53.5%), and definitive radiotherapy (7 patients, 3.5%). The study showed that only patients with intralesional resection benefit from additional radiotherapy.

Management of pelvic EWS also provides unique challenges. A detailed review of 241 patients with pelvic disease demonstrated that the majority of these tumors are large and the most common site is the iliac wing (Hoffmann et al. 1999). Patients with pelvic and other axial primary tumors are at higher risk of having inadequate surgical margins if surgery is attempted (46% inadequate margin rate for axial tumors vs. 15% for appendicular tumors) (Ozaki et al. 1996). In one series of 75 patients derived from INT-0091, 59% did not have attempted surgical resection and instead received definitive radiation alone (Yock et al. 2006). Local failure rates were similar compared to patients treated with either definitive surgery or definitive radiation. However, in a series of 39 patients with pelvic primary tumors, surgery or combined local treat-

ment appeared to be of benefit for patients (Raciborska et al. 2014). Likewise, an updated pooled analysis from the Children's Oncology Group showed that patients with pelvic tumors treated with definitive radiotherapy had the highest local failure rate (Ahmed et al. 2017).

A number of studies have investigated radiotherapy strategies for patients with EWS. One of the few trials to evaluate radiotherapy approaches in a randomized manner was conducted by the Pediatric Oncology Group (Donaldson et al. 1998). In this trial, patients who received definitive radiation for local control were randomized to receive whole bone radiotherapy or involved field radiotherapy. There was no difference in EFS or rate of local failure between randomized arms, and therefore involved field radiotherapy has become the standard approach for patients who require radiotherapy.

The optimal radiotherapy dose has been evaluated in a number of studies. A comparative analysis of CESS-81, CESS-86, and EICESS-92 demonstrated a higher local failure rate after definitive radiation on the CESS-81 trial (Schuck et al. 2003). The CESS-81 trial included a small number of patients with extremity tumors randomized to receive 46 vs. 60 Gy, and no difference in relapse rate was seen (Jurgens et al. 1988). A single-institution experience that utilized a dose of 35 Gy for tumors that responded to induction chemotherapy concluded that this dose provided inadequate local control (Arai et al. 1991). Based upon this experience, contemporary protocols treat most sites selected for definitive radiotherapy with 54 Gy.

9.3 Treatment of the Patient with Newly Diagnosed Metastatic EWS

9.3.1 Systemic Therapy for Metastatic EWS

The majority of patients with metastatic EWS are treated either following regimens utilized in the care of patients with localized disease or on clinical trials seeking to improve outcomes for this group of patients. INT-0091 demonstrated that the addition of IE to VDC did not improve outcomes for patients with metastatic EWS (Grier et al. 2003). Likewise, the EICESS-92 trial included patients with metastatic disease in the randomized comparison between VAIA and EVAIA (Paulussen et al. 2008). A subgroup analysis focused on patients with metastatic disease demonstrated similar outcomes between VAIA and EVAIA (hazard ratio of 0.96).

As the addition of IE or etoposide has not improved outcomes for these patients, another approach has been to intensify dosing of agents standardly used in the care of patients with EWS. This strategy has unfortunately also not improved outcomes. For example, INT-0091 included a cohort of 60 patients with metastatic disease treated with VDC/IE using augmented doses of doxorubicin, ifosfamide, and cyclophosphamide, without improved outcomes compared to patients treated with standard doses of VDC/IE or standard doses of VDC (Miser et al. 2007). Likewise, a single-institution report of patients with metastatic EWS treated with augmented doses of ifosfamide (2.8 g/m^2/day × 5 days) concluded that this approach did not improve outcomes (Magnan et al. 2015). Outcomes following interval compression of chemotherapy cycles have not yet been reported in metastatic EWS, though will be forthcoming from a recently completed trial (AEWS1221).

The use of camptothecins has also been investigated in this population. The COG reported on a trial in patients with metastatic EWS in which patients were treated with window therapy of either topotecan monotherapy or TC (Bernstein et al. 2006). Only 8% of patients treated with monotherapy responded compared to 57% of patients treated with the TC regimen, though overall outcomes were similar to those previously reported for this group of patients. The EE 99 group treated 23 patients with extrapulmonary metastasis with a window of irinotecan monotherapy and reported a 24% response rate after 2 cycles (Morland et al. 2014).

A number of groups have investigated the role of high-dose chemotherapy with autologous stem cell rescue in the treatment of patients with meta-

static EWS. The Children's Cancer Group conducted a prospective trial of post-induction myeloablative therapy with melphalan, etoposide, and total body irradiation (TBI) for patients with newly diagnosed EWS and metastasis involving bone and/or bone marrow (Meyers et al. 2001). Of the 32 eligible patients, 22 met criteria to proceed to myeloablative therapy. The 2-year EFS was 20% for the entire cohort and 24% for patients who received myeloablative therapy. Based upon historic experience with this group of highest-risk patients, the study team concluded that this approach did not improve outcomes.

A European report evaluated the role of TBI in the management of patients with newly diagnosed disease involving the bone and/or bone marrow and of patients with early or multiple relapse (Burdach et al. 2003). Fifty-four patients were treated with one of two conditioning regimens in two sequential trials. In the first trial, TBI was combined with melphalan and etoposide. In the second trial, tandem cycles of melphalan and etoposide were administered. The 5-year EFS estimate was similar between trials (22% for the regimen with TBI and 29% for the regimen with tandem).

A combined Italian and Scandinavian sarcoma group trial included 102 patients with metastatic disease involving the lung, pleura, or no more than one bone metastasis (Luksch et al. 2012). Patients received multiagent chemotherapy and local control followed by busulfan/melphalan conditioning and autologous stem cell rescue. After recovery from high-dose therapy, patients with lung metastasis also received whole lung radiation. Seventy-nine patients received high-dose therapy. The 5-year EFS for the full cohort was 43% in this more favorable group of patients. Responses of the primary tumor and of lung metastases were identified as important prognostic factors.

The French group evaluated 97 patients with newly diagnosed metastatic disease in a single-arm trial using VDC/IE induction followed by busulfan/melphalan high-dose chemotherapy with autologous stem cell rescue (Oberlin et al. 2006). Seventy-five patients met criteria to proceed to high-dose chemotherapy. The 5-year EFS rate for the entire cohort of 97 patients was 37%, suggesting a potential role for this approach compared with prior experience with this population.

The EE 99 trial included a non-randomized arm for patients with extrapulmonary metastatic disease in which all patients were planned to undergo high-dose chemotherapy (Ladenstein et al. 2010). Sixty percent of the 281 patients underwent high-dose chemotherapy, and the 3-year event-free survival rate for the full cohort of 281 patients was 27%. Euro-Ewing 99 also investigated the role of HDC with busulfan/melphalan and stem cell rescue in a randomized fashion in patients with isolated lung metastases. Patients were randomized to either HDC (without lung radiation) or continuation of chemotherapy with lung irradiation at the end. The trial was continued in the successor trial EWING 2008. In this group of patients, there was no benefit to HDC compared to continuation of standard chemotherapy with whole lung radiotherapy (Dirksen et al. 2016).

Another report described outcomes for 18 patients with metastatic EWS and extrapulmonary metastasis planned for therapy with tandem high-dose chemotherapy with thiotepa and busulfan/melphalan conditioning (Loschi et al. 2015). The 3-year overall survival rate was 22%, and the authors concluded that this strategy did not improve outcomes compared to those expected for this population. Another retrospective analysis focused on the value of allogeneic stem cell transplantation. Eighty-seven patients registered in the European Group for Blood and Bone Marrow Transplantation or the Pediatric Registry for Stem Cell Transplantations or in the Asia Pacific Blood and Bone Marrow Transplantation registries have been analyzed. Fifty patients received reduced intensity conditioning (RIC), and 37 received high-intensity conditioning (HIC). Sixty-three patients received marrow from HLA-matched donors and 24 haploidentical or otherwise mismatched donors. In the patients who received RIC, death due to complication occurred in 4 patients and death of disease in 33 patients. In patients who received HIC, the death due to complication rate was significantly higher

and was reported in 16 patients. In contrast, the death of disease rate was slightly lower and occurred in 17 patients. The authors conclude that no survival benefit was achieved in either of the regimens. With a 5-year overall survival of 10–15%, the outcome in the allogeneic setting seemed not improved compared to other studies (Thiel et al. 2011).

In the international EWING 2008 protocol, patients with primary disseminated disease are participating in a randomized comparison between ongoing conventional chemotherapy and ongoing conventional maintenance chemotherapy plus HDC with treosulfan/melphalan with autologous stem cell rescue.

With the aforementioned approaches, the study of novel agents in this population is a high priority. A large body of research has suggested a role for antiangiogenic therapy in EWS (DuBois et al. 2010). In a study, AEWS02P1, the COG studied a metronomic antiangiogenic approach in patients with newly diagnosed metastatic EWS (Felgenhauer et al. 2013). In this non-randomized study, 35 non-eligible patients were treated with VDC/IE with the addition of celecoxib and vinblastine. While the approach was feasible, outcomes were similar to prior studies in this population, with a 2-year EFS rate of 35%. Notably, one third of the patients who received celecoxib plus whole lung irradiation developed pulmonary toxicity > grade 2, including two deaths. The COG has completed a randomized phase III trial of the IGF-1R monoclonal antibody ganitumab added to interval-compressed chemotherapy for patients with newly diagnosed metastatic EWS, with results forthcoming (see Systemic Therapy for Recurrent EWS section for rationale). The St. Jude Children's Research Hospital evaluated the addition of irinotecan, temozolomide, and temsirolimus to standard therapy in this setting, with results pending.

9.3.2 Role of Surgery and Radiation in Metastatic EWS

In addition to management of the primary tumor, surgery and radiation may play a role in the management of metastatic disease. For example, several studies point to a benefit of whole lung radiotherapy in patients with pulmonary metastatic Ewing sarcoma. Perhaps the strongest evidence supporting this approach has been derived from the EICESS experience. In a series of 114 patients with isolated pulmonary metastatic disease, the addition of whole lung radiotherapy was associated with improved outcomes on univariate and multivariate analyses (Paulussen et al. 1998a). In a cohort of patients with pulmonary metastases and other sites of metastatic disease, the addition of whole lung radiotherapy was also associated with improved outcomes (Paulussen et al. 1998b). Beyond these EICESS analyses, a single-institution report described 28 patients with newly diagnosed disease and lung metastasis treated with and without whole lung radiation (Spunt et al. 2001). The group of patients treated with whole lung radiation was enriched for patients with incomplete response to induction chemotherapy and therefore anticipated to have inferior outcomes compared to patients with complete response to induction chemotherapy who were less likely to be selected for whole lung radiation. Overall survival was similar between both groups, suggesting that whole lung radiation may have abrogated the adverse prognostic impact of incomplete chemotherapy response. A series of 26 adults with pulmonary metastatic EWS treated with 12–15 Gy whole lung radiation demonstrated that this modality was tolerable in an adult setting and 45% of patients were free from pulmonary recurrence at 3 years (Casey et al. 2014).

Radiation also plays a role in the management of bone metastasis. One case series reported on 22 patients with EWS and 8 patients with rhabdomyosarcoma and bone metastases managed with a range of radiotherapy regimens (hypofractionation, hyperfractionation, and standard fractionation) (Casey et al. 2015). Local control of irradiated bone metastasis was good, with a 9% cumulative incidence of local failure at 3 years.

The role of surgical resection of metastatic disease in the care of a patient with newly diagnosed EWS has been less well studied. One secondary analysis from the EICESS studies was

unable to demonstrate a survival benefit for resection of pulmonary metastases in this setting (Paulussen et al. 1998a).

In a more recent analysis, the GPOH group demonstrated benefit from local treatment in 120 patients with primary disseminated disease. The 3-year EFS in the entire group was 24%. Forty-seven patients were given local treatment of both the primary tumor and extrapulmonary metastases, whereas 41 patients had either the primary tumor or extrapulmonary metastases treated, and 32 patients did not receive any local therapy. The multivariate analysis considering tumor size, number of bone metastases, age, and application of high-dose chemotherapy showed that absence of local treatment was the only significant adverse prognostic factor (Haeusler et al. 2010).

9.4 Treatment of the Patient with Recurrent EWS

The management of a patient with recurrent EWS requires a careful understanding of the goals of care. For patients continuing to pursue anticancer therapy, a number of options are available that may alleviate symptoms of underlying tumor, prolong life, and, in some cases, achieve long-term disease control even in the recurrent setting. In addition, these patients are often candidates for clinical trials of novel agents.

In order to help inform goals of care, several groups have reported on potential prognostic factors following recurrence (Ferrari et al. 2015; Leavey et al. 2008; Stahl et al. 2011). The most consistent finding across these studies is the importance of time to first relapse as a key determinant of long-term outcomes. Patients who recur within 2 years from initial diagnosis have a very low probability of long-term survival, while approximately 30% of patients with later relapses may be alive 5 years post-relapse (Leavey et al. 2008; Stahl et al. 2011; Shankar et al. 2003). Additional favorable prognostic factors at relapse have included local relapse, younger age, isolated pulmonary recurrence, and low lactate dehydrogenase (LDH) value at initial presentation (Ferrari et al. 2015; Leavey et al. 2008; Stahl et al. 2011). Combined local and distant relapses portend a poor outcome (Leavey et al. 2008; Stahl et al. 2011). In one series, only 39% of all patients with first recurrent disease were able to achieve a second complete remission (Ferrari et al. 2015).

9.4.1 Systemic Therapy for Recurrent EWS

A number of studies have demonstrated activity of camptothecin-based chemotherapy regimens in the care of patients with recurrent EWS. The combination of topotecan and cyclophosphamide has a response rate of approximately 35% in phase II trials in this setting (Hunold et al. 2006; Saylors 3rd et al. 2001). A number of case series have described response rates ranging from 28 to 68% in patients with relapsed EWS treated with irinotecan and temozolomide (with or without vincristine) (Casey et al. 2009; Raciborska et al. 2013; Wagner et al. 2007), though a formal phase II trial has not been reported in this setting.

Some groups have pursued the combination of gemcitabine and docetaxel in the management of patients with relapsed EWS. This combination appears to have some activity in this setting, though response rates have typically been lower than those reported for patients treated with a camptothecin-based regimen. Three single-institution retrospective studies of this combination in patients with relapsed sarcoma included a total of ten patients with EWS, four with objective responses (Mora et al. 2009; Navid et al. 2008; Rapkin et al. 2012). In contrast, a formal phase II trial of this combination included 14 patients with relapsed EWS, with only 2 responses reported (Fox et al. 2012).

A range of other chemotherapy regimens has been employed in this setting, though none has yet been widely adopted. For example, more than 30% of patients treated with high-dose ifosfamide had a partial or complete response, even if previously treated with standard doses of ifosfamide (Ferrari et al. 2009). The combination of etoposide with either carboplatin or cisplatin was described in a retrospective series of 107 patients

with recurrent EWS (van Maldegem et al. 2015). The combination with carboplatin resulted in a higher response rate (51% vs. 29%) and a higher mean progression-free survival time (14.5 vs. 6.3 months) compared to the combination with cisplatin.

The ongoing rEEcur trial seeks to clarify optimal chemotherapy backbone for patients with first recurrent Ewing sarcoma. Patients are randomized to irinotecan/temozolomide, topotecan/cyclophosphamide, high-dose ifosfamide, or gemcitabine/docetaxel.

Several reports have suggested a potential role for high-dose chemotherapy with autologous stem cell rescue in the management of patients with recurrent EWS. A single-institution retrospective study demonstrated that receipt of high-dose chemotherapy was associated with superior outcomes on multivariate analysis (Barker et al. 2005). More recently, a retrospective GPOH analysis evaluated outcomes for 68 patients with recurrent disease and at least a partial response to 4 cycles of conventional chemotherapy (Rasper et al. 2014). Patients treated with high-dose chemotherapy had superior outcomes on univariate and multivariate analyses compared to patients who did not receive high-dose chemotherapy.

Given the unsatisfactory outcomes with these chemotherapy approaches, a number of groups are studying novel targeted agents that may have activity in patients with EWS (Table 9.2). Perhaps the best-studied class of agents is the IGF-1R monoclonal antibodies. Early studies demonstrated a high prevalence of IGF-1R expression on EWS cell lines (Yee et al. 1990), and the negative regulator of the IGF-1 pathway, IGFBP3, is downregulated in the presence of *EWSR1/FLI1* (Prieur et al. 2004). Preclinical studies have demonstrated antitumor activity of antibodies targeting the IGF-1R. In a series of phase I and II clinical trials, monotherapy with anti-IGF-1R monoclonal antibodies yielded objective response rates of 10–15% and another subset of patients with tumor regressions not qualifying as partial responses (Juergens et al. 2011; Malempati et al. 2012; Olmos et al. 2010; Pappo et al. 2011; Tap et al. 2012; Tolcher et al. 2007). To date, biomarkers of clinical benefit to this class of agents have been elusive. These findings have prompted follow-up studies of cixutumumab (formerly known as IMC-A12) in combination with temsirolimus, with mixed results. In 1 adult trial, 2 of 17 patients with recurrent EWS had complete responses, and an additional 3 patients had tumor

Table 9.2 Targeted therapies evaluated in preclinical and early phase clinical studies for patients with recurrent EWS

Agent or class of agents	Stage of development in EWS	Reference(s)
IGF-1R inhibitory monoclonal antibodies	Phase II	Juergens et al. (2011), Malempati et al. (2012), Olmos et al. (2010), Pappo et al. (2011), Tap et al. 2012, Tolcher et al. (2007), Naing et al. (2011), Schwartz et al. (2013), Fouladi et al. (2015), Wagner et al. (2015)
PARP inhibitors	Phase II	Garnett et al. (2012), Brenner et al. (2012), Lee et al. (2013), Norris et al. (2014), Stewart et al. (2014), Choy et al. (2014)
YK-4-279/ TK216	Phase I	Erkizan et al. (2009)
Modified autologous tumor cell vaccine	Phase II	Ghisoli et al. (2015)
Car T cells	Phase I/II	NCT03356782
Pbi-shRNA™ EWS/FLI1 type 1 Lipoplex (LPX)	Phase I	NCT02736565
Immune checkpoint inhibitors	Phase II	NCT02304458; NCT03190174
CDK4/6 inhibitors	Phase I	Kennedy et al. (2015)
MDM2 inhibitors	Phase 1	NCT03654716
177Lu-3BP-227	Phase I	NCT03525392
Transcriptional CDK inhibitors	Preclinical	Iniguez et al. (2018)
LSD 1 inhibitors	Phase I	Sankar et al. (2013, 2014)

regressions >20% (Naing et al. 2011). In another adult trial of the same combination, 4 of 27 patients with recurrent EWS had objective responses (Schwartz et al. 2013). In contrast, phase I and II pediatric trials of this same combination included a total of 16 patients with EWS and reported no objective responses (Fouladi et al. 2015; Wagner et al. 2015). In a European consortium trial, linsitinib (formerly known as OSI -903), an oral small molecule inhibitor of both IGF-1R and the insulin receptor, has been tested in patients with recurrent EWS, with results pending.

An unbiased drug screen identified the presence of *EWSR1/FLI1* as a biomarker of sensitivity to PARP inhibition (Garnett et al. 2012). This finding stimulated follow-up preclinical studies that confirmed this finding and demonstrated additive activity in combination with concomitant DNA damaging agents or radiation (Brenner et al. 2012; Lee et al. 2013; Norris et al. 2014; Stewart et al. 2014). In the clinic, a phase II trial of olaparib monotherapy enrolled 12 patients with recurrent EWS and observed no responses (Choy et al. 2014). Phase I and II trials of PARP inhibitors in combination with temozolomide and/or irinotecan are ongoing.

Targeting lysine-specific demethylase-1 (LSD1) in preclinical models of EWS has been shown to reverse the aberrant gene expression profile associated with *EWSR1/FLI1* (Sankar et al. 2014). Pharmacologic LSD1 inhibition abrogates the growth of EWS in vitro or in vivo (Sankar et al. 2013, 2014). This class of agents has entered the clinic, with a trial for patients with EWS ongoing (NCT03600649).

The aforementioned strategies seek to exploit vulnerabilities associated with the presence of *EWSR1/FLI1*. Attempts to target the EWSR1/FLI1 fusion oncoprotein directly have been hampered by challenges associated with drugging an aberrant transcription factor. However, more recently a small molecule (YK-4-279) has been identified that interferes with the ability of EWSR1/FLI1 to interact with RNA helicase A (Erkizan et al. 2009). This agent has shown antitumor activity in in vitro and in vivo models of EWS. A clinical trial of a clinical grade formulation known as TK216 is ongoing (NCT02657005).

Immunotherapy approaches have also been attempted in the management of patients with recurrent EWS. One trial utilized autologous T cells and dendritic cells pulsed with peptides derived from *EWSR1/FLI1* breakpoints (Mackall et al. 2008). This trial reported that the breakpoint peptides elicited an immune response in a minority of patients and that response was typically only transient. A follow-up trial utilized autologous tumor cells modified to express granulocyte-macrophage colony-stimulating factor and knockdown of furin, thereby reducing transforming growth factor expression (Ghisoli et al. 2015). Among 12 patients with recurrent EWS, 1 patient had a partial response, and overall survival at 1 year was 75%. A follow-up randomized trial of irinotecan/temozolomide with or without the addition of modified autologous tumor cells for patients with first recurrent disease is ongoing (NCT03495921). Trials of immune checkpoint inhibitors with specific strata for patients with EWS are also ongoing.

Several studies focus on the introduction of a personalized medicine approach in pediatric cancers, including EWS (e.g., GPOH-INFORM (Worst et al. 2016), France-ESMART)). The aim of these studies is to identify targets on the individual tumor in order to provide a personalized medicine recommendation. Pilot studies are ongoing and results are pending.

9.4.2 Role of Surgery and Radiation in Recurrent EWS

While there is a clear role for local control strategies in the management of patients with newly diagnosed EWS, the role of surgery and/or radiation is less clear in the management of a patient with recurrent disease. The decision to pursue local control at the time of relapse may depend upon a number of factors, such as response to second-line chemotherapy, time to relapse, and symptom burden associated with sites of relapse disease. However, there are data that patients with recurrent disease treated with a multimodal-

ity approach including chemotherapy with local control measures have superior response rates (Shankar et al. 2003).

Limited data are available on the role of surgical resection of recurrent tumor. One series of 12 patients with recurrent disease involving the lungs only reported outcomes after surgical resection of lung metastasis without additional disease (Bacci et al. 1995). Interestingly, five patients survived without disease for 3 or more years, suggesting a role for surgical management of recurrent disease. A follow-up report by the same group described 24 patients with isolated pulmonary recurrence managed with surgical resection with or without whole lung radiotherapy as a component of therapy. The 5-year overall survival rate for patients selected for surgery was 55% compared to 24% for a comparison group of 34 patients who did not undergo surgery (Briccoli et al. 2004).

Radiation may play a particular role in palliating patients with painful sites of recurrent tumor. Increasingly, there is interest in short-course radiation approaches for this indication. One case series of 8 patients with metastatic EWS with bone metastases treated with a median dose of 40 Gy in 5 fractions demonstrated the feasibility of this approach (Brown et al. 2014). Patients who have not already had whole lung radiation as part of their initial therapy may receive whole lung radiation as part of management of recurrent pulmonary metastatic disease, though the evidence base to support this approach is sparse (Briccoli et al. 2004). A recent analyses on 139 patients registered in the relapse registry of the Cooperative Ewing Sarcoma Study group whole lung irradiation improved survival in patients by 14%, and response of pulmonary lesions to systemic treatment was a significant prognostic factor (Scobioala et al. 2018). No severe pulmonary function disorder or lung toxicities were observed after WLI treatment in both pediatric and adult patients with an isolated pulmonary relapse. WLI was tolerated well in this setting.

9.5 Management of Ewing-Like Sarcoma

Recently, a group of sarcomas has been described that shares some clinical features with EWS but lacks a typical *EWSR1/ETS or FUS/ETS* translocation (such as *EWSR1/FLI1*). These tumors have therefore been termed "Ewing-like sarcomas" but are a heterogeneous group. The two most commonly reported entities have distinct features. Tumors harboring a *CIC/DUX4* translocation have been described in young adults with a male predominance (Italiano et al. 2012; Specht et al. 2014). These tumors are typically soft tissue tumors and appear to have inferior outcomes compared to EWS (Italiano et al. 2012). Tumors harboring a *BCOR/CCNB3* translocation tend to arise in younger patients, also have a male predominance, and are typically primary bone tumors (Cohen-Gogo et al. 2014; Peters et al. 2015; Pierron et al. 2012). These tumors appear to have more favorable outcomes, similar to those reported in patients with EWS.

The evidence base supporting the management of patients with these tumors is sparse. Perhaps due to the name "Ewing-like sarcoma," these patients are commonly treated following regimens established for the care of patients with EWS. Given the completely different biology of these tumors (Specht et al. 2014; Pierron et al. 2012), it is not clear whether systemic and local approaches derived from those used to treat patients with EWS will provide optimal outcomes for patients with Ewing-like sarcomas. For example, tumors harboring *CIC/DUX4* translocations appear to be only variably responsive to standard EWS chemotherapy regimens (Italiano et al. 2012) and seem more aggressive possibly due to high MYC expression (Smith et al. 2015). Due to our lack of understanding of the molecular pathways driving the growth of these distinct tumors, it is also not clear to what extent novel targeted agents being developed for EWS will impact the care of patients with Ewing-like sarcomas.

9.6 Late Effects of Therapy Following EWS Therapy

Improvements in survival rates for patients with localized EWS have come largely as a result of increases in the intensity of cytotoxic chemotherapy. Given the chemotherapy agents commonly used in the management of EWS, survivors are at risk for a host of toxicities shared by other sarcoma survivors, including cardiomyopathy, renal insufficiency, and reduced fertility (Ginsberg et al. 2010; Paulides et al. 2006; Stohr et al. 2006). In addition to these late effects that patients with EWS share with other patients receiving similar agents, there are three classes of risks that merit special emphasis: risk of second malignancy; functional outcomes due to management of the local tumor; and propensity for late recurrence.

As detailed above, chemotherapy regimens for the care of patients with EWS rely heavily upon anthracyclines, alkylating agents, and etoposide. Moreover, a subset of patients requires radiation therapy as a component of local control. It is perhaps not surprising then that a number of reports have established that survivors of EWS are at a particularly high risk for developing second malignant neoplasms (Ginsberg et al. 2010; Garwicz et al. 2000; Goldsby et al. 2008). A population-based study noted that EWS was one of the top three primary pediatric cancer diagnoses (including retinoblastoma and Hodgkin lymphoma) with highest risk of second malignancy (Garwicz et al. 2000). A study of 5-year survivors of EWS followed in the North American Childhood Cancer Survivorship Study (CCSS) reported a 9% cumulative incidence of second malignancy by 25 years after initial diagnosis (Ginsberg et al. 2010). A report from the EICESS-92 trial demonstrated a higher rate of second malignancy in patients treated with etoposide and in patients treated with high-dose chemotherapy (Paulussen et al. 2001). Radiation exposure does not appear to impact risk for secondary leukemia but does appear to impact risk for development of secondary sarcoma (Dunst et al. 1998). A report from the Rizzoli Institute identified a relationship between radiation dose and risk of second malignancy in EWS survivors (Bacci et al. 2005). Patients treated with definitive radiotherapy using standard doses had a cumulative incidence of second malignancy of 20.9% at 20 years compared to 8.9% for patients treated with surgery and reduced dose radiation.

The CCSS has provided important data on the extent of functional limitations in survivors of EWS. Compared to siblings, 5-year survivors of EWS are at a sixfold increased risk of activity limitations or functional impairment (Ginsberg et al. 2010). However, only a minority (<30%) of survivors report functional deficits, disability, or impaired quality of life (Nagarajan et al. 2004). Rates of functional deficits appear similar between patients who underwent limb salvage surgery and patients who underwent amputation (Nagarajan et al. 2004). A recent European study on long-term survivors that used surveys and step watch monitoring showed that, independent from tumor site or local treatment modality, the majority of patients had no major limitations (Ranft et al. 2017).

Patients with EWS and late relapse beyond 5 years from initial diagnosis were first reported in case series (DuBois et al. 2008; McLean et al. 1999). Larger epidemiologic analyses have demonstrated that 5-year survivors of EWS have a higher propensity for late relapse compared to other pediatric solid tumors (Armstrong et al. 2009; Miller et al. 2013; Wasilewski-Masker et al. 2009). Among 5-year survivors of initial EWS, recurrence or progression of the initial disease is the cause of death in the majority of patients (Ginsberg et al. 2010). The biologic basis for this observation is not known, though this finding has implications for the long-term care of patients with a history of EWS.

9.7 Future Directions to Improve Outcomes for EWS

The care of patients with newly diagnosed localized EWS is in many ways a major success story in advancing sarcoma therapies. However, the improvements in survival from intensifying cytotoxic therapies have come at a significant cost in

terms of tremendous burden of late effects. Therefore, this group of patients will require newer targeted therapies, new risk stratification approaches to identify candidates for safe therapy reduction, or a combination thereof to improve not just survival rates but overall clinical outcomes. Strategies to improve outcomes for patients with more advanced (metastatic or recurrent) disease have been largely unsuccessful. For these patients, additional intensification of conventional cytotoxic chemotherapy approaches seems unlikely to advance the field. Instead, cooperation between laboratory and clinical researchers will be needed to identify and test promising new agents targeting *EWSR1/FLI1* or its downstream effectors.

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Experimental Models

10

Susanne A. Gatz, Janet Shipley, Charles Keller, and Corinne M. Linardic

10.1 Introduction

To understand the pathophysiologic mechanisms of human bone and soft tissue sarcomas, and develop interventions to treat them, it became necessary to study sarcomas outside of the human body and deconstruct the events leading to full tumor formation. This has taken the form of human tumor-derived cell lines grown in culture, human tumor-derived cell lines and primary human tumors grown as xenografts in immunocompromised laboratory mice, genetically defined sarcoma cell lines, genetically engineered mouse models, and novel models of sarcoma arising spontaneously in domesticated animals or developed in lower organisms including zebrafish and fruit flies. This chapter reviews the uses of these approaches in understanding bone and soft tissue sarcomas and complements other recent sarcoma model reviews (O'Brien et al. 2012; Kashi et al. 2015).

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10.2 Human Cell Lines

10.2.1 Cell Lines Derived from Human Tumor Tissue

Starting in the 1950s, immortalized cell lines derived from human cancer tissue were cultured ex vivo in sterile tissue culture facilities; the first human sarcoma cell lines were established in the 1960s. Sarcoma cell lines have been used widely to study sarcoma biology and identify new sarcoma treatments. The earliest methods for culturing sarcoma cell lines were as monolayers on tissue culture glass, and later plastic, which were coated to permit cells to adhere and divide. Subsequently, methods were developed to grow or propagate human sarcoma cells as xenografts in immunocompromised laboratory mice. More recently, methods have been developed to culture human sarcoma cells in nonadherent culture

C. A. S. Arndt (ed.), *Sarcomas of Bone and Soft Tissues in Children and Adolescents*, Pediatric Oncology, https://doi.org/10.1007/978-3-030-51160-9_10

conditions as three-dimensional spheres and in bioengineered microphysiologic systems.

(1) Monolayer culture. Human tumor-derived cell lines have been established for rhabdomyosarcoma, non-rhabdomyosarcoma soft tissue sarcomas, chondrosarcoma, osteosarcoma, and Ewing sarcoma. Given the concurrent but independent advances in sarcoma research, the creation of sarcoma cell lines around the globe have developed with different standards. Recent efforts to share cell lines internationally, in addition to describe their risks and benefits, has resulted in the cataloging of sarcoma cell lines including for rhabdomyosarcoma (Hinson et al. 2013; Sokolowski et al. 2014), liposarcoma (Stratford et al. 2012; Mersch et al. 2016), chondrosarcoma (Monderer et al. 2013), and osteosarcoma (Mohseny et al. 2011; Lauvrak et al. 2013; Muff et al. 2015). As of the writing of this chapter, there were no published reviews cataloging Ewing cell lines or synovial sarcoma cell lines.

The uses of monolayer culture in understanding sarcoma biology are extensive and have resulted in findings that are too numerous to reference in this review, but broadly include dissecting signal transduction, performing high-throughput pharmacologic and genetic screens, imaging at the cellular level, in vitro methods of studying metastasis, response to chemotherapy agents, and mechanisms of resistance. A unique biological characteristic of soft tissue sarcomas is their harboring of stable reciprocal translocations that are thought to drive tumorigenesis (Lauer and Gardner 2013). Cell lines have been indispensable for defining this biology. Thus while chromosomal translocations were originally identified in metaphase spreads of human tumor tissue (Ladanyi 1995), monolayer cultures were the first models in which to study the biological effects of the translocations, for example, using RNA inhibition to probe the contributions of the *PAX-FOXO1 (*Kikuchi et al. 2008*)*, *EWS-FLI (*Kovar et al. 2003*)*, and *TLS-CHOP (*Oikawa et al. 2012*)*, fusion genes. With the advent of gene editing technology, cell lines have continued to be the workhorse model for CRISPR-Cas9-engineered chromosomal translocations for Ewing and rhabdomyosarcoma-associated translocations (Torres et al. 2014; Lagutina et al. 2015). Additional development of gene editing technology will continue to rely on cell lines to study impact of gain and loss of function of translocations, tumorigenic function of translocations, gene therapy, and genome-wide screens (Liu et al. 2016).

While a major hurdle in every cancer research area is the misidentification/cross-contamination of cell lines (American Type Culture Collection Standards Development Organization Workgroup ASN 2010), which has led to a call for better quality control and authentication standards (Geraghty et al. 2014), the commonly used sarcoma cell lines at times fall prey to original designation issues when the cell line was established before molecular markers of each sarcoma type were known. For example, A204 was once thought to be a rhabdomyosarcoma cell line, but it is now appreciated to be a rhabdoid tumor cell line (Hinson et al. 2013).

(2) Xenografts. To begin to take into account the contribution of the tumor microenvironment to sarcomagenesis, human sarcoma cells were implanted into immunocompromised laboratory mice, usually subcutaneously after being admixed with growth factors to help the cells become established in the avascular subcutaneous space. These efforts began in the 1980s with the use of surgically, pharmacologically, or radiation-immunosuppressed animals to determine the ability of sarcoma cells to be transplanted and derived as cell lines (Houghton et al. 1982) and soon thereafter to examine the effect of chemotherapy and ionizing radiation on rhabdomyosarcoma, Ewing sarcoma, and osteosarcoma (Floersheim et al. 1986; Rofstad and Brustad 1980; Hoogenhout et al. 1982; Meyer et al. 1990). This later segued to routine use of genetically bred immunodeficient animals (Nanni et al. 1989; Sampson et al. 2013a) and then human-adapted mice (Seitz et al. 2010). Xenograft models for non-rhabdomyosarcoma soft tissue sarcomas had been lagging, largely due to the wide variety of these tumors and the need to create a new model for each type. More recently, a number of pediatric and adult patient-derived xenografts are now emerging from nonprofit and commer-

cial vendors such as The Jackson Laboratory and Champions Oncology. In addition, there have been described xenograft models for low-grade and dedifferentiated liposarcoma (Tilkorn et al. 2011; Smith et al. 2013) and synovial sarcoma (Steinstraesser et al. 2011).

When scaled, this approach supports the systematic pre-clinical evaluation of chemotherapeutic agents on sarcoma tumor growth. The Pediatric Preclinical Testing Program (PPTP), conceived in 2001 from a joint venture between the NCI and the Children's Oncology Group (COG) Phase 1 Consortium, represents one such large-scale effort (Houghton et al. 2007; Kurmasheva and Houghton 2016). Agents have been screened individually for rhabdomyosarcoma, Ewing sarcoma, non-rhabdomyosarcoma soft-tissue sarcoma, and osteosarcoma (Sampson et al. 2013b). In addition, radiation therapy has been applied to PPTP studies, demonstrating its feasibility (Kaplon et al. 2013). Some agents identified in the PPTP program have advanced to human clinical trials. Among targeted agents or new chemotherapies for rhabdomyosarcoma, temsirolimus is notable: in the ARST0921 COG clinical trial for relapsed rhabdomyosarcoma, the study was terminated early after observing that the 6-month event-free survival (EFS) for temsirolimus plus vinorelbine and cyclophosphamide chemotherapy was superior to the 6-month EFS for bevacizumab plus the same chemotherapy (0.5 vs 50%, two-sided p-value <0.01). However, early results of long-term follow-up to determine whether these stepwise changes in 6-month EFS result in improvements to the chemotherapy-only long-term survival rates for metastatic alveolar and embryonal rhabdomyosarcoma suggest no long-term survival benefit of temsirolimus (Mascarenhas et al. 2014). First reports from the COG trial of the IGF1R antibody IMC-A12 were also non-encouraging (Malempati et al. 2015) and akin to the dismal results for RMS patients treated in the R1509 IGF1R antibody phase II trial (SARC Consortium) (Pappo et al. 2014). The results for agents advanced for osteosarcoma and Ewing sarcoma are similarly sobering. Thus, after 40 years of intensifying chemotherapy regimens and a decade of exploring targeted agents via cooperative group trials without change in survival rates for advanced stage sarcomas, new approaches (and perhaps a broader range of preclinical models) are needed more than ever for rhabdomyosarcoma. As of 2015, the PPTP has been transitioned into to the Pediatric Preclinical Testing Consortium (PPTC), http://ctep.cancer.gov/MajorInitiatives/Pediatric_Preclinical_Testing_Program.htm), which represents an even larger-scale program to systematically test new agents in childhood cancer. A key question has been whether this body of models is representative of the human disease; in at least one case where PDGFRA biology was examined, these PPTP models were not (Taniguchi et al. 2008), further emphasizing the need for novel models.

Since it was later recognized that different anatomic sites provided different inputs to tumor cell growth and biology, starting in 2000, investigators began implanting sarcoma cells orthotopically into their presumed anatomic location of origin, so that rhabdomyosarcoma cells could be implanted into muscle beds and osteosarcoma could be implanted into bone (Brekken et al. 2000). Since it was (and is still not) clear what the cell of origin is for Ewing sarcoma, and non-rhabdomyosarcoma soft tissue sarcoma represent a range of anatomic origins, this has been less straightforward, and the earliest studies used gastrocnemius muscles as the site of injection for orthotopic studies (Jaboin et al. 2002).

Finally, xenografts of human sarcomas (or allografts of murine tumors) in immunocompromised mice have lent themselves to the study of metastasis. Addressing the problem of lung metastases, mouse models using tail vein or orthotopic tumor cell injection to permit spontaneously arising lung tumors have been established to develop novel therapeutic strategies for osteosarcoma (Lu et al. 2015; Ratti et al. 2017; Lewis et al. 2017; Li et al. 2018) and Ewing sarcoma (Jia et al. 2003; von Heyking et al. 2017; Satterfield et al. 2017; Wang et al. 2009; Hong et al. 2015). In rhabdomyosarcoma, the most common model to assess lung metastases is tail vein injection of human rhabdomyosarcoma cell lines (Nanni et al. 1989; Taylor et al. 2009; Daniel et al. 2001). Similar to osteosarcoma and

Ewing sarcoma, in non-rhabdomyosarcoma soft tissue sarcomas, both tail vein and spontaneously arising metastases from orthotopic injections have been used (Wang et al. 2010; Cassinelli et al. 2018). Survival surgery, in which sarcoma xenografts are implanted orthotopically in hind limb muscle beds, then surgically amputated when they meet tumor burden, has permitted the study of spontaneous metastases to the lung, a common site of metastasis in sarcoma (Li et al. 2018; Goldstein et al. 2015; Hayashi et al. 2017). The development of cell reporter methods including bioluminescence and fluorescence and advanced in vivo imaging including micro-CT, micro-MRI, and micro-PET to orthotopic and metastatic models has further improved the ability to initiate and track the progress of sarcoma xenografts and their response to experimental therapies (Vormoor et al. 2014; Seitz et al. 2006; Nanni et al. 2007).

(3) Three-dimensional culture. While growth as xenografts in laboratory mice was felt to be a more "natural" study of the growth of human sarcoma cells, it became clear that this was prohibitively costly and there began to be ethical questions about the extensive use of animals in cancer research. Therefore a method to grow cancer cells in three dimensions was sought, and the landmark method for growing human tumor cells in three dimensions was from a breast cancer system, in which normal and malignant breast cancer cells was published in 2007 (Lee et al. 2007). In reality, this "three-dimensional culture" can be growth as spheres in liquid media or growth as spheroids in a matrix-containing semisolid such as methylcellulose. The method is thought to more realistically recapitulate the cellular microenvironment and permit study of interaction with the extracellular matrix, cell-cell interactions, signaling, and resistance to chemotherapy and radiation therapy and, in some (but not all) cases, cancer cell stemness. This approach has been developed for rhabdomyosarcoma (Walter et al. 2011; Bai et al. 2015), fibrosarcoma (Bai et al. 2015), osteosarcoma (Bai et al. 2015), and Ewing sarcoma cells (Lawlor et al. 2002; Leuchte et al. 2014). Whether these sphere culture methods enrich for tumor-propagating cells in the context of stem cell markers is still in question, since definitive identification of alveolar rhabdomyosarcoma tumor stem cells do not follow the traditional marker profiles, even though culturing as spheroids is readily done.

(4) Microphysiologic systems. With the advances in polymer chemistry and biomaterials, it has become possible to culture sarcoma cells in semi-physiologic substrates, more closely mimicking native tumor niches. For osteosarcoma, this has meant the development of microphysiologic systems to study the effects of chemotherapeutic agents on osteosarcoma "microtissues," with the goal of using the system in the future for personalized medicine discovery (Rimann et al. 2014), and to study the role of osteogenic differentiation in bone homeostasis (Prideaux et al. 2014). Similar advances have been achieved with ex vivo tissue culture of normal organs (lung) as a bed for tumor seeding (Mendoza et al. 2010). For Ewing sarcoma, this has meant the development of microphysiologic systems to mimic and therefore study the role of the native bone tumor niche in Ewing sarcoma (Villasante et al. 2014) and to study biochemical stimulation on tumor progression in Ewing sarcoma (Santoro et al. 2015). However, there will be many other uses as outlined in a review of the benefits of culturing in three dimensions (Lamhamedi-Cherradi et al. 2014). There is not yet a microphysiologic system for rhabdomyosarcoma.

10.2.2 Genetically Defined Sarcoma Cell Lines

As opposed to cell lines derived from primary human sarcoma tumor samples, genetically defined cell lines represent the deliberate manipulation and transformation of a nonmalignant primary human (or murine) cell type to an immortalized cell line having some representative qualities of the sarcoma being studied. The landmark study from the Weinberg laboratory generated human tumor cells from the expression of defined genetic elements in normal human epithelial and fibroblast cells (Hahn et al. 1999). This approach has been useful in studying the contributions of

starting cell type/population [although one might disagree that cell of origin can be identified in culture] and oncogenes/tumor suppressors in sarcomagenesis, if correct gene dosage is carefully considered and modeled and promoter constructs are physiologically relevant (Kikuchi et al. 2014). Similar to human tumor-derived cell lines, genetically defined cell lines can be cultured in a variety of conditions or environments to answer different biological questions. There have been genetically defined cell lines generated for rhabdomyosarcoma based on transduction of primary human myoblasts (Linardic et al. 2005; Naini et al. 2008), murine fibroblasts (Xia et al. 2007; Pressey et al. 2011), and murine mesenchymal precursors (Ren et al. 2008; Hettmer et al. 2011). For liposarcoma, the *FUS-CHOP* gene fusion has been introduced into murine and human mesenchymal precursor cells to model myxoid liposarcoma formation (Riggi et al. 2006; Rodriguez et al. 2013). For Ewing sarcoma, both human neural crest and human embryonic stem cells have been used to model sarcoma initiation by introducing *EWS-FLI1* into these cell types (von Levetzow et al. 2011; Gordon et al. 2016). For osteosarcoma, human mesenchymal precursors have been transformed with defined genetic elements, and although they generated sarcoma cell lines, they could not generate osteoid, even when high beta-catenin expression was forced (Li et al. 2009; Piperdi et al. 2012). Therefore, while this approach can be useful, it has limitations.

10.3 Murine-Based Preclinical Models and Their Role in Drug Development

A wide array of mouse-based xenograft, allograft, and genetically engineered model organisms exist for studying bone and soft tissue sarcomas (Kashi et al. 2015; Sokolowski et al. 2014; Davis and Keller 2012; Minas et al. 2017; Riggi et al. 2009; Mutsaers and Walkley 2014; Haldar et al. 2008; Goodwin et al. 2014; Stebbing et al. 2014)—as well as select ex vivo model systems (Mendoza et al. 2010). These model systems, their advantages, and application (and limitations) have been summarized in recent reviews in the literature (summarized in Table 10.1), and considerations for the use of genetically engineered mouse models have been detailed (Davis and Keller 2012). The recent emergence of a large cadre of new patient-derived xenografts, whose tumors are implanted from surgical or autopsy tissue without intermediate cell culture, increases the preclinical toolkit for sarcoma experimental therapeutics. Cell line-derived xenograft models continue to have value for biochemical studies conducted in paired in vitro biochemical studies. This same property makes allografts very useful (i.e., GEM tumors in new mouse hosts). The emergence of orthotopic model xenografts (i.e., intramuscular or intraosseous) instead of solely subcutaneous injection further increased the value of cell line-derived xenograft systems because of the way in which the former more accurately model the tumor microenvironment. In this regard, transgenic models are unique in their ability to study field effects and the normal immune system. Humanized mouse PDX development harboring human or a patient's own immune system is

Table 10.1 Reviews of murine-based sarcoma models

Reference	Perspective
Rhabdomyosarcoma	
Davis and Keller (2012)	– Use and value of transgenic models
Kashi et al. (2015)	– Comparative use of animal models
Sokolowski et al. (2014)	– Model systems as related to therapeutic targets
Ewing sarcoma	
Minas et al. (2017), Riggi et al. (2009)	– Achievements and challenges in making models
Osteosarcoma	
Mutsaers and Walkley (2014)	– Cell-of-origin considerations
Other soft tissue sarcomas	
Haldar et al. (2008)	– Synovial sarcoma transgenic modeling
Goodwin et al. (2014)	– Alveolar soft part sarcoma transgenic modeling
Mixed sarcoma type reports	
Stebbing et al. (2014)	– Compendium of commercially -generated sarcoma PDX models

nascent but likely critical to therapeutic target validation for immunologically based therapeutics. In between fully immunocompetent GEM models and fully immunocompromised *NOD.Cg-Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice are the *Prkdc*^{scid} *Hr*^{SKH1} (SCID-Hairless) host models, which lack B- or T-cells but maintain dendritic cell function. Surprisingly, breeding *Hr*^{SKH1} to homozygosity in GEM models affords hairlessness (improving optical imaging) without impairing T-cell function (Schaffer et al. 2010).

Two questions underlie the use of preclinical models and model systems: (1) the extent to which the models are genetically and phenotypically validated initially and during model propagation, and (2) standards for preclinical studies when presenting therapeutic targets to clinical trials investigators. The former has been a longstanding question and a major issue in preclinical research (Perrin 2014), whereas the latter is very much a work in progress. One possible approach is given in the Appendix. The underlying principles are that the target is representative for the disease, that the model systems are appropriate to address use of the target, and that sufficient independent biological replicates are used to preclinically justify a given target and/or therapeutic response. Whereas examples exist proposing that a drug may have broad applicability when studied only in a single xenograft model derived from a 40+ year old cell line (Renshaw et al. 2013) or only a single transgenic model (Taniguchi et al. 2008), the newer standard might call for at least three (and probably more) biologically independent model systems showing the same result—each model genetically identified and validated at the time of the experiment. The Pediatric Preclinical Testing Consortium and the European-Based Innovative Medicines Initiative 2 (a public-private partnership) are leading these standardization efforts.

10.4 Domesticated Animals

Human cancers generally arise spontaneously and are genetically complex and heterogeneous. Some sarcomas such as osteosarcomas are typically genetically complex, and other sarcomas that arise in childhood are, similar to other pediatric cancers, characterized by relatively few genetic changes, such as those with chromosome translocations including Ewing sarcomas and alveolar rhabdomyosarcomas. Genetically engineered mouse models can recapitulate abnormalities in one or a few genes known to drive tumor development but are dependent on the expression or loss of these genes in an appropriate tissue type at the correct stage of development in order for tumors to develop that are representative of human sarcomas. Genetically engineered mouse models may not clearly represent the human condition; for example, additional genetic changes such as mutation in *TP53* is needed for EWS-FLI1-driven Ewing sarcoma models despite *TP53* mutations being a rare event in human Ewing tumors (Neilsen et al. 2011). Patient-derived xenografts (PDXs) can better capture genetic complexity and heterogeneity but suffer from limitations related to the appropriateness of the site where the tumor is established, and, as PDXs are usually established in immune compromised mice, they will not represent the involvement and response of cells in the immune system.

Sarcomas in domesticated animals are of keen interest as they overcome some of the limitations associated with mouse and other models and are better models of the human condition for certain purposes. Areas where investigations using domestic animals have made a particular impact and are important include: (a) in understanding genetic predisposition to certain types of cancers; (b) for testing treatments in spontaneously arising "pet models" where sarcomas develop in a similar manner to those in humans; and (c) in studying animals that are more closely related in terms of their genetics, biology, and size to humans than other available models.

The dog genome was sequenced in 2005, and around 400 inherited disorders have been characterized in domestic dogs that have been bred to have significantly less genetic variability than found in humans but retain the spontaneous nature of developing cancers (Flisikowski et al. 2015). It is notable that particular breeds of dog are predisposed to specific cancers, with osteosarcomas in

particular occurring 10–30 times more frequently in large breeds of dog than found in humans (Rowell et al. 2011). Several changes in genes predisposing to osteosarcoma in dogs have been implicated including *TP53*, *PTEN*, and *ERB2* (Kirpensteijn et al. 2008; Angstadt et al. 2011). *TP53* gene polymorphisms in dogs with osteosarcomas correspond to the same codons in humans with these tumors (Kirpensteijn et al. 2008). A large and extensive genome-wide association study of large breed dogs with osteosarcomas identified multiple loci associated with tumor, including most prominently an enhancer region likely to affect the expression of CDKN2A/B (Karlsson et al. 2013). Another genome-wide scan for markers associated with osteosarcomas in Scottish deerhounds identified a candidate region containing two tumor suppressor genes (*PPM1L* and *MECOM*) involved in cell proliferation and stem cell maintenance. This region is an orthologue of the human chromosomal region 3q26 that frequently shows loss of heterozygosity in human osteosarcomas, showing strong parallels between human and canine osteosarcoma (Phillips et al. 2010). Parallel involvement of genes also includes *MYC*, *PDGFs/PDGFRs*, *IGF/IGFRs*, and even microRNAs (miR-134 and miR-544) (reviewed in (Fenger et al. 2014)).

Osteosarcomas and other sarcomas can arise spontaneously or can be induced by mutagenic agents including radioactive substances. Older studies investigating therapeutic strategies have focused on investigating induced osteosarcoma formation in beagles, although long latency periods were problematic (White et al. 1994; Lloyd et al. 1994). More recent work has used spontaneously arising sarcomas, particularly in large breeds of dog. Osteosarcomas that spontaneously arise in canines show remarkably strong similarities to those arising in humans both in terms of their clinical presentation and biological features. Osteosarcomas in humans and large canine breeds both show a bimodal age distribution in incidence corresponding to a high incidence of osteosarcoma in children and adolescents (Whelan et al. 2012). This suggests an association between the high growth rate of bones and increased risk of osteosarcoma. Canine and human osteosarcomas present in similar regions of the body and frequently metastasize to the lungs. A difference in clinical aggressiveness has been noted between human and canine osteosarcomas with the latter generally considered more aggressive with median survival times of a few months to a year (Simcock et al. 2012).

At the molecular level, strong similarities between human and dog osteosarcomas have also been identified including gene expression signatures in canines being demonstrably clinically relevant to those in humans (Paoloni et al. 2009; Scott et al. 2011). Other sarcoma types in dogs and humans show strong molecular similarities. A cross-disciplinary review of common soft tissue sarcomas in dogs and humans revealed strong similarities for spindle cell sarcoma with myxoid features/myxofibrosarcoma and undifferentiated pleomorphic sarcoma (UPS) (Milovancev et al. 2015). Poorly differentiated fibrosarcomas in Labrador Retrievers have shown gross chromosomal aberrations and loss of heterozygosity affecting *CDKN2A/B* orthologous to those observed in human fibrosarcomas but not those associated with infantile fibrosarcoma (Sargan et al. 2005). Another example is hemangiosarcomas, which are relatively rare in the pediatric setting but common in particular breeds such as Golden Retrievers, German Shepherds, and Boxers. Hemangiosarcomas in the different breeds show abnormal overexpression of different components of vascular endothelial growth factor (VEGF) signaling, underlying the importance of this pathway and also differences in the components involved that are associated with the unique genetic backgrounds that underlies the susceptibility of the different breeds (Tamburini et al. 2009).

Overall evidence supports these spontaneously arising osteosarcomas and other sarcomas in dogs as ideal models for establishing drug trials to develop new drugs and therapeutic treatment strategies. Translational studies in dogs with osteosarcomas using pharmacokinetic-pharmacodynamic endpoints with serial tumor biopsies and other biological samples before and after treatment have been used to optimize dosing (Rowell et al. 2011). In addition, osteosarcomas in dogs are ideal for studying management

of metastatic disease through exposure to new agents alone and in combination with standard chemotherapy backbones that are equivalent to those used in the human setting (Khanna et al. 2014). Well-conducted multicentered investigations of dogs with spontaneously arising sarcomas are expected to ultimately benefit both dogs and humans.

Sarcomas in other animals equivalent to those of young onset in humans can also be considered useful models for investigations although their low frequencies in most species make translational studies problematic. Similar to predisposition in dogs and humans, cats with mutant *TP53* (codon 261) are prone to osteosarcoma formation (Kirpensteijn et al. 2008). Feline osteosarcomas also show molecular similarities to human and dog tumors such as expression of PDGFRs (Meyer et al. 2015).

Inflammation is a well-established risk factor for cancer in humans, and similar risk factors likely exist in dogs and cats (Morrison 2012; Grivennikov et al. 2010). Cats in particular are associated with vaccination-associated sarcomas, especially fibrosarcomas, and, to a lesser degree, rhabdomyosarcoma. The most widely accepted cause is the inflammatory response of cats to the inactivated feline vaccines (feline leukemia virus and rabies) (Hendrick and Goldschmidt 1991). This is consistent with immunohistochemical detection of p53 protein, fibroblast growth factor-β (FGF-β), tumor growth factor-α (TGF-α), and platelet-derived growth factor (PDGF), and its receptor that have been documented in these feline tumors (Nieto et al. 2003).

10.5 Lower Organisms

Over the last several years, the zebrafish (*Danio rerio*) has emerged as readily manipulatable to create versatile models for cancer research (White et al. 2013; Veinotte et al. 2014; Barriuso et al. 2015). Comparative analysis of protein coding genes revealed zebrafish orthologues for 71.4% of all human genes and 82% of human genes linked to morbidity and listed in Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim/) (Howe et al. 2013). Zebrafish show high fecundity, have large numbers of offspring and embryos that are transparent, are very small, and develop outside the mother. These features enable easy maintenance and the possibility to image cells and organs. An adult pigment-deficient transgenic zebrafish, the casper fish, enables easy in vivo imaging of cancer and other processes in adult zebrafish (White et al. 2008). Together these factors allow high throughput screens of zebrafish at different stages of development following gene editing techniques and drug treatments which are added to the water that the fish are living in (White et al. 2013; Veinotte et al. 2014; Barriuso et al. 2015).

Early models exposed embryos, fry, or adult fish to carcinogens including ethylnitrosourea (ENU), 7,12-Dimethylbenz(a)anthracene (DMBA), and N-methyl-N′-nitro-N-nitrosoguanidine (MNNG). MNNG and DMBA treatment led to a variety of mesenchymal neoplasms in zebrafish following embryo exposure, including rhabdomyosarcomas and a number of sarcomas more prevalent in adults such as chondroma, chondrosarcoma (DMBA only), hemangioma, hemangiosarcoma, and leiomyosarcoma, albeit at a low frequency (Spitsbergen et al. 2000). An ENU-based forward genetic screen was also used to create the first *TP53* mutant zebrafish lines as a useful model and basis for further studies on the role of TP53 in carcinogenesis. Lines with *TP53* missense mutations in the DNA-binding domain showed induction of malignant peripheral nerve sheath tumors (MPNST) in 28% of zebrafish at 8.5 months (Berghmans et al. 2005).

Advances in methods to introduce genetic sequences into zebrafish and genome-editing technologies from early plasmid injections via transposon-mediated transgenesis to transcription activator-like effectors (TALE) nuclease (TALEN) and clustered regularly interspaced palindromic repeats/CRISPR-associated (CRISPR/CAS) technology (Stuart et al. 1988; Auer and Del Bene 2014; White 2015) as well as the decoding and functional understanding of the zebrafish genome, transcriptome, proteome, and, more recently, epigenome have enabled the creation of a vast resource of models and information for

future research (Howe et al. 2013; Freeman et al. 2009; Collins et al. 2012; Kettleborough et al. 2013; Tena et al. 2014; Lee et al. 2015).

Transgenic zebrafish models for several human sarcomas including *KRAS* mutated embryonal rhabdomyosarcomas (ERMS), EWS-FLI1 fusion positive Ewing sarcomas, and *TP53* mutant MPNST have been established (Berghmans et al. 2005; Langenau et al. 2007; Leacock et al. 2012; Amsterdam et al. 2004; Astone et al. 2015). Importantly, these models are both genetically and histologically similar to human disease (White et al. 2013). The *KRAS* mutated ERMS model is the most extensively studied of these models and showcases some of the opportunities that zebrafish have to offer to cancer research. In this model *kRASG12D* is expressed via the recombination activating gene 2 (rag2) promoter which possesses a MyoD-binding E-box motif in zebrafish that is active in mononuclear skeletal muscle cells—satellite cells and differentiating myoblasts. To generate the model, embryos were injected with the *rag2-kRASG12D* transgene at the one-cell stage and 47% of mosaic fish developed zebrafish ERMS (zERMS) by 80 days post-fertilization (dpf). Gene set enrichment analysis (GSEA) showed clustering of zERMS tumors with human fusion negative ERMS and revealed two different gene signatures in zERMS, a signature associated with RMS-specific pathway activation and a signature associated with the kRASG12D status (Langenau et al. 2007). It was also possible to discriminate and assess different cell subpopulations during development and progression of zERMS in vivo based on co-expression of fluorescence labels associated with myogenic factors, including myogenic factor 5 (myf5) and myogenin. zERMS tumors showed significant heterogeneity consisting of at least four cell subtypes which showed distinct expression profiles, proliferation/repopulation, and migratory capacities. In vivo the highly proliferative, myf5+, undifferentiated, tumor-propagating cell subtype requires the more differentiated and nontumor-propagating myogenin + subtype to seed in new metastatic areas. Importantly, comparative histological analysis of human ERMS tissue specimen and mouse xenograft tumors derived from two different ERMS cell lines replicated the tumor heterogeneity identified in zERMS (Ignatius et al. 2012).

Recently, a further development to zebrafish modeling is an optically clear, immunocompromised *rag2*^{E450f} (casper) zebrafish as adult host for cancer transplantation experiments including zERMS (Tang et al. 2016). Together this exemplifies the exciting opportunities in zebrafish models to study of tumor heterogeneity, resistance, metastasis, and relapse which is not yet possible in the mouse (White et al. 2013; Ignatius et al. 2012; Blackburn and Langenau 2014).

Various human cancer cell line xenograft and PDX models have been developed in zebrafish, so far mainly using embryos (reviewed in (Veinotte et al. 2014; Barriuso et al. 2015)). These embryo models have the advantage of lacking a fully developed immune system and are much smaller, facilitating large screening studies. The xenograft technique requires a compromise in maintenance temperature (human tissue/cell lines 37 °C versus zebrafish 28 °C). Typically, injected tumor cells are labeled to enable tracking within the zebrafish embryo organs. Experiments are usually carried out over 2–5 days, which precludes proper tumor formation in the host, and further pharmacokinetic assays cannot yet be performed in zebrafish. Cell line xenograft models of sarcoma include those for Ewing sarcoma, osteosarcoma, and fibrosarcoma (Veinotte et al. 2014; Stoletov et al. 2007; Stoletov et al. 2010; van der Ent et al. 2014; Ban et al. 2014). The versatility of the system with its short-term assessment, comparably low maintenance costs, small number of tumor cells required per xenograft (only a few 100), and the possibility to inject large numbers of host embryos, which may also be genetically modified, makes this an appealing complementary approach to cell line xenografts and PDXs grown in mice.

There is high genetic homology, including oncogenes, between human and invertebrates and specifically *Drosophila melanogaster (*Shilo and Weinberg 1981*)*. Fruit fly models have contributed to understanding the biology of pediatric sarcomas. To explore the pathogenic consequences of the fusion protein associated with alveolar rhabdomyosarcomas, transgenic overexpressing

of the *PAX7-FOXO1* fusion gene in muscle cells of *Drosophila* has been undertaken (Galindo et al. 2006). Investigations took advantage of the ability to fluorescently highlight proteins through reporter constructs combined with real-time visualization of these through the flies' transparent outer cuticle. Results showed that RAS activation acts as a modifier that enhanced the effects of the fusion protein and that PAX7-FOXO1 can promote generation of specific nucleated cells from differentiated myofibers in vivo. This fly model has also been used to investigate the role of downstream targets of the fusion protein (Avirneni-Vadlamudi et al. 2012). The *Drosophila* gene *rols* and the human orthologue *TANC1* appear to be upregulated by the fusion protein and contribute to the undifferentiated phenotype of rhabdomyosarcomas. This is consistent with PAX-FOXO1 phenotypes being dependent on suppression and enhancement of gene products. Through the possibility of multiple F1 generation crosses and screening for lethality in the *Drosophila* PAX7-FOXO1 model system, genetic modifiers that impact on phenotype can rapidly be isolated (Galindo et al. 2015). These modifiers may represent therapeutic targets.

Studies using *Drosophila melanogaster* with mutations in the trithorax group of genes have contributed to identifying and understanding the role of key genes associated with the chromatin remodeling SWI/SNF complex (Tamkun et al. 1992). Aberrant functions of this complex play a key role in some sarcomas. The SS18-SSX fusion protein associated with synovial sarcomas aberrantly forms a complex with SWI/SNF(BAF) competing out SS18 and displacing BAF47 that are normal components of this complex (Kadoch and Crabtree 2013). The majority of rhabdoid tumors are characterized by biallelic inactivation of SMARCB1 that is part of SWI/SNF complex. A comprehensive genetic screen in *Drosophila* identified several genes associated with loss of the *Drosophila* homologue of SMARCB1 (Jeibmann et al. 2014). These genes were confirmed to play a functional role in human rhabdoid tumors. This illustrates the use of *Drosophila* models to identify clinically relevant gene products and pathways in human sarcomas.

10.6 Future Directions

A variety of cell- and animal-based models of sarcomagenesis have contributed to the understanding and treatment of human sarcomas. No single model provides the context to study every aspect of sarcoma formation, progression, metastasis, response to treatment, and relapse, but together the models form a complementary group of tools with which to further understand the origins of sarcomas and improve the outcome of children with this disease. With the globalization of scientific information, there have developed international multidisciplinary groups focusing on the specific sarcomas, whose mission is to unite their efforts in maintaining unpublished results, systems, and models. Sarcoma clinicians and biologists have recognized the need to meet at intervals to discuss in an interdisciplinary fashion the value and use of models and reflecting this; both the Ewing and rhabdomyosarcoma research communities have met and reported on their summits (Hettmer et al. 2014; Kovar et al. 2016).

Appendix. Guidelines for Preclinical Studies

1. Determine the prevalence of a drug's target(s) in the pediatric, adolescent, or young adult sarcoma population (newly diagnosed and/or relapsed populations for that disease):
   (a) Regional, international, and cooperative group tumor banks of surgical- and autopsy-derived samples make these studies possible.
   (b) Types of analyses:
   - RNA expression should be surveyed, which may use public databases. Microarray data should be validated, but RNAseq data would be taken without validation. For each disease, 12–20+ biologically independent patient samples would be evaluated.
   - Protein-level validation would be performed by tissue microarray (to discern tumor cell vs microenvironment

expression of the target). These studies would always survey at least 12 biologically independent patient samples per disease. Western blotting might also be performed on a series of samples for each disease (at least 10 biologically independent patient samples per disease).

2. Determine the functional significance of the target(s) in pediatric sarcomas with high expression of the target in vitro:
   (a) Established cell lines and validated primary cell cultures should be employed.
   (b) Each culture is to be validated for expression of the target(s).
   (c) For each disease, five to eight biologically independent cultures would be evaluated.
   (d) The readout should include a 72-h IC50 determination, except where mechanism of action is different than inhibited cell growth. Genetic knockdown of the target may be employed for proof of concept.
   (e) Every effort should be made to find two-drug combinations (with chemotherapy or with another targeted agent).
3. Determine the functional significance the target(s) in pediatric cancers with high expression of the target in vivo:
   (a) Known cell line xenograft models and validated patient-derived xenograft models would be employed. Genetic model organisms may replace or complement xenograft model systems. Relapse- and autopsy-derived models may be prioritized. Each culture would be validated for expression of the target(s).
   (b) For each disease, three or more biologically independent models would be evaluated.

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11 Strategies for New Agent Development in Pediatric Sarcomas

Emily G. Greengard and Brenda J. Weigel

11.1 Strategies for New Agent Development

Through the use of multimodality therapy including cytotoxic chemotherapy, surgery, and/or radiation therapy, significant progress in decreasing the mortality from sarcomas in children was made between 1975 and 1995. Conventional cytotoxic chemotherapy has had the greatest impact in the treatment of childhood cancers. Despite this, over the past two decades, the rate of decline in mortality has slowed tremendously, making development of novel agents a top priority for researchers in the field of pediatric oncology. Children diagnosed with metastatic sarcomas or those who experience a recurrent sarcoma have a very poor prognosis. Furthermore, our most effective therapies come with significant short-term and long-term toxicities.

The focus of anticancer drug discovery and development has shifted to newer classes of drugs that either selectively target proteins and signal transduction pathways that are directly involved in the development and maintenance of the malignant phenotype in cancer cells or exploit the immune system to eradicate malignant cells. Selection of appropriate molecularly targeted agents for the treatment of sarcomas in children must be based on the role that the drugs' targets play in the pathogenesis of these cancers (Balis et al. 2009). As this varies depending on the tumor type, the agents of interest differ among the various sarcoma subtypes.

In this review, we will focus on the current evidence to support the use of small molecule inhibitors, immunotherapy, and novel targeted chemotherapeutic approaches for the treatment of pediatric sarcomas (Table 11.1).

11.2 Small Molecule Inhibitors

11.2.1 PARP Inhibitors

DNA damage caused by toxins, both naturally occurring and chemotherapy induced, is repaired by multiple mechanisms. Poly (ADP-ribose) polymerases (PARPs) are a family of enzymes involved in DNA repair through the recruitment and activation of proteins that use several of these repair mechanisms (de Murcia et al. 1997; Ruscetti et al. 1998). Inhibition of PARP has gained much attention, particularly for the treatment of Ewing sarcoma. It has been known for some time that Ewing sarcoma cells typically express high levels of PARP; however the relationship between PARP and EWS-FLI1, the chromosomal translocation most commonly observed in Ewing sarcoma, is still not clear (Soldatenkov

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C. A. S. Arndt (ed.), *Sarcomas of Bone and Soft Tissues in Children and Adolescents*, Pediatric Oncology, https://doi.org/10.1007/978-3-030-51160-9_11

Table 11.1 Agents in development for the treatment of pediatric sarcomas

Drug class	Target	Therapeutic agents	Study (reference or clinical trial #)
Small molecule inhibitors	PARP	Olaparib Niraparib Talazoparib	Choy et al. (2014) NCT02044120 NCT02392793
	MET	Crizotinib Cabozantinib	Mosse et al. (2013) NCT02867592
	VEGF	Pazopanib	NCT01956669 NCT02180867
	PDGR	Pazopanib	NCT01956669 NCT02180867
	mTOR	Temsirolimus	NCT02567435 NCT01222715
		Nab-rapamycin	NCT0297588
	XPO1	Selinexor	Gounder et al. (2016) NCT02323880
	TRK	Larotrectinib	NCT02637687
		Entrectinib	NCT02650401
	PI3K	Copanlisib	NCT03458728
	Wee1	MK-1775	NCT02095132
	CHK1/2	Prexasertib	NCT02808650
	FGFR	Erdafinitib	NCT03210714
	CDK	Palbociclib	NCT03526250
Monoclonal antibodies	SEM4D	VX15/2503	Patnaik et al. (2016a, b)
	IGF-1R	Cixutumumab Ganitumab	Weigel et al. (2014) Tap et al. (2012) NCT02306161
	PDGFR	Olartumab	Tap et al. (2016) NCT02451943 NCT02659020 NCT02677116
	VEGF	Ramicurimab Bevacizumab	NCT02564198 NCT01492673 NCT00667342
	CD56	Lorvotuzumab	NCT02452554
	GD2	Dinutuximab	NCT02484443
	GPNMD	Glembatumumab vedotin (CDX-011)	NCT02487979
Bone signaling	RANKL	Zoledronic acid	Goldsby et al. (2013)
		Denosumab	NCT02470091
Epigenetic targeting	HSP90		
	HDAC	Vorinostat	Fouladi et al. (2010)
	LSD1		

et al. 1999, 2002. Despite this, preclinical data evaluating PARP inhibitors and the relationship of EWS-FLI1 expression and sensitivity to the PARP inhibitor olaparib was statistically significant, similar to the relationship between imatinib and BCR-ABL (Garnett et al. 2012). Additional preclinical studies utilized a candidate gene approach to evaluate olaparib in Ewing sarcoma cells and again showed increased sensitivity of Ewing sarcoma cell lines as opposed to osteosarcoma and rhabdomyosarcoma cell lines. In addition, EWS-FLI1 gene transfer experiments seemed to increase the sensitivity to olaparib. Preclinical studies have demonstrated that the activity of PARP inhibitors in Ewing sarcoma cell lines is potentiated by temozolomide, and in tumor xenografts, the combination resulted is significant tumor growth reduction (Brenner et al. 2012). The synergy is likely due to the fact that methylated DNA bases are repaired by pathways that are dependent on PARP. The pediatric preclinical testing program (PPTP) studied the PARP inhibitor talazoparib (BMN 673) both alone and in combination with temozolomide. The single agent in vitro testing showed Ewing sarcoma cell lines to be the most sensitive; however in vivo testing of the single agent showed little activity in Ewing sarcoma xenografts. Despite this, when combined with temozolomide, responses were seen, with high level synergy in two Ewing sarcoma xenografts (Smith et al. 2015).

The PARP inhibitor, olaparib, was studied as a single agent in adult patients with relapsed or refractory Ewing sarcoma. The agent was found to be safe and tolerable in a very heavily pretreated population; however no significant responses or durable disease control was observed (Choy et al. 2014). Despite these results, there are several ongoing studies of PARP inhibitors in combination with chemotherapy for sarcomas given the encouraging preclinical data when PARP inhibitors are combined with chemotherapy. The combination of PARP inhibition with temozolomide was studied through a children's oncology group (COG) phase 2 study in recurrent or refractory Ewing sarcoma. The trial has completed accrual and analysis is pending (NCT02044120). In addition, St. Jude has a cur-

rently enrolling study examining a PARP inhibitor in combination with irinotecan with and without temozolomide (NCT02392793).

11.2.2 MET Inhibitors

Aberrant signaling of c-MET, the receptor for hepatocyte growth factor (HGF) has been linked to the development of cancer in murine models (Birchmeier et al. 2003). This is supported by the fact that lentiviral transfection leading to the overexpression of MET transforms primary human osteoblasts to become osteosarcoma (Patane et al. 2006). c-MET overexpression occurs in 60% of osteosarcoma patient's tumor samples, from both primary and metastatic sites of disease (Ferracini et al. 1995). In addition, MET has an important role in the biology of rhabdomyosarcoma, with overexpression and hyperactivity correlating with metastatic potential. In preclinical models of rhabdomyosarcoma, MET inhibition leads to decreased migration and formation of metastatic disease (Miekus et al. 2013). Similarly, several potent and both highly specific and less specific tyrosine kinase inhibitors of c-MET have been effective in suppressing the metastatic phenotype in osteosarcoma cells (Christensen et al. 2005; MacEwen et al. 2003). Both crizotinib, designed to inhibit anaplastic lymphoma kinase (ALK), and cabozantinib, designed to inhibit vascular endothelial growth factor (VEGF), have off-target effects of inhibiting c-MET. Each of these small molecule inhibitors has completed phase I studies in pediatrics, and cabozantinib is currently in phase 2 testing in pediatric patients with select solid tumors (Mosse et al. 2013) (NCT02867592). Further studies are needed to better understand their utility in pediatric sarcomas that overexpress c-MET.

11.2.3 Mammalian Target of Rapamycin (mTOR) Inhibitors

The mammalian target of rapamycin (mTOR) is a ubiquitous serine threonine kinase involved in the regulation of the cell cycle, angiogenesis, and apoptosis through interactions with mitogen-activated protein kinase and AKT. The cell signal pathways that activate mTOR are known to be altered in osteosarcoma, rhabdomyosarcoma, and Ewing sarcoma (Scotlandi et al. 2005). Activation of the PI3K/AKT/mTOR pathway is characterized by upregulated phosphorylated AKT levels and has been observed frequently in Ewing sarcoma samples. Everolimus, rapamycin, and temsirolimus are mTOR inhibitors that have been studied in preclinical models of pediatric sarcomas. When studied in the pediatric preclinical testing program (PPTP), rapamycin induced objective responses in osteosarcoma and rhabdomyosarcoma xenografts (Houghton et al. 2008). Furthermore, rapamycin was shown to reduce metastases in a murine model of osteosarcoma (Wan et al. 2005). Unfortunately, in response to the inhibition of mTOR, AKT, another serine-threonine kinase upstream mTOR, becomes hyper-phosphorylated and activated. AKT activation leads to resistance to apoptosis and decreased cell death (Wan et al. 2007). This effect can be abrogated by inhibition of IGF-1R; however when this strategy was studied in a COG phase 2 clinical trial, no objective responses were demonstrated. More encouraging, the COG performed a phase 2 trial for patients with recurrent rhabdomyosarcoma, randomizing patients to receive temsirolimus in combination with chemotherapy or bevacizumab in combination with chemotherapy. Patients randomized to the temsirolimus arm had a superior objective response rate to those randomized to the bevacizumab arm (47.4% vs. 27.5%) (COG Study Progress Report, Spring 2014, NCT01222715). Based on these results, the COG recently opened a phase 3 study incorporating temsirolimus into the upfront treatment for patient with intermediate-risk rhabdomyosarcoma (NCT02567435). A novel mTOR inhibitor, ABI-009 or nab-rapamycin, is currently being studied in combination with irinotecan and temozolomide through a COG phase 1 trial for relapsed and refractory solid tumors (NCT0297588). Nab-rapamycin is a human albumin bound preparation of intravenous rapamycin. Through this albumin nanotechnology, lipophilic drugs such as rapamycin can be encapsulated, enhancing permeability and retention and allow-

ing the molecules to accumulate in solid tumors. This theoretically allows for increased drug delivery to the tumor, increased efficacy, and decreased toxicity. Nab-rapamycin was studied in adult patients with non-hematologic malignancies, and the RP2D was found to be 100 mg/m^2/dose weekly, every 3 out of 4 weeks (Gonzalez-Angulo et al. 2013). As some patients required the day 15 dose to be held due to toxicities, an alternate schedule of dosing weekly for 2 weeks followed by 1 week off is now being studied in adults and is the schedule adopted in the pediatric phase 1 trial.

11.2.4 Tropomyosin Receptor Kinase Inhibitors

The tropomyosin receptor kinases (TRKs) are involved in nervous system development, and NTRK1, NTRK2, and NTRK3 encode TRKA, TRKB, and TRKC, respectively. Gene fusions involving NTRK have been seen in a wide range of malignancies including infantile fibrosarcoma. NTRK fusions have also been reported in isolated cases of undifferentiated sarcoma and radiation-induced sarcomas (Vaishnavi et al. 2015). Larotrectinib, an orally bioavailable, potent, ATP-competitive, selective inhibitor of TRKA, TRKB, and TRKC has shown significant tumor regression in preclinical models of tumors harboring NTRK gene fusion proteins. Larotrectinib has been studied extensively in adults as well as pediatrics, and the adult RP2D is 100 mg BID. The drug is very well tolerated and the majority of adverse events that have occurred have been grade 1 or 2 (Drilon et al. 2018). A pediatric phase 1 trial of this drug used a physiologically based PK approach to determine the starting dose of the drug needed to match the exposure (AUC) to a dose that has been previously tested in adults. This, along with not meeting DLT criteria and resulting in sufficient unbound drug concentration to produce 98% inhibition of TRK A/B/C, was how the RP2D was determined. Through this approach, the RP2D was determined to be 100 mg/m^2/dose (capped at 100 mg BID) (Laetsch et al. 2018). The efficacy of larotrectinib in treating children with recurrent solid tumors harboring actionable NTRK fusions is now being studied through the pediatric MATCH trial (NCT02637687). In addition, the COG is developing a phase 2 clinical trial to evaluate larotrectinib for the upfront treatment of infantile fibrosarcoma and other solid tumors harboring NTRK fusions. Entrectinib, another TRK inhibitor with potent activity against TRKA, TRKB, and TRKC as well as ROS1 and ALK, is also being studied in children and adolescents with recurrent solid tumors that harbor NTRK alterations (NCT02650401). Two adult phase 1 trials of entrectinib have been completed and established 400 mg/m^2/dose and 600 mg/dose to be the BSA based and fixed dose RP2Ds, respectively (Drilon et al. 2017).

11.2.5 Phosphatidylinositol-3-Kinase (PI3K) Inhibitors

Phosphatidylinositol-3-kinases (PI3Ks) play critical roles in cell proliferation and survival signaling, and the PI3K pathway is a critical regulator of multiple signal transduction pathways that promote cell survival and cell proliferation. The PI3K-AKT axis is among the most critical of these pathways and has been shown to be dysregulated in many tumor types via several different mechanisms. PI3K pathway alterations have been demonstrated in 60–80% of both osteosarcomas and rhabdomyosarcoma and approximately 85% of Ewing sarcoma. Many inhibitors have been developed targeting the PI3K pathway including mTOR inhibitors. Copanlisib is a novel pan-class I PI3K inhibitor and has been evaluated for adults with a wide variety of advanced malignancies both as a single agent and in combination with other investigational agents. The preclinical data for copanlisib is robust, demonstrating potent activity in Ewing sarcoma and rhabdomyosarcoma cell lines. Copanlisib has been studied in multiple adult oncology trials, and the RP2D is 60 mg given intravenously in a 3-week-on and 1-week-off schedule (Patnaik et al. 2016a). A trial of copanlisib in pediatric patients with relapsed/refractory solid tumors is ongoing (NCT03458728).

11.2.6 Wee1 and CHK1 Inhibitors

Checkpoint kinase proteins 1 (CHK1) and 2 (CHK2) are conserved serine/threonine kinases and are key effectors of multiple checkpoint responses when cells are exposed to genotoxic stress. Wee1 is a tyrosine kinase that is activated in response to DNA damage and plays a role in chemo-resistance and tolerance of oncogene-induced cellular stress. In the presence of DNA damage or replication stress by chemotherapy, radiation, or oncogenes, cyclin-dependent kinase 1 (CDK1) activity is restrained by both CHK1 and wee1, allowing for repair of DNA prior to mitosis and toleration of replication stress and maintenance of tumor cell viability. Inhibition of Wee1 or CHK1 leads to replication fork collapse or mitotic catastrophe, the generation of double-strand DNA breaks, and ultimately cellular death (Zhang and Hunter 2014).

The wee1 inhibitor, MK-1775, was developed to overcome this checkpoint and render cells more sensitive to chemotherapy. In both preclinical cell lines and patient-derived xenografts of soft tissue sarcoma, MK-1775 leads to tumor inhibition and enhances the efficacy of gemcitabine (Kreahling et al. 2013). Additionally, Wee1 inhibition has been shown to sensitize osteosarcoma cell lines to radiotherapy (PosthumaDeBoer et al. 2011). The children's oncology group continues to study MK-1775 in combination with irinotecan for children with relapsed and refractory solid tumors and includes an expansion cohort for rhabdomyosarcoma (NCT02095132).

Prexasertib is a novel, second-generation ATP-competitive, selective, dual inhibitor of CHK1/CHK2. As a single agent in vitro, prexasertib acts as (1) a DNA-damaging agent by causing double-stranded DNA breakage, (2) a checkpoint inhibitor, and (3) an inhibitor of DNA replication and mitosis. Preclinical cell lines including Ewing sarcoma, osteosarcoma, and rhabdomyosarcoma were all highly sensitive to prexasertib with IC_{50} values for growth inhibition of 1 nM or lower. In pediatric tumor xenograft models, prexasertib was well tolerated and resulted in an objective response in 43% of solid tumors including Ewing sarcoma and rhabdomyosarcoma. Prexasertib has been studied in early phase clinical trials in adults with the RP2D being 105 mg/m^2 once daily on days 1 and 15, every 28 days (Hong et al. 2016). A phase 1 trial of prexasertib for pediatric patients with relapsed/refractory solid tumors is ongoing through the COG (NCT02808650).

11.2.7 Fibroblast Growth Factor Receptor (FGFR) Inhibition

The fibroblast growth factor receptor (FGFR) family of receptor tyrosine kinases regulates several aspects of growth and development and, when inappropriately activated, results in abnormal development and disease. Dysregulated, constitutive FGFR signaling secondary to amplifications, translocations, and point mutations in FGFR genes has been shown to mediate oncogenic downstream signaling. There are four different known FGFRs (FGFR1, FGFR2, FGFR3, FGFR4), and within sarcomas, activating mutations of FGFR1 and FGFR4 are seen in 3% and 8% of rhabdomyosarcomas, respectively (Missiaglia et al. 2009). Erdafitinib is a potent, oral pan-FGFR tyrosine kinase inhibitor that has demonstrated potent inhibition of cell proliferation in FGFR pathway-activated cancer cell lines. Erdafinitib has been studied extensively in adult patients with malignancies, and the RP2D is 8 mg oral once daily (Perera et al. 2017). It is currently being studied in pediatric patients with tumors harboring FGFR 1/2/3/4 alterations through the NCI-COG Pediatric MATCH Treatment Trial (NCT03210714).

11.2.8 Cyclin-Dependent Kinase (CDK) Inhibition

Cyclin-dependent kinases (CDKs) are members of the serine/threonine protein kinase family and play crucial roles in tumor cell proliferation and growth by controlling the cell cycle, transcription, and RNA splicing. Several CDKs influence downstream targets and phosphorylate transcription factors involved in tumorigenesis. Many sarcomas express CDK proteins at high

levels, and inhibition of various CDKs results in decreased cell growth and apoptosis. CDK4 and CDK6 are two of the kinases important for cell cycle phase progression and have been demonstrated to phosphorylate and regulate the activity of the tumor suppressor protein Rb. CDK4 and CDK6 have been shown to be upregulated in 85% of liposarcomas. Furthermore, in biopsy samples of 45 human osteosarcomas, amplification of chromosome 12q14 (harboring the CDK4 gene) was one of the most frequent genomic alterations identified through high-density SNP arrays (Liao et al. 2016). Additionally, human synovial sarcomas highly express CDK4 with high level expression correlating with clinical stage and TNM grade. Knockdown of CDK4 with specific siRNAs inhibits cell proliferation and enhances apoptotic effects in synovial sarcoma cells. Several specific small-molecule inhibitors of CDK4/CDK6 have been identified including palbociclib, abemaciclib, and ribociclib. In preclinical models, palbociclib suppresses synovial sarcoma cell proliferation and growth in a dose-dependent manner (Li et al. 2018). Palbociclib is currently being studied in adults patients with sarcomas that overexpress CDK4 (NCT03242382) and will be studied in the pediatric MATCH trial for patients with relapsed/refractory Rb-positive advanced solid tumors with activating alterations in cell cycle genes (NCT03526250).

11.2.9 Selective Inhibitors of Nuclear Export (SINE)

One of the newer targets in cancer therapy is exportin 1 (XPO1), also known as chromosomal region maintenance protein 1 (CRM1). XPO1 is the primary component of a nuclear export protein complex that tumor suppressor (TSP) and growth regulatory (GRP) proteins use to exit the nucleus. Once TSP/GRPs exit the nucleus, they are unable to perform their work of preventing the development and progression of carcinogenesis. Thus, active nuclear export of TSP/GRP is a very efficient and rapid way of overcoming normal cell cycle regulation and the genomic stability assessment mediated by these proteins (Sharpless and DePinho 2007). XPO1 overexpression, which occurs in many cancer types including osteosarcoma, is correlated with poor prognosis, suggesting that alterations in nuclear cytoplasmic transport, and hence mislocalization of TSP, cell cycle regulators, and/or pro-apoptotic proteins, could promote oncogenesis and resistance to chemotherapy (Huang et al. 2009; Shen et al. 2009; Yao et al. 2009).

Selinexor is an orally bioavailable selective inhibitor of nuclear export (SINE) that binds and inactivates XPO1 in a reversible manner, thereby forcing the nuclear retention of key TSP/GRP. Transient retention of TSP/GRP in the nucleus at high levels via XPO1 blockade activates cell cycle checkpoint and genomic surveying. This leads to the death of nearly all types of malignant cells, whereas normal cells undergo transient cell cycle arrest and recovery when the export block is released.

The NCI supported Pediatric Preclinical Testing Program (PPTP) tested selinexor again their panel of pediatric cell lines and in vitro found Ewing Sarcoma cell lines to have a greater sensitivity (median rIC_{50} = 57 nM) to the agent than other cell lines (Attiyeh et al. 2016). Other in vitro studies have demonstrated sensitivity of various soft tissue sarcoma cell lines including undifferentiated sarcoma, RMS, liposarcoma, and leiomyosarcoma to selinexor with IC_{50}s ranging from 28.8 nM to 218.2 nM (median: 66.1 nM) (Nakayama et al. 2016). When these tumor types were studied in vivo in sarcoma xenografts, selinexor at 15 mg/kg twice weekly resulted in significant tumor growth delay in all sarcoma subtypes (Nakayama et al. 2016). In the PPTP's murine solid tumor models, selinexor was dosed at 10 mg/kg orally 3 days per week. Tumor growth inhibition met criteria for intermediate or high activity in 34% of the solid tumors, most frequently Wilms' tumor and Ewing sarcoma (Attiyeh et al. 2016). Extensive animal toxicology studies in Sprague Dawley rats and cynomolgus monkeys have demonstrated dose-dependent reductions in food intake and body weight to be the predominant DLT (Pharma I 2012).

A phase 1B study of selinexor in adult patients with advanced refractory bone or soft tissue sarcoma found the most commonly reported drug-related adverse events to be nausea, vomiting, anorexia, and fatigue. In addition, grade 3–4 cytopenias were not uncommon. Although none of the evaluable patients had an objective response by RECIST criteria, 33% had durable stable disease suggestive of anticancer activity in sarcoma (Gounder et al. 2016).

The appropriate dosing of selinexor, as well as the toxicity profile in children, will be defined via a COG phase 1 study that is currently enrolling children with relapsed or refractory solid tumors (NCT02323880).

11.3 Targeting Cell Surface Molecules

Monoclonal antibodies have been successfully developed for several malignancies including B-cell hematologic malignancies (anti CD-20), breast cancer (anti-HER2), and neuroblastoma (anti-GD2). To date there has been little success in developing monoclonal antibodies for the treatment of pediatric sarcomas; however, many new targets and strategies are currently in development.

11.3.1 SEMA-4D Inhibition

Semaphorins consist of a family of soluble and transmembrane proteins, originally defined as axonal-guidance factors (Giraudon et al. 2005). They can also induce cytoskeletal changes in immune, endothelial, and tumors cells and guide their migration in the tumor microenvironment (TME) (Mendes-da-Cruz et al. 2009; Takamatsu et al. 2010a, b). Semaphorin 4D (SEMA4D, CD100) has been implicated in the regulation of leukocyte infiltration into tumors and tumor growth and has been shown to inhibit immune-cell migration to the tumor.

Immunohistochemical analysis of SEMA4D expression on tissues from several human tumor types has shown that SEMA4D is overexpressed in multiple malignancies (Basile et al. 2006). Expression of SEMA4D may be regulated by the tumor microenvironment, including inflammatory cells and tumor-associated macrophages. Additionally, strong expression of SEMA4D at the invasive margins of actively growing tumors influences distribution of leukocytes in the tumor microenvironment and correlates with invasive disease and poor prognosis (Leonard et al. 2015). Investigators have studied the expression of SEMA4D in a variety of soft tissue sarcomas, and two separate analyses have shown high level expression to correlate with poor prognosis, overall, and disease-free survival (Ch'ng et al. 2007). Recently, it was reported that osteosarcoma tumors demonstrated upregulation of SEMA4D compared to normal human osteoblasts with high level expression in over half of the tumors (Moriarity et al. 2015). Overexpression of SEMA4D in osteosarcoma cell lines led to activation of MET or ERBB2 and subsequent increased phosphorylation of AKT and/or ERK.

The role of SEMA4D and potential application of an anti-SEMA4D antibody is likely much broader than osteosarcoma. SEMA4D is expressed on cells within the tumor stroma and modulate the activity of the immune system. Treatment with anti-SEMA4D antibody leads to increased levels of interferon γ and tumor necrosis factor α and tumor-specific cytotoxic T-cell activity. This immunologic response is localized to the tumor, with minimal T-cell and cytokine activity in the peripheral lymphoid organs. This is important because it has been reported that efficiency entry of functional tumor-specific T cells into the tumor correlated with improved survival and response to immunotherapy (Evans et al. 2015a, b).

The combined role of SEMA4D in tumorigenesis from both the tumor cells and tumor microenvironment and the fact that it is a cell surface receptor makes it an attractive therapeutic target. Similar to checkpoint inhibitors, its role in controlling the tumor microenvironment by modulating interactions with key players in the immune system make it an attractive target for many pediatric malignancies; however its additional roles

in osteosarcoma tumorigenesis make it particularly applicable to this disease.

A phase 1 trial of the SEMA4D-humanized monoclonal antibody, VX15/2503, has been completed in adults with advanced solid tumors demonstrating the antibody to be well tolerated (Patnaik et al. 2016b). An early phase trial of VX15/2503 through the COG is ongoing. The trial has a phase 1 component for pediatric patients with relapsed or refractory solid tumors and a phase 2 component for children and young adults with relapsed or refractory osteosarcoma (NCT03320330).

11.3.2 Insulin- Like Growth Factor Receptor 1 (IGF-1R) Inhibition

A large body of preclinical and early clinical data suggests that IGF1 and IGF2 might play an important role in the initiation and progression of a variety of cancers, including pediatric sarcomas (Pollak 2008; Benini et al. 2001). That being said, early phase clinical trials in the use of single agent IGFR1 monoclonal antibodies have not been quite as encouraging. The largest study executed thus far was performed by the COG and enrolled 114 eligible patients to receive cixutumumab. Of the sarcoma patients enrolled, only one had a response; however 15% had prolonged stable disease (Weigel et al. 2014). A smaller phase 2 study of the IGFR1 inhibitor, ganitumab, included 18 patients with Ewing sarcoma with one patient having a partial response but 33% having regression of at least 10% (Tap et al. 2012). The use of ganitumab in combination with chemotherapy is currently being explored through a COG randomized phase 2 trial in patients with newly diagnosed metastatic Ewing sarcoma (NCT02306161).

11.3.3 Platelet-Derived Growth Factor Receptor (PDGFR) Inhibition

Platelet-derived growth factor receptor (PDGFR) has an important role in tumorigenesis and tumor progression. Through binding of PDGR to its receptor, autophosphorylation is induced resulting in cell proliferation, chemotaxis, increased intracellular calcium, and apoptosis inhibition (Heldin and Westermark 1999). Many tumors overexpress PDGFRα, and overexpression is associated with cancer progression, reduced survival, and metastatic disease (Carvalho et al. 2005). In addition, the PDGF/PDGFRα axis is required for the production of VEGF, which is an important angiogenic regulator (Dong et al. 2004). Olaratumab (IMC-3G3; LY3012207) is a fully monoclonal antibody that selectively binds human PDGFRα with high affinity and was recently FDA approved for the treatment of soft tissue sarcomas in adults. Preclinical data of both olaratumab alone and in combination with chemotherapy have demonstrated antitumor activity in human soft tissue sarcoma xenograft models (Loizos et al. 2005). Based on these results, a phase 1b and randomized phase 2 trial of olaratumab and doxorubicin versus doxorubicin alone was performed in adults with soft tissue sarcomas. Olaratumab with doxorubicin met its predefined primary endpoint for progression-free survival and achieved a highly significant improvement of 11.8 months in median overall survival without an increase in serious toxicity, suggesting a positive risk-benefit profile for the addition of this agent for the treatment on soft tissue sarcomas (Tap et al. 2016). There are several ongoing studies of olaratumab in combination with chemotherapy in adults (NCT02451943, NCT02659020) and one currently enrolling pediatric clinical trial (NCT02677116).

11.3.4 Targeting Tumor Antigens: CD56

CD56 is expressed on all rhabdomyosarcomas, malignant peripheral nerve sheath tumors, synovial sarcoma, and several other pediatric tumors (Olsen et al. 2006). Lorvotuzumab (IMGN901) is a monoclonal antibody to CD56 conjugated to a microtubule inhibitor, DM1. The PPTP tested IMGN901 against a panel of solid tumor models. In vitro experiments demonstrated

low nM IC50s against RMS, and in vivo experiments demonstrated complete sustained remission in 2/7 RMS models, both of alveolar histology (Wood et al. 2013). Given these encouraging preclinical results, IMGN901 was studied in a COG phase 1/2 study, with phase 2 cohorts including patients with RMS, malignant peripheral nerve sheath tumor, and synovial sarcoma (NCT02452554). Accrual to this trial is complete and the analysis of results is in progress.

11.3.5 Targeting Tumor Antigens: GD2

GD2, a cell surface disialoganglioside, is expressed in more than 95% of osteosarcoma samples, making it a potential therapeutic target for osteosarcoma. The anti-GD2 chimeric antibody, dinutuximab, was recently approved in combination with IL-2 and GM-CSF, for the treatment of high-risk neuroblastoma. Preclinical studies evaluating GD2 expression in osteosarcoma have demonstrated strong immunoreactivity in 15/17 primary and metastatic osteosarcoma tumors (Heiner et al. 1987). Furthermore, 24 patient-derived osteosarcoma cell lines had strong expression of GD2 with only 4.3% negative for GD2 by immunohistochemical staining (Roth et al. 2014). A phase 2 study of dinutuximab in combination with GM-CSF in patients with recurrent osteosarcoma is ongoing (NCT02484443).

11.3.6 Targeting Tumor Antigens: GPNMB

Glycoprotein non-metastatic B (GPNMB) is a type I transmembrane glycoprotein; this is normally expressed in a variety of cell types including osteoblasts and osteoclasts (Maric et al. 2013) and is thought to play a role in tissue repair, cellular adhesion, and regulation of cell growth and differentiation. Aberrant expression and overexpression has been demonstrated in a variety of cancers, including osteosarcoma with overexpression correlating with tumor invasiveness and metastases (Rich et al. 2003). Glembatumumab vedotin (CDX-011) is a fully human IgG2 monoclonal antibody conjugated to the microtubule inhibitor, monmethyl auristatin (MMAE). Antitumor activity of CDX-011 has been demonstrated in xenograft models of osteosarcoma, and a COG phase 2 study evaluated its role in the treatment of patients with recurrent or refractory osteosarcoma with results currently pending (NCT02487979).

11.4 Anti-angiogenesis

Anti-angiogenesis as a strategy for cancer therapy was identified over 40 years ago and has been studied extensively in both adult and pediatric cancers. Several anti-angiogenic agents have been approved for treatment of colon, renal, and other adult malignancies. The role of anti-angiogenic agents in pediatric cancers is complicated by unique toxicities in a growing and developing human. Vascular endothelial growth factor's (VEGF) role in tumor neo-angiogenesis was identified in the 1960s when it was isolated from tumors including neuroblastoma, hepatoblastoma, and Wilms' tumor. The endothelial cell mitogenic and survival functions of VEGF are mediated primarily by the tyrosine kinase receptor VEGFR-2 (FLK-1/KDR). VEGF-VEGFR-2 binding, auto-phosphorylation, and downstream SRC family kinase activation mediate disruption of the endothelial barrier, resulting in increased vascular permeability. Signaling through VEGF-1 is more complex and has been associated with preclinical metastatic niches. VEGF expression is induced by hypoxia via the transcriptional activator hypoxia-inducible factor (HIF)-1α, which is negatively regulated by the von Hippel-Lindau (VHL) tumor suppressor gene (Glade Bender et al. 2011).

VEGF expression has been demonstrated in several pediatric sarcomas and often correlates with prognosis. Overexpression has been demonstrated in primary tumors from patients with Ewing sarcoma, and in experimental models, the isoform $VEGF_{165}$ is a critical driver of vasculogenesis. There is some evidence that the

EWS-ETS fusion protein may indirectly upregulate VEGF expression (Glade Bender et al. 2011). Osteosarcoma tumors that highly express VEGF and VEGF receptor tend to carry a poorer prognosis than those that don't express VEGF (Khanna et al. 2014). $VEGF_{165}$ appears to be necessary for development of pulmonary metastases in osteosarcoma (Khanna et al. 2014).

VEGF signaling can be targeted through inhibition of the ligands, receptors, or protein. Agents that target ligands include bevacizumab (Avastin; Genentech Inc., South San Francisco, CA), a humanized monoclonal neutralizing antibody that binds with high affinity to all five human VEGF isoforms and aflibercept (VEGF Trap; Regeneron, Tarrytown, NY), a potent composite decoy receptor, in which the extracellular domains of VEGFR-1 and VEGFR-2 are fused to an Fc segment of IgG_1. Antibodies that block VEGF2 include ramucirumab (IMC-1121B; ImClone Systems, New York, NY) and its murine counterpart for preclinical studies. There are several small molecule inhibitors that targets the VEGF signaling pathway including cediranib, pazopanib, sorafenib, sunitinib, semaxanib, vandetanib, and most recently, cabozantinib and axitinib. Sorafenib, sunitinib and cediranib have been tested through the PPTP in an in vivo pediatric solid tumor panel. Sorafenib demonstrated significant differences in event-free survival (EFS) in 27/36 (75%) of solid tumor xenografts tested as compared to controls (Keir et al. 2010). Similarly, Sunitinib demonstrated prolonged EFS in 19/35 (54%) of solid tumor models, with intermediate to high activity in 4/6 RMS models and 4/5 Ewing sarcoma models (Maris et al. 2008; Morton et al. 2012). The relatively more selective anti-VEGF RTK inhibitor, cediranib, demonstrated activity in 78% of solid tumor models, with activity noted in 3/3 Ewing, 5/5 RMS, and 4/5 osteosarcoma models. Pazopanib, a multi-targeted kinase inhibitor, including targets to VEGF and PDGR, was also studied through the PPTP. In a panel of pediatric RMS and Ewing sarcoma xenografts, no objective responses were observed; however it did induce statistically significant differences in EFS compared to controls in approximately one-half of the sarcoma xenograft models tested (Keir et al. 2012).

Pazopanib was subsequently studied through a randomized, double-blind, placebo-controlled phase 3 trial in adults with metastatic soft tissue sarcoma and significantly increased progression-free survival compared with placebo (van der Graaf et al. 2012). Based on these results, the FDA approved pazopanib for the treatment of adults with advanced soft tissue sarcomas in 2012.

Most of the VEGF inhibitory agents entering clinical trials in pediatrics have only completed phase I evaluation, so limited data on antitumor activity is available. There have been early signals of single-agent activity, including partial and minor responses and stable disease for >6 months in soft tissue sarcoma, Ewing sarcoma, and osteosarcoma. Pazopanib is currently being studied in a phase 2 COG trial for children with relapsed and refractory solid tumors (NCT01956669). However, monotherapy with TKIs is likely not sufficient to produce a significant measurable response, and several phase 2 and 3 studies are underway to evaluate combining these agents with conventional cytotoxic chemotherapy. The COG is currently enrolling patients to a phase 2/3 study of pazopanib in combination with chemoradiation in pediatric non-rhabdomyosarcoma soft-tissue sarcomas (NCT02180867).

11.5 Bone Signaling

There is substantial experience with the use of bisphosphonates for treatment of bone metastases in adults with malignancies, with FDA approval for its use in combination with systemic therapy (Green 2004; Ibrahim et al. 2003). Originally developed for the treatment of osteoporosis, bisphosphonates act by blocking the RANK-RANK ligand interaction, which is the primary pathway associated with osteoclast activation. By inhibiting osteoclast activity and bone resorption, zoledronic acid (ZA), a third-generation bisphosphonate, has been shown to target the bone microenvironment, improve bone

strength, and reduce tumor-related pain and skeletal-related events in several adult cancers. Additionally, in pediatrics sarcomas, there may be a role for preventing tumorigenesis. Although the malignant cell in osteosarcoma is an osteoblast or osteoblast-like cell, it is believed that the cross-talk or interaction between malignant osteoblasts and osteoclasts results in the release of bone-associated growth factors in the microenvironment of osteosarcoma. Interrupting this relationship may result in tumor regression. Preclinical studies of ZA in osteosarcoma have demonstrated inhibition of primary tumor growth, reduction in lung metastases, and prolonged survival in animal models (Heymann et al. 2005; Ory et al. 2005). Synergy with conventional chemotherapeutic agents including doxorubicin and ifosfamide has also been demonstrated in preclinical models (Heymann et al. 2005).

The COG performed a feasibility and dose discovery study of ZA with concurrent chemotherapy (combination of cisplatin, doxorubicin, methotrexate, ifosfamide, and etoposide) in the treatment of newly diagnoses metastatic osteosarcoma. ZA was given for a total of eight doses over 36 weeks, and a dose escalation was performed with three dose levels studied. The dose limiting toxicities encountered were hypophosphatemia, hypokalemia, hyponatremia, mucositis, limb pain, and limb edema, and the maximum tolerated dose (MTD) was defined as 2.3 mg/m^2 (max 4 mg). Ultimately, the studied deemed the combination of ZA with conventional chemotherapy to be safe for patients with metastatic osteosarcoma (Goldsby et al. 2013). Future studies are still needed to determine the clinical benefit of ZA in patients with osteosarcoma.

Denosumab is a fully human monoclonal antibody to the receptor activator of nuclear factor-κB ligand (RANKL) and is FDA approved for prevention of skeletal-related events in adults with solid tumor bone metastases (Xgeva) and adults and skeletally mature adolescents with giant cell tumor of bone that is unresectable or where surgical resection is likely to result in severe morbidity. It is also approved for the treatment of hypercalcemia of malignancy refractory to bisphosphonate therapy. Preclinical studies of denosumab are limited as the antibody does not recognize murine or canine RANKL. However, in vivo studies utilizing other approaches to inhibit RANKL activity including osteoprotegerin (OPG), a decoy receptor for RANKL, decreased primary and metastatic tumor burden with a significant decrease in the development of lung metastases (Lamoureux et al. 2007a, b). Additionally, xenograft studies using RANK-Fc, a chimeric protein that efficiently binds RANKL, demonstrated decreased number of lung metastases and improved survival (Akiyama et al. 2010; Lamoureux et al. 2008).

Denosumab is generally well tolerated with common toxicities including hypocalcemia and hypophosphatemia. Osteonecrosis of the jaw is observed in similar rates to that seen with the use of bisphosphonates. Femoral fractures, with little or no trauma, can also occur, commonly in patients with other comorbid conditions (Boyce et al. 2012; Ellis et al. 2008; Lipton et al. 2007; McClung et al. 2006). Finally, there have been reports of rebound severe hypercalcemia after discontinuation of denosumab (Gossai et al. 2015). The currently accruing COG phase 2 study is attempting to determine whether denosumab therapy either increases the disease control rate as compared to historical controls or produces an objective response rate greater than 5% in patients with recurrent osteosarcoma (NCT02470091).

Given that osteosarcoma is characterized by formation of bone by malignant cells, bone-seeking radiopharmaceutical agents are also a potential targeted therapy for this disease. These therapies can offer a potential means to simultaneously treat multiple osseous and osteoblastic non-osseous sites of osteosarcoma. The bone scan with avid uptake of ^{99m}Tc-MDP is the best screening to identify candidates that may benefit from this approach. Samarium-153-EDTMP, an alpha emitting radiopharmaceutical, has been studied since the 1980s and is available for the palliative treatment of bone metastases, including osteosarcoma, since the mid-1990s. It has been used in combination with docetaxel successfully and in combination with radiotherapy (Anderson

et al. 2008; Hobbs et al. 2011). In addition, once samarium-153-EDTMP is administered and unbound drug is eliminated in the urine (within 6 hours), a radiosensitization chemotherapeutic agent can be given in order to enhance efficacy (Hobbs et al. 2011). With the support of stem cells to overcome prolonged thrombocytopenia, high-dose samarium-153-EDTMP has been studied extensively in osteosarcoma, and although responses have been demonstrated, they are not durable responses (Franzius et al. 2001). At this point, samarium-153-EDTMP's utility is really limited to palliation. A newer beta particle emitting agent, radium-223, has been studied in preclinical models of osteosarcoma demonstrating avid skeletal deposition, relative sparing of the bone marrow, and nearly no soft tissue uptake. Radium-223 has a longer half-life than samarium-153-EDTMP and fast radon daughter decay providing for less off-target toxicity. Early phase studies in men with metastatic prostate cancer demonstrated excellent activity against bone metastases with a high therapeutic index, and efficacy was confirmed in a randomized phase 3 trial, leading to FDA approval of radium 223 for this indication. The role of radium-223 in osteosarcoma is still being investigated and a phase I trial at MD Anderson Cancer Center is ongoing (NCT01833520).

11.6 Epigenetic Targeting

11.6.1 Heat Shock Protein 90

Heat shock protein 90 (Hsp90) is a molecular chaperone of specific "client" proteins that in many cases are linked to oncogenic and metastatic cancer phenotypes (Pearl et al. 2008). The client proteins of interest to sarcomas include IGF-1 receptor, AKT, and c-MET, and these hsp90-client protein interactions protect these proteins from degradation. Preclinical data have demonstrated that Hsp90 inhibition results in impaired cell growth, apoptosis, and angiogenesis suppression, presumably through degradation of the client proteins previously protected by Hsp90 (Workman et al. 2007). Hsp90 inhibitors have been studied in several preclinical models of sarcomas. In these preclinical models, the Hsp90 inhibitor, geldanamycin, induced autophagy and apoptosis in osteosarcoma and rhabdomyosarcoma cell lines (Lukasiewicz et al. 2009; Mori et al. 2015). When a novel Hsp90 inhibitor, PU-H71, was studied in Ewing sarcoma cells lines, depletion of critical proteins including AKT, cMYC, ERK, RAF1, IGFR-1, and EWS-FLI1 was observed. In addition, xenograft models of Ewing sarcoma injected with PU-H71 had significantly decreased primary tumor and metastatic disease burden compared with mice injected with a control (Ambati et al. 2014). There are several ongoing clinical trials of Hsp90 inhibitors in adult cancers, however no currently open trials for pediatric malignancies.

11.6.2 Histone Deacetylase (HDAC)

A subgroup of sarcoma characterized by chromosomal translocations may be particularly vulnerable to HDAC inhibitors. Histones participate in DNA packaging in eukaryotic cells to form nucleosomes and organize chromatin. HDAC plays a role in carcinogenesis by deacetylating histone tails, leading to gene repression. Many of the fusion oncoproteins in translocation-associated sarcomas are associated with epigenetically silenced genes involving HDAC. In preclinical models, treatment with HDAC inhibitors reversed these changes and resulted in apoptosis through PI3K/mTOR/AKT pathway inhibition (Chu et al. 2015). The COG performed a phase 1 trial of vorinostat, and although only a few sarcoma patients were treated, no responses were demonstrated (Fouladi et al. 2010). A newer generation HDAC inhibitor, entinostat, is currently being studied through a COG phase 1 trial for children with relapsed/refractory solid tumors (NCT02780804). The potency and selectivity for specific HDAC isoforms may separate entinostat from other HDAC inhibitors in clinical development. In contrast to other HDAC inhibitors in clinical development, entinostat inhibits class I HDACs more potently than class II HDACs. As there is some in vitro data that inhibition of class

I HDACs is sufficient to induce apoptosis in cancer cell lines, entinostat may demonstrate antitumor activity with limited clinical toxicity (Saito et al. 1999).

11.6.3 Histone Lysine-Specific Demethylase 1 (LSD1)

Lysine-specific demethylase 1 (LSD1) is a flavin adenine dinucleotide (FAD)-dependent amine oxidase with important epigenetic eraser function, specifically catalyzing oxidative demethylation of mono- and dimethyl-lysine at histone H3 lysines 4 and 9, generating formaldehyde and hydrogen peroxide (Shi et al. 2004). LSD1 is also reported to demethylate-modified lysines on a myriad of non-histone proteins such as DNMT1, E2F1, MYP1, p53, and STAT3. The epigenetic effects of LSD1 are implicated in diverse biologic features pertinent to cell proliferation, chromosome segregation, and regulation of the stem cell pluripotency, to name a few (Scoumanne and Chen 2007). LSD1 has been found to be highly expressed in several sarcomas including Ewing sarcoma, chondrosarcoma, osteosarcoma, rhabdomyosarcoma, and synovial sarcoma (Bennani-Baiti et al. 2012; Schildhaus et al. 2011). Overexpression of LSD1 results in cell proliferation, migration, and metastases (Ding et al. 2013). Tranylcypromine (TCP) is a monoamine oxidase inhibitor that also inhibits LSD1 and has been shown to inhibit the proliferation of Ewing sarcoma, osteosarcoma, rhabdomyosarcoma, and chondrosarcoma cell lines, albeit in millimolar ranges which cannot be reasonably achieved in clinical settings (Bennani-Baiti et al. 2012). In preclinical models, treatment of Ewing sarcoma cell lines with the LSD1 inhibitor HC1-2509 reversed the transcriptional profiles driven by EWS-FLI1 and significantly delayed tumorigenesis in vivo (Sankar et al. 2014). Several LSD1 inhibitors are in early phase clinical trials for myeloid malignancies and have yet to be studied in clinical trials of sarcomas. Studies are needed to understand the role of LSD1 inhibitors in the treatment of sarcomas, and certainly an interesting approach to consider would be combining LSD1 inhibitors with HDAC inhibitors.

11.7 Summary and Conclusions

Pediatric sarcomas remain one of the challenges within pediatric oncology due to the fact that they are a heterogeneous group of diseases without clearly defined molecular targets that are readily accessible for therapeutic intervention. The cure rates have increased dramatically over the last decades for patients with non-metastatic disease; however, the highest-risk patients have not experienced such a benefit from optimization of conventional cytotoxic chemotherapy. This review provides an overview of the many and varied strategies that are being evaluated to improve the treatment of pediatric sarcoma ranging from targeted drugs to immunotherapy to drugs of unique mechanisms of action. The real challenge rests in how to study these varied agents in a wide variety of tumor types. The agents reviewed all had preclinical and/or clinical data in pediatric sarcoma relevant models or from adult studies justifying the evaluation of the agents in pediatrics. This will be key in prioritizing agents to move forward for study. This review focused mainly on single agents in development; however, it will be important to think carefully about combination strategies and how agents may be able to be combined to maximize efficacy. This is truly an exciting time for the development of new agents for pediatric sarcoma with many possibilities that will hopefully lead to improved outcomes for these patients.

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Immunotherapy for Pediatric Sarcomas

12

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12.1 Introduction

The impetus to harness the immune system against sarcomas dates back to observations of sarcoma tumor regression in the setting of erysipelas by Wilhelm Busch in Germany and William Coley in New York in the late 1800s and the subsequent study of sarcoma treatment with Coley's toxin (Coley II 1891; McCarthy 2006; Johnston and Novales 1962; Nauts and McLaren 1990; Que et al. 2017). Over a century later, anti-tumor activity related to bacterial infection in both canines (Lascelles et al. 2005) and humans (Jeys et al. 2007) continues to inspire active research and development of immune-based therapies (Murakami et al. 2017; Tsung and Norton 2006; Sottnik et al. 2010). Early in the twentieth century, Paul Ehrlich postulated that the immune system could surveil against cancer, but it was not until more than 50 years later that scientists discovered interferon, the first immune protein discovered capable of regressing tumors (Isaacs and Lindenmann 1957; Gresser et al. 1969). The eventual discovery of the structure and role of monoclonal antibodies (Raju 1999) and how to produce them at scale (Kohler and Milstein 1975) would result in the most important tool for scientists and physicians to target cancer cells in order to unleash immune responses. A deep understanding of the role of T cells was uncovered during the later decades of the twentieth century, setting the stage for the modern era of immunotherapy that includes both adoptive cell transfer and checkpoint inhibition. Over the past two decades, cancer immunotherapy has grown from a scientific endeavor largely carried out by academic scientists to a new pillar of cancer therapy as novel methods to harness and unleash the immune system have become standard for the treatment of cancer.

While pediatric oncology saw great advances in patient survival throughout the second half of the twentieth century with the adoption of combination chemotherapy and multimodal therapy (Smith et al. 2014), this effect has mostly plateaued over the past two decades. This is especially noticeable in high-risk metastatic and relapsed sarcomas, where there has been little progress in improving patient outcomes for over 30 years. Therefore, the success of immunotherapy across a broad spectrum of cancers, including malignancies resistant to other forms of

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C. A. S. Arndt (ed.), *Sarcomas of Bone and Soft Tissues in Children and Adolescents*, Pediatric Oncology, https://doi.org/10.1007/978-3-030-51160-9_12

conventional cytotoxic chemotherapy or targeted therapies (Brahmer et al. 2015; Postow et al. 2015; Brown et al. 2016; Robert et al. 2015), has sparked an interest in harnessing these therapies to treat pediatric sarcomas. In pediatric oncology in general, immunotherapy has already altered the landscape of relapsed B cell acute lymphoblastic leukemia (anti-CD19 CAR T cells (Lee et al. 2015; Maude et al. 2014, 2018; Gardner et al. 2017) and bispecific antibodies (Gore et al. 2018; von Stackelberg et al. 2016)) and high-risk neuroblastoma (anti-GD2 monoclonal antibodies (Yu et al. 2010; Kushner et al. 2018)). To date, the efficacy of immunotherapy in the treatment of pediatric sarcomas has been disappointing, and this approach has not yet benefited large numbers of patients. However, early results from several trials indicate that this powerful strategy will eventually bear fruit, adding to the multimodal armament needed to cure high-risk malignancies.

Potential challenges to effective immunotherapy in pediatric sarcomas include a low number of nonsynonymous somatic mutations (NSSM), the infiltration of the tumor microenvironment with immunosuppressive immune cells, and modulation of tumor cell surface molecules that can dampen the anti-tumor effects of immune cells. The adaptive immune system is largely dependent on presentation of mutated or foreign peptides as presented in the major histocompatibility complex (MHC). Therefore, presentation of tumor-specific epitopes is dependent on a high number of NSSMs (Grobner et al. 2018; Alexandrov et al. 2013). Many pediatric sarcomas, however, are driven by single gene fusions, compared to adult cancers which often are driven by a lifetime of mutagenic exposures such as ultraviolet sunlight or cigarette smoke (Alexandrov et al. 2013). Even osteosarcoma, which has a highly dysregulated genome among pediatric cancers, has low numbers of NSSMs, despite high levels of copy-number variation and gene deletions (Campbell et al. 2017). Sarcomas have also been reported to be infiltrated by inhibitory immune cells, including regulatory T cells, myeloid-derived suppressor cells, and tumor-associated M2 macrophages among others (Diaz-Montero et al. 2014; Laoui et al. 2014; Nishikawa and Sakaguchi 2014; Zhang et al. 2013). Additionally, in response to immune infiltration, downregulate human leukocyte antigens (HLA) to avoid detection by cytotoxic T cells. In both osteosarcoma and Ewing sarcoma (EWS), patients with tumors with decreased or absent HLA class I expression have inferior survival compared to those with high expression (Tsukahara et al. 2006; Yabe et al. 2011). Further, lung metastases consistently lacked HLA I expression in EWS tumors, and matched relapsed specimens demonstrated a tendency towards decreased HLA expression upon disease progression (Berghuis et al. 2009). In response to immune attack, tumor cells also upregulate immune checkpoint molecules such as PD-L1 and CD47 that are suppressive to T cells and macrophages. While each of the mechanisms of immune evasion represents a major barrier to successful immune control of cancers, they also represent therapeutic opportunities that can potentially be targeted.

Immunotherapies broadly can be divided into two classes of therapeutics, namely, those that amplify a native tumor response and those that initiate a new immune response where previously there was none. In this chapter, we will review the experience with both strategies in pediatric sarcomas, exploring areas of current investigation and future directions in the laboratory and the clinic.

12.2 Amplifying Natural Immune Responses

Immune response amplifiers aim to enhance the native immune response which is often present in cancer patients, but blunted by a suppressive tumor microenvironment and escape mechanisms initiated by tumor cells. The earliest immune response amplifiers were vaccines, which have been deployed for pediatric sarcomas, but more recently the use of monoclonal antibodies to block immune checkpoint molecules has come to the forefront of adult oncology, mediating complete responses in patients with metastatic can-

cers such as melanoma and lung cancer (Brahmer et al. 2015; Postow et al. 2015; Robert et al. 2015). However, sarcomas present several particularly difficult barriers to using these agents given their minimal mutation burden and tendency to be infiltrated by suppressive immune cells.

12.2.1 Checkpoint Blockade

Tumors can take advantage of T cell checkpoints such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or programmed cell death 1 (PD-1) to evade immune surveillance by expressing their ligands, resulting in inhibition of tumor-infiltrating T cells. Using monoclonal antibodies to block these interactions can result in significant and durable anti-tumor responses in certain diseases that carry high mutational burden like melanoma and non-small cell lung cancer (Brahmer et al. 2015; Postow et al. 2015; Robert et al. 2015). In sarcomas, however, clinical responses with these agents have been much more limited.

Merchant et al. (2016a) published the first trial of checkpoint blockage in pediatric patients. The researchers reported on the treatment of 33 patients with solid tumors, 17 of whom had relapsed or refractory sarcomas, with ipilimumab, a CTLA-4 blocking antibody. No objective clinical responses were observed. The adverse events included immune-related significant adverse events (irSAEs) including transaminitis, endocrinopathies, colitis, and pancreatitis. Increased overall survival in patients experiencing grade 2 or greater immune-related adverse events mirroring the adult experience, suggests that the mechanism of action of disinhibiting T cells was achieved in some patients who may have derived some clinical benefit (Merchant et al. 2016a).

Single-agent PD-1/PD-L1 inhibition has generally had higher response rates in adult malignancies such as melanoma than CTLA-4. However, PD-1 inhibition has not been successful in the treatment of most sarcomas common in children. Treatment of adult patients with advanced sarcoma using the anti-PD-1 antibody pembrolizumab showed objective responses in 7 out of 40 patients with soft tissue sarcoma, but most responders fell within two histologies, undifferentiated pleomorphic sarcoma and liposarcoma. In contrast, response rates in bone sarcomas were poor (1/22 responses in osteosarcoma and 0/13 responses in Ewing sarcoma) (Tawbi et al. 2017). Single-agent anti-PD1 antibody nivolumab similarly did not show clinical activity in adult patients with locally advanced, unresectable, or metastatic sarcoma (D'Angelo et al. 2018a). In pediatric specific trials, single agent anti-PD1/anti-PD-L1 were not efficacious in patients with osteosarcoma, Ewing sarcoma, or rhabdomyosarcoma (Davis et al. 2020; Geoerger et al. 2020). Perhaps this is not surprising given the very infrequent expression of PD-L1 in pediatric tumors (Majzner et al. 2017). Combination trials of both anti-PD1 and anti-CTLA4 antibodies in pediatrics are underway but, given the limited efficacy of either single agent, are unlikely to significantly alter the treatment of pediatric sarcomas.

While likely multifactorial (Conway et al. 2018), an important determinant of response to checkpoint inhibition is the presence of neoantigens for native T cells to recognize, often closely linked to tumor mutational burden. Pediatric tumors in general, and sarcomas in particular, have markedly lower mutational rate as compared to adult tumors (Campbell et al. 2017), which may at least in part explain the near absence of response to this treatment in pediatric sarcomas to date. Notable exceptions are tumors arising in children with DNA repair deficiency syndromes who amass high numbers of tumor NSSMs, where checkpoint inhibition has induced remarkable clinical responses (Bouffet et al. 2016; Lewin et al. 2018). Evidence of efficacy in these hypermutated tumors suggests that combination treatment strategies of checkpoint inhibitors with agents or modalities that increase the number of mutations in the tumor (alkylating agents, radiotherapy) (Brown et al. 2018) and/or enhance the presentation of neoantigens (epigenetic modifiers) (Magner et al. 2000) could enhance clinical activity.

12.2.2 Innate Immune Activators

Pediatric tumors, including sarcomas, have been reported to contain fewer T cells and more macrophages than adult cancers (Vakkila et al. 2006). While cytotoxic T cells require mutated or foreign peptides as targets, macrophages exert their anti-tumor activity in response to a variety of pro- and anti-phagocytic signals. They do not rely on mutational burden. Therefore, the innate immune system may be more easily unleashed in pediatric tumors. This can be achieved with Toll-like receptor (TLR) agonists or antibodies that block macrophage checkpoint molecules such as CD47.

TLRs are pattern-recognition receptors that can initiate both innate and adaptive immune responses, and TLR agonists can be exploited for active immunotherapy against cancer (Adams 2009). The context of TLR activation is critical however, as TLRs have been associated with both immune system activation and tumor cell apoptosis as well as tumor progression and immunosuppression. Despite this hurdle, TLR agonists have been brought to the clinic as adjuvants in vaccine approaches or combined with radiotherapy and/or chemotherapeutic agents. Indeed, intratumoral immunotherapy with Bacillus Calmette–Guérin (BCG) is now commonly used to treat early-stage urothelial carcinoma. More recently, the synthetic TLR4 agonist GLA-SE has been tested in patients with sarcoma. Fifteen adult patients with soft tissue sarcoma received GLA-SE via intratumoral injection, which in 12 patients was combined with concurrent radiotherapy. This treatment approach was well tolerated and resulted in complete regression of the injected tumor in one patient and stable disease in six patients. Encouragingly, TLR4 agonist treatment was associated with changes in the tumor microenvironment including increased T cell infiltration and a switch from immunosuppressive M2 to the immunostimulatory M1 phenotype of tumor-associated macrophages (Seo et al. 2017). This observation suggests that further development of this treatment approach is warranted possibly in combination with other immune modulators. This approach has not yet been tested in pediatric trials.

CD47, which has been described a “Don’t Eat Me” signal, is a macrophage checkpoint molecule, expressed on tumor cells and normal tissues to avoid phagocytosis. CD47 interacts with the myeloid inhibitory immunoreceptor SIRPα, transmitting an anti-phagocytic signal to the macrophage (Jaiswal et al. 2009; Matlung et al. 2017). Blocking this interaction has been shown to promote the phagocytosis of cancer cells by macrophages and neutrophils. CD47 is overexpressed in osteosarcoma as compared to normal osteoblastic cells, and blocking CD47 decreased the development of lung metastasis in an in vivo preclinical model of osteosarcoma (Xu et al. 2015). While a first-in-human trial of the anti-CD47 antibody Hu5F9-G4 resulted in few clinical responses (Sikic et al. 2019), combination of this agent with rituximab, a CD20-targeting monoclonal antibody, resulted in a high complete response rate in non-Hodgkin lymphoma (Advani et al. 2018). Given the predominance of macrophages in pediatric solid tumors, there may be a strong role for CD47 blockade combined with tumor-specific monoclonal antibodies in the future for pediatric sarcoma patients. Phase I trials of this agent have yet to be initiated in children but are currently being planned.

12.2.3 Cytokine-Based Immunotherapy

Cytokines are soluble proteins produced by immune cells that can regulate both innate and adaptive immune responses. The use of exogenous cytokines to stimulate immune responses has been studied and remains an area of active research in pediatric sarcoma patients, though there have not yet been trials leading to clear clinical benefit.

Interleukin-2 (IL-2), which can activate cytotoxic T lymphocytes and natural killer (NK) cells, is FDA approved for metastatic renal cell carcinoma and melanoma in adults and as part of a regimen of the anti-GD2 antibody dinutuximab, GM-CSF, and IL-2 for children with high-risk neuroblastoma. One study of high-dose IL-2 in children with metastatic and refractory solid

tumors included four patients with osteosarcoma and two patients with Ewing sarcoma (Schwinger et al. 2005). Two osteosarcoma patients remarkably achieved a complete response (CR), while the two patients with Ewing sarcoma developed progressive disease. As in adult patients, toxicity was significant including capillary leak syndrome. Given the poor prognosis of patients on the study, the results are intriguing but difficult to interpret given the small number of patients treated (Schwinger et al. 2005). The Rizzoli Institute published results from a cohort of pediatric osteosarcoma patients diagnosed with metastatic disease that were treated upfront with IL-2, high-dose methotrexate, doxorubicin, cisplatin, ifosfamide, autologous lymphocyte infusion, and surgery (Meazza et al. 2017). The 3-year overall survival of 45% was modestly increased compared to historic controls, but survivors included only those patients who achieved complete resection of their primary and metastatic lesions, making the outcomes difficult to interpret (Meazza et al. 2017). Thus, while IL-2 infusion may benefit some osteosarcoma patients, further study is required to determine if it can be safely integrated into upfront therapy and benefit a larger number of patients. New, safer formulations of IL-2, such as a pegylated version NKTR212, have shown promise in clinical trials in adult malignancies (Bentebibel et al. 2019) and could potentially be deployed in such trials.

Interferon therapy for pediatric sarcomas has been studied primarily in osteosarcoma. A single-institution trial of interferon-α and surgical resection in 89 consecutive localized osteosarcoma patients demonstrated promising 10-year metastases-free and sarcoma-specific survival rates (39% and 43%, respectively) as compared to historic controls undergoing surgery alone (Müller et al. 2005). Importantly, this study was initiated prior to the adoption of high-dose chemotherapy as part of the standard of care for osteosarcoma. Integration of interferon-α (IFN-α) into upfront therapy for osteosarcoma was then definitively studied in a randomized trial as maintenance therapy in patients who had a good histologic response to neoadjuvant chemotherapy (EURAMOS-1). Unfortunately, the addition IFN-α to standard chemotherapy backbone did not enhance progression-free or overall survival, calling into question the role of interferon for patients who receive highly active chemotherapy (Bielack et al. 2015).

Inhaled GM-CSF has been evaluated for the treatment of pulmonary metastases of sarcomas. In a phase I study of aerosolized GM-CSF in patients with malignant metastases to the lungs and a subsequent expanded report of patient outcomes in a single institution, disease stabilization or partial regression was noted in 8 of 13 patients with a sarcoma (Anderson et al. 1999; Rao et al. 2003). However, a Children's Oncology Group (COG) trial (AOST0221) evaluated aerosolized GM-CSF in first pulmonary recurrence of osteosarcoma and found no benefit compared to historical controls (Arndt et al. 2010). Overall, it is questionable whether adjuvant or neoadjuvant cytokine therapy alone can provide a benefit to patients with metastatic osteosarcoma.

12.2.4 Oncolytic Viruses

Oncolytic viruses (OVs) selectively replicate in and cause lysis of cancer cells, resulting in the activation of a native immune response against the tumor and, potentially, the release of neoantigens to the microenvironment (Lawler et al. 2017). Recent advances in genetic engineering have led to the development of "armed" oncolytic viruses that can secrete cytokines and other immune stimulants (Fukuhara et al. 2016). T-VEC (talimogene laherparepvec), a second-generation oncolytic herpes simplex virus type 1 (HSV-1) armed with GM-CSF, was the first OV therapy approved by the FDA in 2015 for treatment of advanced malignant melanoma and is now being investigated for treatment of advanced non-CNS pediatric solid tumors including sarcomas. Bolstered by safety data in adults, including several sarcoma trials, the study of OVs for pediatric sarcomas is expanding but remains in its infancy with uncertain potential for efficacy (Lettieri et al. 2012). At least three completed trials enrolling pediatric patients including sarcomas utilizing different OVs to date have demon-

strated safety but no objective responses (Streby et al. 2017; Burke et al. 2015; Kolb et al. 2015).

12.2.5 Anti-tumor Vaccines

Despite being one of the oldest immunotherapy strategies for the treatment of cancer, there has been limited success in developing efficacious anti-tumor vaccines, and only one is FDA approved to date (sipuleucel-T for metastatic prostate cancer). Anti-tumor vaccine strategies include targeting defined antigens, inoculation of patient-specific tumor lysate, or administering dendritic cells pulsed with tumor antigens. Sarcoma-specific fusion proteins such as EWS-FL1 (Ewing sarcoma), PAX3-FOXO1 (rhabdomyosarcoma), and SS18-SSX (synovial sarcoma) make particularly attractive targets given their tumor specificity, but clinical trials of vaccines against these targets have not been successful (Dagher et al. 2002). Other vaccine targets include cancer–testis antigens (NY-ESO-1, BAGE, MAGE, GAGE, LAGE, SSX, LIPI, PRAME) as well as gangliosides (GD2/3) and WT1 (Pender et al. 2018; Ohta et al. 2009).

Trials to enhance vaccine efficacy have focused on using pulsed dendritic cells (which can present antigens to endogenous T cells) and treating patients with lower disease burdens after the completion of chemotherapy. Vaccine potency appears limited, and it may be easier to initiate an effective immune response against minimal residual disease versus bulk tumor. For instance, among newly diagnosed metastatic or recurrent pediatric sarcoma patients on a phase I trial who were treated with standard antineoplastic therapy followed by a vaccine of autologous lymphocytes and tumor lysate-pulsed dendritic cells ± recombinant human IL7, a 5-year intent-to-treat (ITT) OS of 63% was observed for Ewing sarcoma and rhabdomyosarcoma (vs. 0% in other sarcomas) (Merchant et al. 2016b). Notably, among patients with newly diagnosed metastatic EWS/RMS, 5-year ITT OS was 77%, which is higher than previously reported in this population, suggesting that adjuvant immunotherapy in newly diagnosed metastatic patients could improve outcomes for this high-risk population (Merchant et al. 2016b).

Additional positive data from vaccine studies in Ewing sarcoma has been reported. In a trial of patients with recurrent or progressive Ewing sarcoma who received Vigil, an autologous tumor lysate-based vaccine transduced to achieve GM-CSF secretion and TGF-beta knockdown, overall survival was 73% at 1 year for Vigil-treated patients. This compared favorably to 23% overall survival at 1 year for non-randomly selected patients who were not treated with the vaccine (as a result of production failure or other choice of management) (Ghisoli et al. 2016). A complete response in a single patient with metastatic Ewing sarcoma to this vaccine has also been reported (Ghisoli et al. 2017). Given the selection bias and lead time bias inherent in all of these trials, it is important that promising approaches are studied in a randomized fashion. The Vigil vaccination approach is currently being studied in a phase III trial in combination with irinotecan and temozolomide in subjects with relapsed or refractory metastatic Ewing sarcoma. While it is difficult to enroll patients in a sufficiently powered clinical trial, it is essential to determine if therapy such as a vaccine given in an adjuvant setting is truly efficacious.

12.3 Synthetic Immunotherapies to Generate Immune Responses

In order to enact an anti-tumor response against an immunologically silent cancer, researchers have learned to generate synthetic immune responses against cancer cells. These developments, largely drawing on monoclonal antibodies and their derivatives, have allowed for the redirection of the immune system to target cancers that it otherwise could not. Given the low immunogenicity of pediatric sarcomas, this approach has a higher potential for success, with initial signs of clinical efficacy already apparent in early-phase clinical trials.

12.3.1 Monoclonal Antibodies

Monoclonal antibodies (moAb) were the first synthetic immunotherapies to demonstrate clear clinical benefit, highlighted by the experience with trastuzumab in HER2-overexpressing breast carcinomas (Slamon et al. 2001) and rituximab in CD20-positive hematologic malignancies (Lim and Levy 2014). In pediatric oncology, dinutuximab, a monoclonal antibody targeting GD2, has become part of the standard of care of multimodal therapy for newly diagnosed high-risk neuroblastoma (Yu et al. 2010), opening the door for other pediatric applications.

Monoclonal antibodies bind specific tumor-associated surface antigens and engage immune cells, primarily macrophages and natural killer (NK) cells via their Fc receptors (FcγR), to activate effector functions. These effector cells either then phagocytose or kill the target cell, a process known as antibody-dependent cellular cytotoxicity (ADCC), which is thought to be the principal method of cell killing achieved through treatment with tumor-specific antibodies. Antibodies can also be conjugated to drugs, toxins, and radioactive isotopes to specifically and directly deliver a cytotoxic load to the tumor that can further enhance their efficacy. This approach has been successful for antibodies targeting CD30 (brentuximab vedotin for Hodgkin lymphoma) (Cole et al. 2018) and CD33 (gemtuzumab ozogamicin for AML) (Pollard et al. 2016). It is important to note that not all monoclonal antibodies are generated to initiate ADCC, as many are engineered to block targets important in other pathways (e.g., ganitumab, bevacizumab, etc.), and such therapies are discussed elsewhere.

12.3.1.1 GD2

GD2 is a diaganglioside overexpressed on cancer cells including neuroblastoma and many pediatric sarcomas. Dinutuximab, an anti-GD2 moAb for the treatment of high-risk neuroblastoma, was the first FDA-approved tumor-reactive moAb for use specifically in treatment of a childhood malignancy. Almost all osteosarcoma cases express GD2, and a smaller number of Ewing sarcoma and rhabdomyosarcoma samples do as well (Chang et al. 1992; Dobrenkov et al. 2016; Long et al. 2016).

Among pediatric sarcomas, GD2 most is highly expressed in osteosarcoma, and expression is maintained at the time of recurrence (Roth et al. 2014; Poon et al. 2015), making it an attractive target for treatment of this disease. There were several osteosarcoma patients enrolled on the initial phase I trials of dinutuximab (ch14.18) or its parental murine antibody, 14g2a. Best responses have included both a CR and a mixed response, demonstrating that GD2 targeting is possible in osteosarcoma patients (Murray et al. 1994; Frost et al. 1997; Yu et al. 1998). However, no trial has demonstrated clear clinical benefit to a larger number of patients, although clinical trials of anti-GD2 antibodies in osteosarcoma are continuing. Recently, several trials demonstrated an enhancement of the anti-tumor activity of anti-GD2 antibodies in neuroblastoma through combination with systemic chemotherapy (Federico et al. 2017; Mody et al. 2017), and this approach could eventually be successful in osteosarcoma or other GD2-expressing sarcomas.

12.3.1.2 HER2

Human epidermal growth factor-2 (HER2), a proto-oncogene encoded by the ERBB2 gene, is overexpressed on several sarcomas including osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma (Scotlandi et al. 2005). Trastuzumab is a Her2-specific antibody that has been successfully employed in the treatment of Her2-amplified breast cancer, where levels of Her2 expression are much higher than on sarcomas (Thomas et al. 2002; Kilpatrick et al. 2001). Her2 was initially thought to be correlated with poor prognosis in osteosarcoma (Gorlick et al. 1999; Hynes 1993), although this was not borne out in a prospective trial (Gorlick et al. 2014). The Children's Oncology Group conducted a phase II trial of children with newly diagnosed metastatic OS treated with chemotherapy ± trastuzumab (Ebb et al. 2012). Patients whose tumors overexpressed Her2 received chemotherapy plus trastuzumab, while those whose tumors were Her2 negative

received chemotherapy alone. No significant differences in survival were seen in patients who received trastuzumab + chemotherapy compared to those who received chemotherapy alone (event-free survival of 32% in both arms, OS of 50% for chemotherapy alone compared to 59% for chemotherapy + trastuzumab) (Ebb et al. 2012). The reasons for failure are unclear but could relate to lower-level expression of Her2 on osteosarcoma versus carcinomas. However, this does not preclude the targeting of Her2 on osteosarcoma using next-generation synthetic immunotherapeutics such as antibody drug conjugates (i.e., trastuzumab emtansine, a Her2-targeted antibody drug conjugate FDA approved for the treatment of breast cancer) or CAR T cells (discussed below).

12.3.1.3 B7-H3

B7-H3 (CD276) is an immune checkpoint molecule overexpressed in a multitude of human malignancies including pediatric sarcomas (Wang et al. 2014; Picarda et al. 2016; Suh et al. 2003; Ma et al. 2016; Leitner et al. 2009; Castellanos et al. 2017; Ma et al. 2019). This protein was first targeted by the monoclonal antibody 8H9 by researchers from Memorial Sloan Kettering Hospital (Modak et al. 2001). It has since been developed into an antibody radioconjugate, in which ^{131}I is linked to the antibody for targeted delivery to the tumor (Kramer et al. 2015). This agent is currently under study for desmoplastic small round cell tumor (in addition to several CNS malignancies), and early safety data has been presented in abstract form (Modak et al. 2018). While the researchers present promising event-free survival data for patients who are treated with the antibody radioconjugate after they are rendered into a remission, this data is difficult to interpret in a small, single-institution trial that is highly susceptible to selection bias (Modak et al. 2018). A phase I trial of a different anti-B7-H3 antibody, enoblituzumab, demonstrated tumor regressions in adult malignancies (Powderly et al. 2015), and was recently studied in pediatric patients with solid tumors including sarcomas, but clinical data has not yet been presented.

12.3.2 T Cells Expressing Affinity Matured T Cell Receptors (TCRs)

The clinical experience with tumor-infiltrating lymphocytes (TILs) (Dudley et al. 2002) and PD-1/CTLA-4 checkpoint inhibitors (Postow et al. 2015) in metastatic melanoma clearly demonstrates that T cells are potent enough to eradicate advanced malignancies. However, pediatric tumors often lack the mutational load required for neoantigen presentation and a successful antitumor response (Campbell et al. 2017). Fortunately, researchers have learned how to endow T cells with tumor specificity by transducing them to express a tumor-specific T cell receptor. These engineered TCRs most often target cancer–testis antigens or oncofetal antigens, overexpressed on some sarcomas with limited expression on normal tissues (Lettieri et al. 2012).

This approach was first successfully applied in patients with melanoma; administration of T cells engineered to express a MART-1 TCR resulted in clinical responses in 6 of 20 (30%) patients with metastatic melanoma (Morgan et al. 2006). From these pioneering studies and those performed with TILs, immunotherapists learned that lymphodepletion (pretreatment with chemotherapy, most often cyclophosphamide and fludarabine) prior to administration of tumor-specific T cells greatly enhances the engraftment and expansion of adoptively transferred T cells (Dudley et al. 2008). While the mechanism of lymphodepletion is not fully understood, it is thought to be through depletion of regulatory T cells and suppressive myeloid cells, increasing the concentrations of homeostatic cytokines that drive T cell expansion, and making space in the bone marrow niche for successful engraftment (Dudley et al. 2008).

Engineered TCRs recognizing the cancer–testis antigen, NY-ESO-1, have been successfully deployed to treat adolescents and adults with synovial sarcoma. In the first trial of NY-ESO-1 TCRs, tumor regressions were observed in 11/18 (61%) synovial sarcoma patients (10 partial response (PR) and 1 CR) (Robbins et al. 2011;

Robbins et al. 2015) treated with this TCR and exogenous IL-2. No long-term persistence of the adoptively transferred TCRs was observed, and additional NY-ESO-1 peptide vaccination did not prolong administered T cell persistence (Robbins et al. 2015). A follow-up clinical study of the same NY-ESO-1-specific TCR explored its clinical activity without co-administration of IL-2, and a high response rate of 50% was maintained (6/12 patients, 1 CR and 5 PR) (D'Angelo et al. 2018b). The CR occurred in a patient with diffuse pulmonary metastasis, and transient radiographic worsening of disease was seen 48 h after administration of TCR-transduced T cells (pseudoprogression), but disease response was observed a few weeks later. The TCR-transduced T cells persisted over 6 months in responding patients, demonstrating that long-term T cell expansion and persistence is required for durable anti-tumor activity.

A notable limitation of utilizing a transduced TCR is genetic restriction by HLA-type. Most engineered TCRs are restricted to HLA-A*02, which, while common in Caucasians, represents only approximately 45% of the US population. Therefore, it is difficult to broadly apply these therapies to larger patient populations. In one study, researchers screened 120 synovial sarcoma patients and found only 37 that both were HLA-A*02 positive and expressed NY-ESO-1 (D'Angelo et al. 2018b).

12.3.3 Chimeric Antigen Receptor (CAR)

In order to overcome the genetic restriction of TCRs, researchers developed chimeric antigen receptors (CARs), which combine the specificity of a monoclonal antibody with the cytolytic capacity of a T cell in an MHC-independent manner (Gross et al. 1989). CARs are synthetic receptors consisting of an antigen-binding domain, commonly an antibody-derived single-chain variable fragment (scFv), linked to a transmembrane domain, CD3ζ, and, most often, additional costimulatory domains. Unlike TCRs, CARs recognize cell surface antigens that are often shared with similar tissues of origin. Therefore, it is important to identify targets that are not highly expressed on vital tissues that could result in on-target, off-tumor toxicity. The B cell lineage-restricted CD19 has emerged as an ideal target for hemtologic malignancies. Treatment with CD19 CAR T cells is revolutionizing the care of patient with relapsed and refractory B cell malignancies including childhood B-ALL (Lee et al. 2015; Maude et al. 2014, 2018; Gardner et al. 2017). While normal B cells are also eliminated by CD19 CAR T cells, patients can be supported through B cell aplasia with IVIG supplementation.

Successful deployment of CAR T cells to sarcomas requires an appropriate target antigen that is highly and homogeneously expressed on tumor cells, but absent or present only at low levels on normal tissues. The same molecules targeted with monoclonal antibodies, such as Her2 and GD2, have also been targeted with CAR T cells. While Her2 is expressed on sarcomas and other pediatric tumors at levels too low for effective monoclonal antibody therapy, Her2 CAR T cells can recognize and kill Her2 low-expressing cancer cells (Ahmed et al. 2009). A potential danger of this approach is on-target, off-tumor toxicity against healthy cardiac and pulmonary tissues that also express low levels of Her2. The first patient to receive Her2-targeted CAR T cells suffered respiratory collapse within hours of her cell infusion and ultimately died. While this was initially assumed to be due to targeting of Her2 on lung epithelium (Morgan et al. 2010), it now appears more consistent with cytokine release syndrome, a known toxicity of CAR T cells (Majzner and Mackall 2018). That patient was given a dose of CAR T cells that was 100 times what was eventually deemed the safe dose of CD19 CAR T cells.

Researchers at Baylor School of Medicine have since demonstrated the safety of targeting Her2 with CAR T cells. Using a CAR containing a different scFv, they carried out a phase I/II dose escalation study in patients with relapsed or refractory HER2-positive sarcomas. Patients were treated with increasing doses of HER2 CAR-transduced T cells without prior lymphodepletion. All dose levels were well tolerated with-

out any signs of on-target, off-tumor toxicity. T cell trafficking to the site of tumor was observed, and one patient had 90% necrosis of his tumor which was surgically removed after cell infusion. There was minimal CAR T cell expansion and persistence observed (Ahmed et al. 2015). After establishing the safety of their Her2 CAR T cells, the researchers then added lymphodepletion to a subsequent clinical trial. In this trial, in vivo expansion of CAR T cells was observed, and a complete response was achieved in a patient with metastatic alveolar RMS limited to the bone marrow (Hegde et al. 2017). While overall response rates were low, these studies demonstrate the safety and potential efficacy of Her2 CAR T cells for children with sarcoma despite some normal tissue expression of the target. This likely results from a therapeutic window in which the level of antigen on cancer cells is higher than that on normal cells, and thus CAR T cells preferentially target the tumor, leaving normal tissues largely intact.

GD2 is another possible target antigen for CAR T cell therapy in pediatric sarcomas. As discussed above, GD2 is frequently expressed on osteosarcoma and occasionally on Ewing sarcoma and rhabdomyosarcoma (Chang et al. 1992; Dobrenkov et al. 2016; Long et al. 2016). GD2 CAR T cells have been tested in clinical trials, largely for children with neuroblastoma. The first clinical study used an early design CAR without incorporation of costimulatory molecules (Pule et al. 2008; Louis et al. 2011). Instead, the CAR was expressed in Epstein–Barr virus (EBV)-specific T cells that could allow for physiological costimulation through the TCR. While in vivo expansion and persistence of administered CAR T cells was limited, complete responses were observed in 3/11 (27%) evaluable patients. In a subsequent clinical study, the design of this GD2-CAR was altered to incorporate CD28 and OX40 costimulatory domains, and stepwise addition of lymphodepletion prior to CAR T cell administration was introduced. While in vivo expansion of CAR T cells was observed, no clinical responses were reported (Heczey et al. 2017). Preliminary results of a clinical study using a GD2 CAR similarly incorporating a costimulatory domain (CD28) but based on a humanized anti-GD2 binder show some clinical activity (partial responses) in two of four neuroblastoma patients receiving $>10^8/m^2$ CAR T cells. These responses were associated with cytokine release syndrome, indicating clinical activity of CAR T cells (Straathof et al. 2018). Importantly, despite expression of GD2 on normal peripheral nerve cells and brain tissues, GD2 CAR T cells have never been associated with peripheral or central neurotoxicity in human trials (Pule et al. 2008; Louis et al. 2011; Heczey et al. 2017; Straathof et al. 2018). Therefore, GD2 is a possible target for CAR T cell treatment of both neuroblastoma and pediatric sarcomas (Long et al. 2016). While trials of GD2 CAR T cells in children with osteosarcoma have been carried out, results have not yet been reported. Other potential CAR T cell targets for pediatric sarcomas include B7-H3 (Ma et al. 2019; Du et al. 2019), folate receptor (Lu et al. 2019), and ROR1 (Huang et al. 2015).

12.3.4 Bispecific Antibodies

An additional method for generating a T cell response against tumor cells is using a bispecific antibody that targets a tumor-specific antigen as well as CD3, which is expressed by T cells. These bispecific antibodies are able to draw T cells and tumor cells into close proximity and drive T cell activation through crosslinking of the TCR complex. This approach has demonstrated clear success in pediatric B-ALL, where the CD19 × CD3 bispecific blinatumomab is FDA approved (Gore et al. 2018; von Stackelberg et al. 2016). Similar agents have been generated for sarcomas and other solid tumors, including those targeting Her2 (Lopez-Albaitero et al. 2017), GD2 (Hoseini et al. 2017), and B7-H3 (Ma et al. 2019). Thus far, only a GD2 x CD3 bispecific has been tested in children with sarcomas, with one PET only response being observed in a patient with osteosarcoma (Yankelevich et al. 2019).

12.4 Conclusions and Future Directions

Immunotherapy for the treatment of pediatric sarcomas remains early in development and faces unique challenges such as low neoantigen expression in many pediatric tumors. While T cell checkpoint blockade has revolutionized the care of some adult malignancies, single agent activity has not been observed in children or adults with sarcomas. Thus, it is likely that alternative forms of immunotherapy will be necessary. Success may be achieved by combining traditional therapies, such as chemotherapy and radiation, with immune activators such as checkpoint antibodies, but it is more likely to come about as a result of therapeutics that can initiate new immune responses in otherwise immunologically cold tumors. Such therapies as monoclonal antibodies and their derivatives, engineered TCRs, and CARs can generate anti-tumor activity in tumors to which immune cells are otherwise silent or suppressed. These novel therapeutics have already altered the care of pediatric patients with hematologic malignancies and neuroblastoma and are slated to drive further advancement over the years to come that will hopefully benefit larger numbers of children with high-risk sarcomas.

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