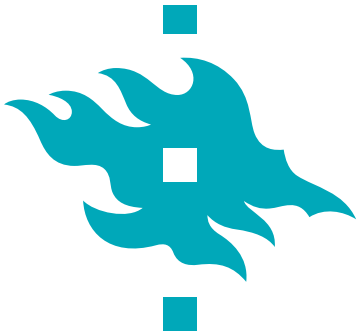

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KIRSI SANTTI

DESMOID TUMOR: ONCOLOGICAL MANAGEMENT AND PROGNOSTIC BIOMARKERS

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DESMOID TUMOR

ONCOLOGICAL MANAGEMENT AND PROGNOSTIC BIOMARKERS

Kirsi Santti

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To my family

Abstract

Desmoid-type fibromatosis, also known as aggressive fibromatosis or desmoid tumors, are very rare neoplasms, accounting for 0.03% of all newly diagnosed neoplasms and less than 3% of all soft tissue tumors (Escobar et al. 2012). Desmoid tumors occur in different anatomic locations in musculoaponeurotic tissues and may be painful, although they are seldom fatal. Approximately 10% of desmoid tumors are associated with an inherited condition called familial adenomatous polyposis (FAP) while the majority of desmoid tumor patients harbor a somatic mutation in the *CTNNB1* gene. Indolent tumors are surveilled; however, progressing and symptomatic desmoid tumors are managed with surgery, radiotherapy, or systemic therapy. Different systemic approaches include non-steroidal anti-inflammatory agents, endocrine therapy, tyrosine kinase inhibitors, and chemotherapy.

This thesis evaluated the outcome of oncological treatments at Helsinki University Hospital. We tried to seek novel molecular markers to identify different risk groups. We also aimed to illuminate the underlying pathobiological mechanisms in desmoid tumors.

The patients were treated at Helsinki University Hospital between 1987 and 2010 in study I (49 radiotherapies) and until 2011 in studies III (n = 76) and IV (n = 83). The patterns of recurrences after radiotherapy were analyzed using image co-registration. Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. were utilized for response evaluation in studies I and II; additionally, World Health Organization (WHO) criteria were used in study II. Study II examined the effect of cyclin-dependent kinase inhibitor ribociclib together with endocrine treatment in a patient with multifocal desmoid tumors and FAP. A tissue microarray was built of the formalin-fixed paraffin-embedded desmoid tumor specimen. The slides were immunohistochemically stained with Ki67, cyclin D1, cyclin A, and estrogen receptor β antibodies. Digitally assisted evaluation of the slides was carried out using Pannoramic Viewer software (3DHistech, Budapest, Hungary).

Radiation dose was independently associated with time to progression in patients treated with surgery combined with radiotherapy or radiotherapy alone (hazard ratio 0.71, p = 0.02). Local control rate was 75% at five years. The majority of recurrences after radiotherapy occurred at the margin of radiotherapy target (82%, 9/11), two were in-target (18%, 2/11), but none was out-of-target. Ribociclin, goserelin, and letrozole reduced symptoms and stabilized multiple desmoid tumors in a patient with treatment-resistant multiple desmoid tumors for ten months. High expression of cyclin A predicted poor outcome after surgery (hazard ratio 1.9, p = 0.02) whereas Ki67 or cyclin D1 expression rate did not reach statistical significance. Estrogen receptor β expression level had a positive association with proliferation.

This thesis is a comprehensive investigation of a rare disease entity. The results demonstrate that radiotherapy is an effective treatment in desmoid tumors. High cyclin A expression is a novel risk factor for recurrence after surgery.

Finnish summary

Desmoidit ovat erittäin harvinaisia kasvaimia, jotka käsittävät 0.03% kaikista kasvaimista ja alle 3% kaikista pehmytkudoskasvaimista. Desmoidit kasvavat eri ruumiinosissa lihaksissa ja kalvojänteissä. Ne voivat aiheuttaa kipua, mutta johtavat vain harvoin kuolemaan. Noin 10%:lla desmoidipotilaista on familiaalinen adenomatoottinen polypoosi (FAP), kun suurimmalla osalla potilaista on somaattinen *CTNNB1* geenimutaatio. Rauhallisesti käyttäytyviä kasvaimia seurataan, mutta kasvavia ja oireisia desmoideja voidaan hoitaa leikkaamalla, sädehoidolla tai lääkehoidoilla. Käytettyihin lääkehoitoihin kuuluvat tulehduskipulääkkeet, hormonaalinen hoito, tyrosiinikinaasin estäjät ja solunsalpaajat.

Tämän väitöskirjan tavoitteena oli arvioida onkologisten hoitojen tuloksia Helsingin yliopistollisessa keskussairaalassa. Pyrimme etsimään uusia biomerkkiaineita, joiden avulla voisimme erotella ennusteellisia ryhmiä. Lisäksi tarkoituksemme oli valaista desmoidien kehittymisen ja kasvun taustalla vaikuttavia patologisia ja biologisia tapahtumia.

Potilaat hoidettiin Helsingin yliopistollisessa sairaalassa vuosien 1987 ja 2010 välillä tutkimuksessa I (49 sädehoitoa) ja vuoteen 2011 saakka tutkimuksissa III (n = 76) ja IV (n = 83). Sädehoidon jälkeisiä uusiutumia analysoitiin yhdistämällä kuvantamistutkimuksia sädehoitosuunnitelmiin. RECIST 1.1. kriteeristöä käytettiin hoitovasteiden arvioimisessa tutkimuksissa I ja II, lisäksi käytimme WHO kriteeristöä tutkimuksessa II. Tutkimuksessa II selvitettiin solusyklin estäjä ribosiklibin vaikutusta yhdessä hormonaalisen hoidon kanssa potilaalla, jolla oli FAP:iin liittyen useita desmoideja. Kudossirukokoelma koottiin parafiiniin valetuista ja formaliinilla kiinnitetyistä desmoidikudosnäytteistä. Objektilasit värjättiin immunohistokemiallisesti Ki67-, sykliini D1-, sykliini A- ja estrogeenireseptori β -vasta-aineilla. Näytteet arvioitiin tietokoneavusteisesti käyttäen Pannoramic Viewer -ohjelmistoa (3DHistech, Budapest, Hungary).

Sädehoitoannos oli itsenäisesti yhteydessä etenemisaikaan leikkauksen ja sädehoidon jälkeen tai yksin sädehoidon jälkeen (vaarasuhde 0.71, p = 0.02). Paikalliskontrolli oli 75% viiden vuoden kohdalla. Sädehoidon jälkeen suurin osa uusiutumista ilmaantui hoitokohteen reunalle (82%, 9/11), kaksi oli sädehoitokohteessa (18%, 2/11), mutta yksikään ei kasvanut täysin kohdealueen ulkopuolella. Ribosiklibi, gosereliini ja letrotsoli vähensivät oireita ja vakauttivat monipesäkkeiset desmoidit kymmeneksi kuukaudeksi. Korkea sykliini A:n immunopositiivisuus ennusti nopeampaa uusiutumista leikkauksen jälkeen (vaarasuhde 1.9, p = 0.02), kun taas Ki67:n tai sykliini D1:n ilmentymisellä ei havaittu tilastollisesti merkittävää vaikutusta desmoidien uusiutumiseen. Estrogeenireseptori β:n korkeampi immunopositiivisuus oli yhteydessä solujen jakautumisnopeuteen.

Tämä väitöskirja sisältää perusteellisen selvityksen harvinaisesta kasvaintyypistä. Tuloksemme selvästi osoittavat, että sädehoito on desmoidien tehokas hoitomuoto. Korkea sykliini A:n immunopositiivisuus on uusi lisääntynyttä uusiutumisriskiä ennustava tekijä leikkauksen jälkeen.

Contents

List of original publications

This thesis is based on the following publications:

I Santti K, Beule A, Tuomikoski L, Rönty M, Jääskeläinen A. S., Saarilahti K, Ihalainen H, Tarkkanen M, Blomqvist C. Radiotherapy in desmoid tumors: treatment response, local control, and analysis of local failures. Strahlenther Onkol, 193(4), 269-275, 2017.

II Santti K, Beule A, Rönty M, Ihalainen H, Tarkkanen M, Blomqvist C. The CDK 4/6 inhibitor ribociclib has activity in the treatment of inoperable desmoid tumor. A case report. Acta Oncol, 58, 897-900, 2019.

III Santti K, Ihalainen H, Rönty M, Böhling T, Karlsson C, Haglund C, Tarkkanen M, Blomqvist C. High cyclin A expression but not Ki67 is associated to early progression in desmoid tumors. J Surg Oncol, 118(1), 192-8, 2018.

IV Santti K, Ihalainen H, Rönty M, Karlsson C, Haglund C, Sampo M, Tarkkanen M, Blomqvist C. Estrogen receptor beta expression correlates with proliferation in desmoid tumors. J Surg Oncol, 119, 873-9, 2019.

The publications are referred to in the text by their Roman numerals.

Abbreviations

APC	adenomatous polyposis coli
cdk	cyclin-dependent kinase
CI	confidence interval
cki	cyclin-dependent kinase inhibitor
CR	complete response
CT	computed tomography
CTV	clinical target volume
EORTC	European Organisation for Research and Treatment for Cancer
ERα	estrogen receptor α
ERβ	estrogen receptor β
FAP	familial adenomatous polyposis
FDG-PET	fluorodeoxyglucose positron emission tomography
GnRH	gonadotropin-releasing hormone
GTV	gross tumor volume
HER2	human epidermal growth factor 2
HR	hazard ratio
LCR	local control rate
LRFS	local recurrence-free survival
MER	magnetic resonance imaging enhancement ratio

MRI	magnetic resonance imaging
N/A	not applicaple
NSAIDs	nonsteroidal anti-inflammatory drugs
ORR	objective response rate
PFS	progression-free survival
PD	progressive disease
PDGFR	platelet-derived growth factor receptor
PgR	progesterone receptor
PR	partial response
RR	response rate
SD	stable disease
SERM	selective estrogen receptor modulator
tcf-lef	T-cell factor, lymphoid enhancer factor
TMA	tissue microarray
TTP	time to progression
TTR	time to recurrence
VEGFR	vascular endothelial growth factor receptor
y	year

1. Introduction

Desmoid tumors are mesenchymal neoplasms classified as intermediately malignant tumors (Fletcher, World Health Organization., and International Agency for Research on Cancer. 2013). Desmoid-type fibromatosis arises in deep soft tissues in muscles and fascial tissues in various anatomic sites, including the extremities, trunk, and head and neck area. Higher incidence in fertile females indicates hormonal influence in desmoid tumor development and growth. Desmoid tumors occur sporadically or are inherited in the context of familial adenomatous polyposis (FAP). The main challenge is the local morbidity due to the occasionally aggressive behavior of these tumors and the high recurrence rate following surgery. The natural course of the disease varies from indolent to life-threatening; however, desmoid tumors lack the propensity to metastasize.

The rarity of the disease and the variable biological behavior has led to difficulties in the formulation of treatment guidelines. Today treatment protocols recommend a first-line wait-and-see policy with close radiological monitoring (Kasper, Baumgarten, et al. 2017). Many of these patients require a change in the treatment strategy during the follow-up. Enlarging or symptomatic tumors should be managed, either operated or treated with radiotherapy or systemic therapy (Mehren et al. 2019). Clinical factors associated with worse outcomes after surgery include large tumor size and extremity location, young patient age, and positive resection marginals, although the results in different series have been inconsistent (Yao et al. 2014; Crago et al. 2013). Quality of life should be considered a top priority when selecting treatment modality. A multidisciplinary sarcoma team should be responsible for the treatment design of this complex disease. The management of pediatric patients suffering from desmoid tumor has not been included in this study.

In this thesis, we investigated the efficacy of radiotherapy and explored the activity of a novel combination of ribociclib and endochrine therapies in taper

desmoid tumors. We also examined the underlying pathobiology and evaluated the expression and the predictive role of different proliferation biomarkers and estrogen receptor β (ERβ). A better understanding of desmoid tumor pathobiology could help in separating differing risk groups and finding novel therapies.

2. Review of the literature

2.1. GENERAL

Desmoid tumors are uncommon fibroblastic neoplasms constituting less than 0.03% of all tumors. The word desmoid originates from the Greek word "desmos" describing the characteristic tendon- or band-like tissue of the tumor. In scientific literature abdominal wall desmoid tumor was first reported by MacFarlane in 1832 and subsequently named by Muller in 1838 (MacFarlane 1832; Muller 1838). The clinical behavior of these soft tissue tumors varies from spontaneous resolution to aggressive, infiltrative growth.

2.2. EPIDEMIOLOGY

Desmoid tumors' estimated incidence is 2.4 to 4.3 per million inhabitants annually in Finland (Reitamo et al. 1982). The incidence might be slightly underestimated due to underdiagnosis. A Dutch study, for example, observed a rising incidence from 2.1 to 5.4 per million people per year between 1993 and 2013 (van Broekhoven, Grunhagen, et al. 2015). Desmoid tumors can occur in all age groups with a peak incidence from 30 to 40 years with female-male ratio of approximately 2:1 (Penel et al. 2016). The vast majority of desmoid tumors occur sporadically, but at least 7.5% is associated with FAP (Nieuwenhuis, Casparie, et al. 2011). Between 10% and 20% of FAP syndrome patients develop desmoid tumor(s), and the risk is approximately 800-fold higher compared with the general population (Heiskanen and Jarvinen 1996; Nieuwenhuis, Lefevre, et al. 2011). Female predominance has not been observed in FAP carriers, and sporadic and FAP-related desmoid tumors are regarded as separate disease entities (Nieuwenhuis, Lefevre, et al. 2011).

2.3. PATHOPHYSIOLOGY

The vast majority of desmoid tumors show characteristic positive nuclear β-catenin staining in immunohistochemistry. Activation of the Wnt/β-catenin

signaling pathway is caused by a mutation either in the β-catenin gene *CTNNB1* or the tumor suppressor gene *APC* in up to 95% of sporadic desmoid tumor patients. In previous genomic studies, the occurrence of these mutations was lower, 85%; however, the next generation of sequencing techniques revealed nearly uniform alterations in the *APC* and *CTNNB1* genes (Crago et al. 2015). Germline *APC* gene mutation causes FAP, a syndrome that is characterized by the development of hundreds of colon adenomas, and if left untreated, it can account for virtually 100% lifetime risk for the development of colorectal cancer. Gardner's syndrome is a FAP subtype with typical manifestations of skull osteomas and skin and soft tissue tumors such as desmoid fibromatosis. The loss of the *APC* gene function disrupts the β-catenin degradation complex formation, which subsequently causes the β-catenin accumulation, as illustrated in Figure 1. Mutation in the *CTNNB1* gene prevents β-catenin phosphorylation and further degradation in proteasome leading to β-catenin stabilization. In both *APC* and *CTNNB1* mutations, the abundant cytoplasmic β-catenin translocates into the nucleus where it acts as a coregulator of TCF/LEF family of transcription regulators. They can further activate oncogenes.

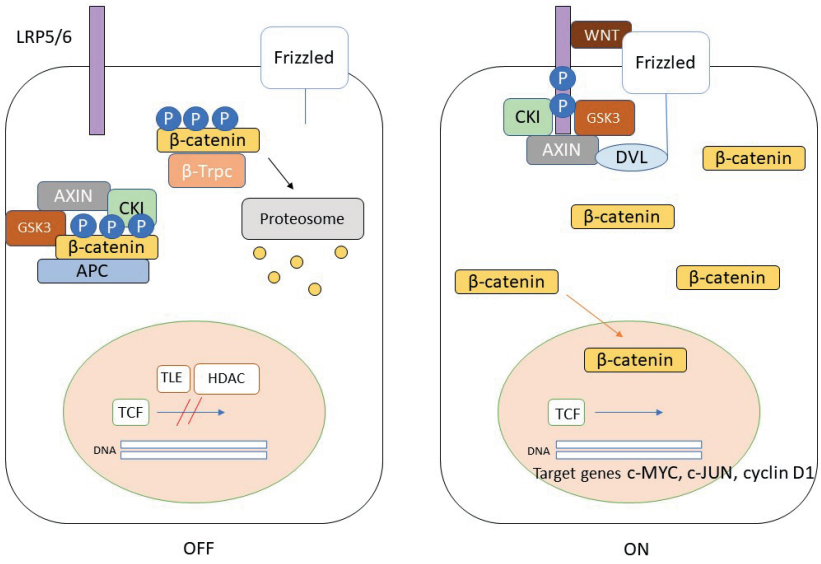

Figure 1. In sporadic desmoid tumors mutation in the CNNB1 gene prevents phosphorylation of β-catenin and therefore its proteosomal destruction. In patients with familial adenomatous polyposis, deficiency of the adenomatous polyposis coli (APC) protein inhibits formation of β-catenin destruction complex. In both occasions accumulated cytoplasmic β-catenin translocates into the nucleus to activate target genes. Adapted from (Martinez Trufero et al. 2017).

2.4. CLINICAL FEATURES

The desmoid tumor is often presented as an asymptomatic soft tissue mass, which can cause pain or pressure when invading into adjacent tissues. Depending on the anatomic site, desmoid tumors can grow into nerves and vessels or cause compression and obstruction of ureters or small intestine. In the head and neck area, the desmoid tumor can induce dyspnoea. Tumor growth can lead to functional and cosmetic impairment, and in more complicated conditions, it can cause absence from work, retirement, or even death. After prophylactic colon surgery, desmoid tumors constitute a relevant risk for morbidity in FAP patients, given the frequent multifocal and intra-abdominal presentation (Koskenvuo et al. 2016).

Desmoid tumors can appear virtually in all parts of the body. Sporadic tumors are more common in the trunk and limb girdles, whereas FAP-related tumors often arise in the abdominal wall, mesenterially and multifocally (Figure 2.

Desmoid tumors lack the propensity to metastasize; however, it has been hypothesized that the circulation of mesenchymal progenitor cells could explain the multifocal appearance lesions in sporadic desmoid tumor patients (Bekers et al. 2018; Wu et al. 2010). This conception is based on an observation of the same gene mutation in different lesions per patient (Bekers et al. 2018).

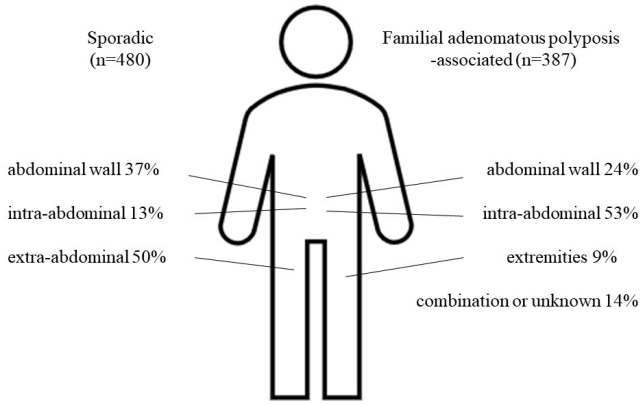

Figure 2. Desmoid tumor localization in patients with the sporadic and FAP-associated disease (Nieuwenhuis, Lefevre, et al. 2011; Nieuwenhuis, Casparie, et al. 2011).

2.4.1. RISK FACTORS

Trauma, including surgery, is a risk factor for desmoid tumor development. FAP patients who have undergone prophylactic colorectal surgery have a higher risk for desmoid tumor presentation in the years following the abdominal operation (Nieuwenhuis, Lefevre, et al. 2011). The growth factors could explain the phenomenon in the initial phase of wound repair, which activates β-catenin mediated signaling. This development subsequently induces proliferation in wound fibroblasts (Cheon et al. 2004). Consequently, desmoid-type fibromatosis has been described as the uncontrolled growth of a scar. In FAP patients, prophylactic colon surgery may be postponed a few years to delay surgery-induced desmoid tumor development (ML et al. 2017). For FAP carriers, positive familial history and a mutation in the *adenomatous polyposis coli* (*APC)* gene 3' codon increase the risk of desmoid tumors (Nieuwenhuis, Lefevre, et al. 2011).

Another risk factor is pregnancy, during and shortly after which desmoid tumors typically emerge in the abdominal wall, particularly the rectus abdominis muscles. The elevated risk has been connected to hormonal influence and pregnancy-induced aponeural stretching. These tumors usually behave indolently and can, in many cases, be either observed or successfully resected. On the contrary, nearly half of women with existing sporadic desmoid tumor experience disease progression during or after gestation. Therefore, careful monitoring during pregnancy is required. The complication risk depends on the tumor location, and for most patients, desmoid tumors can be treated successfully during pregnancy, although data of intra-abdominal or retroperitoneal tumors are limited. Generally, pregnancy is not considered contraindicated in desmoid tumor patients (Fiore et al. 2014).

2.4.2. SCREENING FOR FAP

Desmoid fibromatosis may be the first manifestation of FAP, and colonoscopy should be considered for newly diagnosed desmoid tumor patients. In literature, endoscopic screening has revealed undiagnosed FAP in 1.3–3.7% of these patients (Koskenvuo et al. 2016; van Houdt et al. 2019). Diagnostic yield was higher in patients below 40 (11%), with intra-abdominal, retroperitoneal (5.4%), or multifocal tumors (29%), and in patients with a family history of FAP (8%). Tumoral *CTNNB1* gene alteration seems to exclude *APC* mutation, and therefore, patients harboring *CTNNB1* tumor mutation may not require endoscopic screening (van Houdt et al. 2019).

2.5. IMAGING

In the initial diagnostic phase in primary health care ultrasound can be feasible for patients presenting with a soft tissue mass. Subsequently, soft tissue lesion can be visualized with computed tomography (CT) or with magnetic resonance imaging (MRI) to evaluate the tumor diameter and adherence to adjacent structures. For intra-abdominal tumors, CT is the primary choice, whereas in other locations, due to the superior soft tissue contrast, MRI is the gold standard in desmoid tumor imaging. In non-contrast

CT often low attenuation of these tumors is close to attenuation of skeletal muscles. Contrast enhancement varies from mild to medium and only infrequently desmoid tumors show prominent enhancement. Low MRI signal on T1-weighed images is a common feature for these tumors, whereas, in T2-weighed images the signal intensity varies depending on lesion cellularity and collagen content (Figure 3) (Braschi-Amirfarzan et al. 2016). 18F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET) has been investigated not only as a diagnostic imaging modality but also as an evaluative tool for the role of ^{18}F-FDG uptake changes in the prediction of therapy response in desmoid tumors (Kasper et al. 2013). In clinical practice, ^{18}F-FDG-PET is seldom used in desmoid tumors.

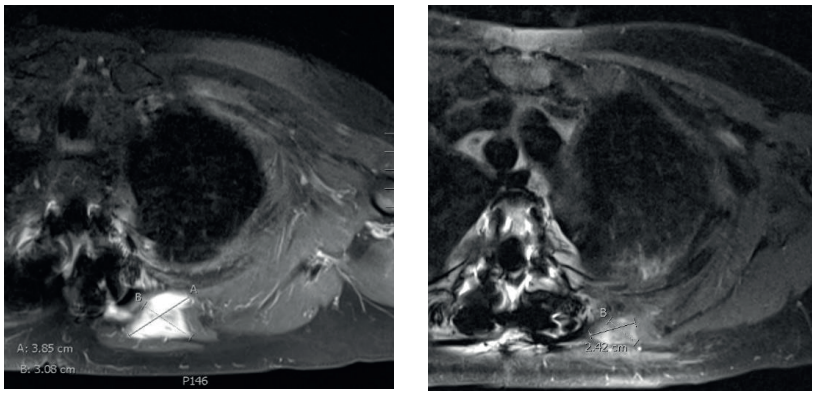

Figure 3. Desmoid tumor growing in the upper back adjacent to the region previously operated because of osteoporosis. Concurrent unspecified findings in the lungs and thyroid gland proved to be a metastatic follicular thyroid carcinoma. The desmoid causing pain and discomfort was treated with 60 Gy in 2 Gy fractions radiotherapy. Six months after radiotherapy magnetic resonance imaging (MRI) displayed decreased T2 signal intensity and stable disease.

2.6. HISTOPATHOLOGY

Extra-abdominal desmoid tumors arise from musculoaponeurotic or facial tissues, and intra-abdominal tumors stem from mesenteric folds or retroperitoneally. A percutaneous core needle biopsy, examined by an expert sarcoma pathologist, is useful to confirm the diagnosis. Macroscopically desmoid tumors are composed of pale tissue mass, strands, or plaques. Tumor cells show fibroblastic or myofibroblastic differentiation. They consist of

elongated spindle-like cells with often abundant collagen and vasculature with perivascular edema (Figure 4). Mitosis is generally rare, nuclear atypia is absent and cellularity is sparse (Fisher and Thway 2014; Fletcher, World Health Organization., and International Agency for Research on Cancer. 2013).

Nuclear β-catenin expression is utilized in differential diagnostics as a diagnostic tool to distinguish these tumors from morphologically similar lesions and to confirm the diagnosis. The differential diagnosis includes other myofibroblastic lesions, perineurinomas, low-grade fibromyxoid sarcomas, gastrointestinal stromal tumors, and spindle cell liposarcomas (Fisher and Thway 2014). Between 82% and 100% of desmoid tumors show positive β-catenin staining; however, a few other soft tissue lesions, such as solitary fibrous tumors and fibrosarcomas, also occasionally express β-catenin. Moreover, sample representativity, immunohistochemistry procedure (antibody clones, fixation, staining) and interpretation may affect the outcome in immunohistochemistry (Ng et al. 2005). Gene mutation analysis may be a more sensitive and specific method than immunohistochemistry in differential diagnostics, although not as commonly available and associated with higher costs (Koike et al. 2019). In large series with up to 191 potential morphologic mimics, *CTNNB1* mutation has not been detected in lesions potentially imitating desmoid tumors (Amary et al. 2007; Le Guellec et al. 2012). In patients treated with a non-surgical approach, the evaluation of β-catenin expression from a core needle biopsy may be challenging, and especially for these patients, *CTNNB1* gene analysis may prove useful. In desmoid-type fibromatosis, the most frequently identified *CTNNB1* mutations are located in exon 3 codons 41 or 45, including three point mutations: T41A (substitutes threonine with alanine), S45F (substitutes serine with phenylalanine), and S45P (substitutes serine with proline) (Crago et al. 2015; Salas et al. 2010).

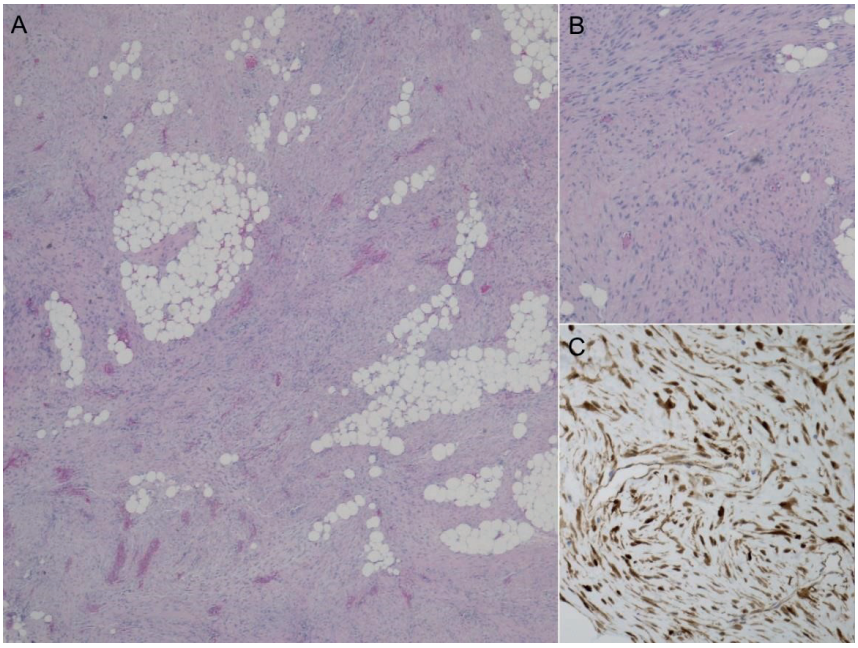

Figure 4. (A) Desmoid tumors tumors often invade into adjacent tissues. (B) Tumors consist of spindle-shaped cells with abundant vasculature. (C) Typical nuclear staining for β-catenin by immunohistochemistry.

2.7. PREDICTORS OF POSTOPERATIVE RECURRENCE

Desmoid tumors have the propensity to recur even after a complete resection with negative surgical margins. Local relapse most commonly occurs within two years following the initial treatment; however, recurrences after decades from primary treatment have been reported (Fiore et al. 2009).

The significance of surgical margins has long been debated. Positive surgical margins predicted faster recurrence in many retrospective series (Nuyttens et al. 2000; Ballo et al. 1999; Merchant et al. 1999) but not in others (Lev et al. 2007; Salas et al. 2011; Merchant et al. 1999). In a meta-analysis of 1295 patients with a resected extra-abdominal desmoid tumor, the positive surgical margin increased the risk for recurrence almost twofold (hazard ratio [HR] 1.78, 95% confidence interval [CI] = 1.4–2.26) (Janssen et al. 2017). Besides marginals, young age under 25 and upto to 37 years, tumors size over 4–10 centimeters, and limb location have independently predicted local recurrence following surgery (Yao et al. 2014; Crago et al. 2013; Salas et al. 2011). To aid

decision-making, a nomogram to predict postsurgical relapse was created and validated using age, tumor localization, and size as variables (Crago et al. 2013). Moreover, in sporadic desmoid tumors, a specific *CTNNB1* mutation S45F predicted elevated risk for recurrence after surgery (Colombo et al. 2013; Lazar et al. 2008; van Broekhoven, Verhoef, et al. 2015; Domont et al. 2010). However, not all reports confirmed these results (Romero et al. 2012; Mullen et al. 2013). A meta-analysis combining all these studies might clarify the role of S45F mutations in desmoid tumor biology.

2.8. MANAGEMENT

During recent years, the management of desmoid tumors has changed from initial radical surgery into a more conservative approach. The National Comprehensive Cancer Network guidelines suggest therapy including surgery, systemic therapy, or radiotherapy for progressive or symptomatic desmoid (Mehren et al. 2019). Individualized therapy design in multi-professional collaboration is recommended. Patient preference should be taken into account after being informed about the risks and benefits of different treatment policies.

2.8.1. SURVEILLANCE

The European Consensus Initiative advocates an initial follow-up period before further management of desmoid tumor patients (Kasper, Baumgarten, et al., 2017). The watchful waiting policy originates from observations that even without any treatment, desmoid tumors may undergo unpredictable phases of tumor growth, stabilization, and spontaneous regression (20–30% of tumors) (Colombo et al. 2015). In retrospective surveillance studies, the local control rate has varied from 60% to 92%, with the highest rate of prolonged stabilization in abdominal wall tumors (Briand et al. 2014; Barbier et al. 2010; Fiore et al. 2009; Huang et al. 2014; Salas et al. 2011). The advantage of follow-up strategy is that a portion of patients avoids the morbidity related to surgery, radiotherapy, or medical therapy. The observational strategy requires frequent imaging to detect rapid growth or

impending infiltration or compression to adjacent vulnerable structures (Braschi-Amirfarzan et al. 2016). During follow-up, clinical control and imaging may be performed every 3 to 6 months for the first 2 to 3 years and after that every six months or annually (Mehren et al. 2019). The optimal length of surveillance continues to be an open question.

The factors responsible for progression at surveillance of desmoid tumors have remained unknown. Predictive factors for unfavorable outcome in surgically treated patients cannot be applied to patients who are observed. A few studies have examined whether changes in MRI signal intensity predict progression during follow-up with contradictory results (Castellazzi et al. 2009; Healy et al. 1997). In a recent series of 37 observed patients, hyperintense T2 signal in ≥ 90% of tumor volume predicted progression (Cassidy et al. 2018). Currently, the wait-and-see strategy is explored in several prospective trials (NCT01801176, NCT02547831, and NTR4714) (van Broekhoven et al. 2016).

2.8.2. SURGERY

A decade ago, the mainstay of the treatment was surgery. The accumulating evidence of observational policy led to a change of management guidelines. Upfront surgical therapy may be necessary if desmoid tumor impairs function or in case of emergencies, such as intestinal obstruction or perforation. The goal of surgical resection is to obtain clear margins (R0) (Table 1), preserving function and esthetic appearance. In some occasions, reconstructive surgery may be needed. Considering the infiltrative growth pattern, obtaining negative margins may be a challenge, especially in specific locations such as the limb girdles, head and neck area, and the small bowel mesenterium. Negative margins should not be attempted sacrificing neurovascular or other critical structures, given that not all tumors recur even after margin positive surgery. For extra-abdominal desmoid tumors, the local control rate of after surgery has varied from 47% to 86% whereas the outcome following abdominal wall tumor resection seems superior with a recurrence rate of only 5% (Wilkinson et al. 2014; Smith et al. 2018). An abdominal mesh can be used to repair the defect in the resection site.

***Table 1**. Classification of surgical margins. (Adapted from The eighth edition AJCC Cancer Staging manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging) (Amin et al. 2017)*

R0	No residual tumor	Negative/free/clear margins
R1	Microscopic residual tumor	Intralesional resection/
R2	Macroscopic residual tumor	positive margins

The vast majority of tumors in the abdominal cavity grow in the root of mesenteric vessels. Depending on the tumor site and size, resection of an intra-abdominal desmoid tumor may lead to small bowel resection and cause significant morbidity (Latchford et al. 2006). A stable and symptomless intra-abdominal tumor can be followed; however, in other cases, surgical resectability should be assessed, and potential benefits and disadvantages should be weighed against those of systemic therapy.

2.8.3. RADIOTHERAPY

In the adjuvant setting, radiation has been used after margin-positive resection. However, similar to resection marginals, adjuvant radiotherapy has been a controversial issue in desmoid tumor management (Crago et al. 2013; Yao et al. 2014). In a meta-analysis, adjuvant radiotherapy following resection with R1 or R2 margins reduced the risk for relapse; however, the effect was more prominent in the group with recurrent desmoid tumors. Radiotherapy after surgery with R0 margins was not associated with improved prognosis (Janssen et al. 2017). Taking into account long-term radiation-related toxicity and given that only about one third of the primary tumors after a resection with R1 margins recur, postoperative radiotherapy is recommended in recurrent desmoid tumors after intralesional resection if re-resection of a relapse would be potentially harmful (Janssen et al. 2017; Kasper, Baumgarten, et al. 2017).

Surgery combined with adjuvant radiotherapy and radiotherapy as the sole treatment have demonstrated similar local control rates (Guadagnolo, Zagars,

and Ballo 2008; Keus et al. 2013). Therefore, if inadequate surgical margins appear likely after an upfront operation, radiotherapy alone can be delivered to avoid surgery-related morbidity. For intra-abdominal desmoid fibromatosis, radiation is seldom used due to the proximity of radiosensitive organs and a high risk of radiation-induced enteritis.

2.8.3.1. Treatment planning and dose

The goal of radiotherapy is to target the tumor volume or the operative bed with an adequate dose sparing the adjacent healthy tissues. Treatment planning simulation is performed with either CT or MRI patient fixed in the treatment position. The clinical target volume (CTV) includes the microscopic disease surrounding the gross tumor volume (GTV). Usually, 5-cm margins have been utilized vertically, and 2-cm ones have been used in other directions, excluding the natural barriers, such as bones and fascial planes (Keus et al. 2013; Guadagnolo, Zagars, and Ballo 2008). The infiltrative growth pattern emphasizes the significance of wide margins in desmoid tumors (Zlotecki et al. 2002). Given the tumor location, both the photon beam and electrons can be implemented using individually appropriate techniques.

A favorable dose-response has been described (Choi et al. 2018; Spear et al. 1998). Radiotherapy alone for inoperable progressing desmoid tumors using moderate dose radiation with fractionation scheme of 56 Gy in 28 fractions yielded a local control rate of 82% at three years in a prospective multicenter phase II study (Keus et al. 2013). Higher doses from 60 to 65 Gy have been employed; however, doses higher than 56 Gy were associated with a higher rate of radiation-induced complications (Guadagnolo, Zagars, and Ballo 2008). In the case of microscopic residual tumor, 50 Gy is considered a sufficient dose, whereas, for tumors with a macroscopic residual or gross tumor, 50–56 Gy is recommended. Radiation therapy can be delivered in two phases using the shrinking field technique, first attending to 50 Gy and then boosting the gross tumor volume.

2.8.3.2. Radiation-related toxicity

In the treatment of a semi-malignant tumor, the risk for secondary cancer and long-term sequelae should be marked, especially in the young patient group. Toxicity depends on the irradiated site, volume, dose, and individual sensitivity for radiation. Acute radiation-related toxicity, such as skin irritation, is often reversible, whereas late toxic effects can be long-lasting. Late toxic effects include joint and muscle stiffness, lymphedema, fibrosis, skin effects, neuropathy, bone fracture, and osteonecrosis, for example. Second malignancy in the irradiated volume is a rare event. (Guadagnolo, Zagars, and Ballo 2008; Yao et al. 2014) Continuously improving imaging and radiation techniques reduce the irradiation of healthy tissues.

2.8.4. OTHER LOCOREGIONAL THERAPIES

Percutaneous cryoablation and radiofrequency ablation are minimally invasive local treatment investigated in desmoid tumors (Ilaslan et al. 2010; Schmitz et al. 2016). Percutaneous cryoablation and radiofrequency ablation allow the management of lesions with diameters approximately up to 10 cm or 5 cm, respectively, or larger tumors within multiple treatment times. Involvement or proximity of critical tissues limits the use of both treatment modalities. Ultrasound-guided or magnetic resonance-guided high-intensity focused ultrasound is a non-invasive treatment suitable also for larger desmoid tumors (Ghanouni et al. 2017; Zhao et al. 2016). If tumor eradication is sought, the tumor should be separated from skin and nerves. In a desmoid tumor series, the most common reported adverse effect was skin burn, and the most challenging complication was nerve injury (Ghanouni et al. 2017). The advantages of these locoregional treatments include repeatability and safety. The initial results in small retrospective series have been promising, although more extensive prospective trials and longer follow-up time are warranted.

Isolated limb perfusion with tumor necrosis factor α and melphalan showed clinical benefit in locally advanced extremity desmoid tumors where surgery would have resulted in mutilation (van Broekhoven et al. 2014). The response

rate (RR) was 68% (19/28), and after seven years of median follow-up time, disease progression was observed following 39% (11/28) of initial treatment with isolated limb perfusion. Toxicity was mostly modest, including erythema and edema, although there is a risk for more severe complications. The role of these locoregional techniques in treatment algorithms is unclear, although, in specialized centers, they offer an additional alternative.

2.8.5. SYSTEMIC THERAPY

Desmoid tumors are uncommon, and their behavior is unpredictable, including periods of growth and regression, which makes the evaluation of medical therapies demanding. Various therapeutic agents have been used in desmoid tumor management; however, these therapies have rarely been compared with each other in clinical trials. There is no consensus about the sequence of medical agents. Despite being challenging, it has highly been encouraged to enroll desmoid tumor patients in prospective and randomized clinical trials. This approach is needed to provide high-level evidence-based treatment guidelines in the future.

Table 2. *Response evaluation criteria in solid tumors (e.g., RECIST 1.1. guidelines) (Adapted from Eisenhauer et al. New response criteria in solid tumours: revised RECIST guideline (version 1.1.) Eur J Cancer 2009) (Eisenhauer et al. 2009)*

Complete response (CR)	Disappearance of all target lesions
Partial response (PR)	At least a 30% decrease in the sum of diameters of target lesions, taken as reference for the baseline sum diameters
Progressive disease (PD)	At least a 20% increase in the sum of diameters of target lesions, taken as reference for the smallest sum on study
Stable disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taken as reference for the smallest sum diameters while on study

2.8.5.1. Non-steroidal anti-inflammary drugs

The complete shrinkage of an inoperable thoracic desmoid tumor during the treatment with indomethacin for pericarditis led to the use of NSAIDs in desmoid tumors (Waddell and Gerner 1980). Pericarditis was induced by preceding radiotherapy, and when assessed afterward, a late response for radiotherapy may have contributed to the tumor response. After that, NSAIDs were utilized in the management of desmoid tumors. NSAIDs, such as meloxicam, sulindac, or celecoxib, can be administered as first-line therapy due to their minimal toxicity. Additionally, NSAIDs prevent the development of premalignant colonic polyps in patients with FAP.

NSAIDs inhibit cyclooxygenase (COX) 1 and 2 enzymes. In desmoid tumors, the upregulation of COX 2 may be due to stabilized β-catenin acting as a coactivator for T-cell factor/lymphoid enhancer factor (tcf-lef) family of transcription factors. These transcription factors promote genes that regulate proliferation, differentiation, and apoptosis, including COX 2 (Signoroni et al. 2007). In desmoid tumors, the immunohistochemical expression of COX 2 has varied from 50% to 100% (Cho et al. 2018; Nishida et al. 2010; Signoroni et al. 2007). Furthermore, COX 2 inhibition in desmoid tumor cell cultures resulted in reduced proliferation, and *in vivo* mouse models COX 2 inhibition reduced desmoid tumor sizes (Poon et al. 2001).

The activity of NSAIDs has been documented in successful case reports, and the RRs in minor series have varied from 25% to 57% (Cho et al. 2018; Janinis et al. 2003; Nishida et al. 2010; Tanaka et al. 2008). In combination with endocrine therapy, NSAIDs have yielded similar RRs as in monotherapy with these agents (Quast et al. 2016). A retrospective study of 20 patients with an extra-abdominal desmoid tumor found no correlation between the outcome of meloxicam and the expression of COX 2 in tumor cells (Cho et al. 2018). Moreover, the results were compared with active surveillance studies in extra-abdominal desmoid tumors. Thirteen of 20 patients (65.0%) in the meloxicam study reached SD or PR, whereas, in the surveillance studies, 80.3% (167/208) of desmoid tumor patients experienced either tumor growth arrest or regression (Cho et al. 2018). Today the position of NSAIDs in desmoid tumor

therapy is restricted to patients with slowly growing desmoid tumors and mild symptoms.

2.8.5.2. Hormonal therapy

Endocrine treatment is often a well-tolerated and affordable option for frontline therapy in desmoid tumors. Selective estrogen receptor modulators (SERM) such as tamoxifen, toremifene, and more uncommonly raloxifene have been used for desmoid tumors in doses parallel to breast cancer and tamoxifen, as high-dose therapy can reach up to 160 mg daily (Brooks et al. 1992; Fiore et al. 2015). The efficacy of hormonal therapy has been varied, and it is based on case reports and uncontrolled series. A systematic review summarized endocrine treatments with a RR of 51% with a 9-month median duration of treatment, whereas, in a prospective phase II study on children, the RR remained at 8% and progression-free survival (PFS) was 36% at two years (Bocale et al. 2011; Skapek et al. 2013). Only anecdotal cases of treatment with aromatase inhibitors and gonadotropin-releasing hormone (GnRH) analog have been reported (Bauernhofer et al. 1996; Debled et al. 2012; Wilcken and Tattersall 1991).

2.8.5.3. Chemotherapy

Chemotherapy is usually used for inoperable desmoid tumors in case of rapid growth. Both low dose agents and traditional dose chemotherapy have been administered, including anthracyclines and methotrexate in combination with vinca-alkaloids. A retrospective Italian study of methotrexate plus vinca-alkaloid yielded a RR of 48% and a median PFS of 75 months with 75 patients (Palassini et al. 2017). In a retrospective study, the French Sarcoma Group reported a higher 54% RR with anthracycline-based chemotherapy (n = 13) compared with a 12% RR with non-anthracycline regimens (e.g., the combination of methotrexate and vinblastine) (n = 27). The median PFS of 41 months did not differ significantly between the groups (Garbay et al. 2012). Adverse events such as severe myelotoxicity, anthracycline-induced

cardiotoxicity, and elevated risk of secondary leukemia limit the use of chemotherapy. Liposomal doxorubicin is often recommended to avoid cardiac toxicity.

2.8.5.4. Tyrosine kinase inhibitors

Tyrosine kinase signaling pathways regulate cell growth and proliferation. Imatinib was the first tyrosine kinase inhibitor with described activity in desmoid tumors (Mace et al. 2002). Imatinib inhibits a limited number of tyrosine kinases, such as platelet-derived growth factor receptor (PDGFR)-α, PDGFR-β, stem cell receptor c-KIT, and nonreceptor tyrosine kinase ABL. In prospective trials of imatinib with 141 patients overall, the RR has varied from 6% to 18%, and 1-year PFS has varied from 37% to 66% with tolerable side effects (Chugh et al. 2010; Heinrich et al. 2006; Kasper, Gruenwald, et al. 2017; Penel et al. 2011). No significant correlation between the response and c-KIT, PDGFR-β, or other candidate target proteins were detected (Chugh et al. 2010). With sunitinib, a higher RR of 26% and a 2-year PFS of 74% was documented in a prospective phase II study with 19 patients (Jo et al. 2014). Pazopanib is another multitarget tyrosine kinase inhibitor investigated in small series of desmoid tumor patients (Agresta et al. 2018; Martin-Liberal et al. 2013; Szucs et al. 2017). Moreover, a non-comparative randomized phase II trial evaluated the activity of pazopanib (n=46) or methotrexate combined with vinblastine (n=20) in progressive desmoid tumors. Treatment with pazopanib demonstrated a RR of 37% and a 2-year PFS of 67% whereas in the chemotherapy group the RR was 25% and 2-year PFS was 79%. (Toulmonde et al. 2019) Compared with imatinib, sunitinib and pazopanib target a broader spectrum of tyrosine kinases, including PDGFR, c-KIT, vascular endothelial growth factor receptor (VEGFR), and receptor tyrosine kinase RET with differing affinity to the receptors. VEGFR overexpression has been linked with aggressive growth in desmoid tumors (Matono et al. 2011)

Sorafenib is a multikinase inhibitor, which besides targeting PDGFR, VEGFR, and RET, also inhibits RAF- and MAP-kinase pathways. A retrospective study of sorafenib with 62 evaluable patients obtained a similar RR of 18% with a

differing affinity to the receptors. VEGFR overexpression has been linked with aggressive growth in desmoid tumors (Matono et al. 2011)

Sorafenib is a multikinase inhibitor, which besides targeting PDGFR, VEGFR, and RET, also inhibits RAF- and MAP-kinase pathways. A retrospective study of sorafenib with 62 evaluable patients obtained a similar RR of 18% with a median PFS of 48 months (Munhoz et al. 2016). In a recent randomized prospective phase III trial with 87 patients, the RR was 33%, and the 2-year PFS was 81% in the sorafenib group compared with RR of 20% and 2-year PFS of 36% in the placebo group (HR 0.13) (Gounder et al. 2018). The start dose for sorafenib was 400 mg once daily, which is lower than in other indications. The results of the biomarker study based on 25 sets of paired biopsy specimen are awaited.

The challenge with a rare disease entity is generally the unavailability of financial reimbursements for systemic agents. This concerns not only tyrosine kinase inhibitors but also other outpatient medical therapies. At present, no medicines are accepted for reimbursement for desmoid tumor patients in Finland.

2.8.5.5. Other therapies

The activity of interferon alpha is based on single case reports (Fernberg et al. 1999; Stengel et al. 2008). Adverse effects of especially the non-pegylated formulation are common and often severer, including asthenia, flu-like symptoms, hematologic toxicity, and depressive symptoms. Pegylated interferon with a more favorable side-effect profile is not available in Finland. Besides, the subcutaneous administration, at least three times per week, may limit the use of interferon alpha. The biological basis for the use of interferon in desmoid tumors is unclear.

In a phase I dose-escalation study, a novel therapeutic agent, oral γ-secretase inhibitor PF-03084014, demonstrated a promising activity with RR of 71% (5/7) in desmoid tumors (Messersmith et al. 2015). γ-Secretase activates Notch signaling, which is commonly deregulated in cancer and interacts with

the Wnt/β-catenin pathway (Rodilla et al. 2009). The long-term follow-up study showed no disease progression in five patients who achieved PR at follow-up from 48 to 73+ months (Villalobos et al. 2018). In a phase II study with 17 patients, γ-secretase inhibitor PF-03084014 achieved a PR rate of 29%. Furthermore, during the median follow-up time of 25 months, eleven patients had SD (Kummar et al. 2017). With a dose of 150 mg twice per day, the most common grade 1/2 adverse effects were diarrhea and skin toxicity. The only grade 3 side effect was reversible hypophosphatemia (Kummar et al. 2017). A randomized placebo-controlled phase III study is underway (NCT03785964).

Table 3. *Outcome of local and systemic therapies in desmoid tumors.*

Treatment	Study	Type of study	*n*	*ORR %*	*Local control*
Surgery	(Crago et al. 2013)	Retrospective	*413*		5-y LRFS 72%
	(Huang et al. 2009)	Retrospective	126		10-y LCR 81%
	(Mankin, Hornicek, and Springfield 2010)	Retrospective	177		LCR 83%
	(Merchant et al. 1999)	Retrospective	74		LCR 77%
Surgery and radiotherapy	(Crago et al. 2013)	Retrospective	82		5-y LRFS 68%
	(Guadagnolo, Zagars, and Ballo 2008)	Retrospective	74		10-y LCR of 78%
	(Mankin, Hornicek, and Springfield 2010)	Retrospective	39		LCR 87%
Radiotherapy	(Guadagnolo, Zagars, and Ballo 2008)	Retrospective	41	N/A	10-y LCR of 65%
	(Keus et al. 2013)	Phase II study	44	50	3-y LCR 81.5%
High intensity focused ultrasound	(Ghanouni et al. 2017)	Retrospective	25	N/A[a]	N/A
Radiofrequency ablation	(Ilaslan et al. 2010)	Retrospective	5	N/A	N/A
Percutaneus cryoablation	(Schmitz et al. 2016)	Retrospective	26	N/A	N/A
Isolated limb perfusion	(van Broekhoven et al. 2014)	Retrospective	28	65	LCR 61%, 7-y median follow-up
Sulindac	(Tsukada et al. 1992)	Retrospective	14	57	N/A
Tamoxifen or toremifen	(Brooks et al. 1992)	Retrospective	26	42	N/A
Toremifen	(Fiore et al. 2015)	Retrospective	44	25	2-y PFS 90%
Anthracycline based chemotherapy	(de Camargo et al. 2010)	Retrospective	35	37	LCR 88%
	(Garbay et al. 2012)	Retrospective	13	54	Median PFS 41 months
Methotrexate and vinka-alkaloid	(Azzarelli et al. 2001)	Phase II study	30	40	10-y PFS 67%
	(Li et al. 2017)	Retrospective	71	35	2-y PFS 80%
	(Palassini et al. 2017)	Retrospective	75	48	Median PFS 75 m
Imatinib	(Chugh et al. 2010)	Phase II study	51	6	1-y 66%, 3-y 58%
	(Penel et al. 2011)	Phase II study	40	10	1-y 67%, 3-y 40%
Sunitinib	(Jo et al. 2014)	Phase II study	19	26	1-y PFS 80%
Pazopanib	(Toulmonde et al. 2019)	Phase II study	46	37	1-y PFS 86%, 2-y PFS 67%
Sorafenib	(Gounder et al. 2018)	Phase III study	87	33	2-y PFS 81%
	(Munhoz et al. 2016)	Retrospective	62	18	Median PFS 4 years
γ-secretase inhibitor PF-03084014	(Kummar et al. 2017)	Phase II study	17	29	59% remain on study ≥ 2 years

[a]Median total tumor volume decreased by 52%

ORR = objective response rate, LRFS = local recurrence-free survival, LCR = local control rate, PFS = progression-free survival, N/A = not applicaple, y= year

2.9. CYCLINS AND CYCLIN-DEPENDENT KINASES IN CELL CYCLE REGULATION

Disturbances in the cell division are a hallmark of cancer-driving cells, which leads into malignant proliferation. Cyclins and cyclin-dependent kinases (cdk) are critical players in the strictly regulated cell cycle. Peaking of specific cyclins activate cdks, which are present throughout the cell cycle. As a complex, they promote cell cycle progression via phosphorylation, whereas cdks are negatively regulated by naturally occurring cyclin-dependent kinase inhibitory proteins, such as the INK4, CIP, and KIP families (Sherr and Roberts 1999).

Cells enter the cell cycle from the quiescent phase G0 or straight after the previous cell division. In the first gap phase, G1 retinoblastoma tumor suppressor protein (Rb) serves as a regulatory restriction point (Figure 5). Before the restriction point, mitogenic signaling, such as RAS, estrogen, and Wnt-β-catenin, promotes cell cycle progression by inducing an increase in cyclin D expression level. D-type cyclins bind to cdk 4, which then phosphorylate and partly inactivate Rb. After that, Rb unbinds E2F family of transcription factors, which promotes cyclin E, A, and cdk2 and other factors associated with mitosis. In the late G1 phase, cyclin E and cdk2 complex completely deactivates Rb by hyperphosphorylation and driving the transition from G1 checkpoint to DNA synthesis (S) phase. After passing the restriction point, cell division proceeds independently of external growth signals. Following DNA replication in S phase, cyclin A peaks and pairs with cdk 2, leading to the second gap phase (G2). The cell continues growing and prepares itself for mitosis in the G2 phase where it exits once cyclin B and its partner cdk 1 are activated in the second checkpoint. In the mitotic phase (M), the nucleus is divided, followed by cytokinesis. After cyclin B-cdk1 complex degradation, the cell exits the M phase to form two daughter cells. In addition to the canonical functions, cyclins and cdks have cell-cycle-independent roles, such as the modulation of transcriptional activity via activation of RNA polymerase II (Diaz-Moralli et al. 2013).

If a phase is not properly completed, cell division is stopped in order to prevent the development of defected and unviable cells. If repairment is not achieved,

this can lead to either apoptosis or shifting the cell into the resting phase Go (Diaz-Moralli et al. 2013).

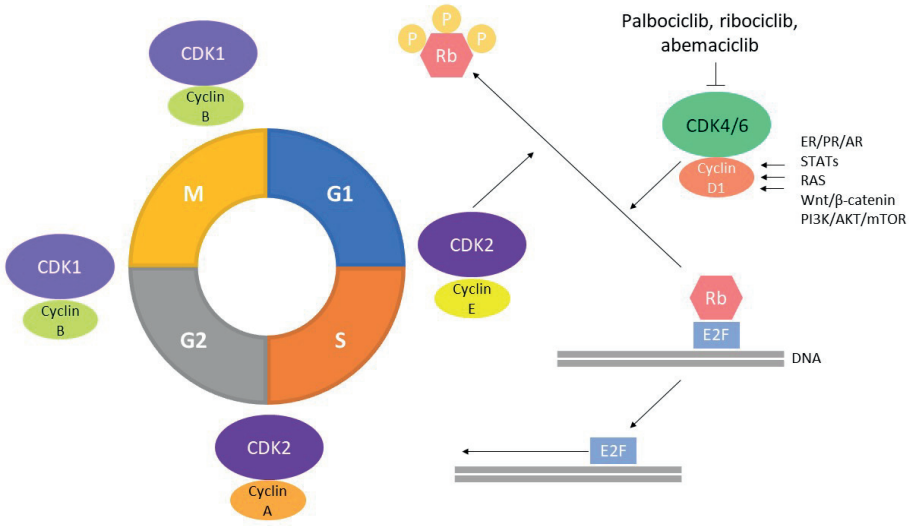

Figure 5. In the first gap phase (G1) of the cell cycle cyclin-dependent kinases (cdk) 4 and 6 bind with D-type cyclins and phosphorylate retinoblastoma susceptibility protein Rb which inhibits E2F family of transcription factors. Growth factors, such as sex hormones, increase the expression of cyclin D. Rb is further phosphorylated by cdk2-cyclin E complex leading to increased transcription of factors involved in mitosis and finally to cell cycle progression into phase S. Modified from (Ribnikar, Volovat, and Cardoso 2019)

In neoplasms, cell division can be dysregulated in many ways. $P16^{INK4A}$-cyclin D-cdk4/6-Rb pathway aberrations frequently occur in various cancer types, including the inactivation of inhibitory proteins INK4 and CIP/KIP and the overexpression or amplification of *CDK4/6*, *CCND1*, and Rb mutations. D-type cyclins include cyclin D1, D2, and D3, of which cyclin D1 expression is the most frequently altered and also the most widely investigated (Qie and Diehl 2016; Ingham and Schwartz 2017). Cyclin D1 overexpression has been associated with poor prognosis in several cancer types, such as breast and prostate (Aaltonen et al. 2009; Fleischmann et al. 2011; Xu et al. 2013). Data on the clinical significance of cyclin A protein expression in cancer is comparatively limited. In renal cell carcinoma, endometrial carcinoma, and soft tissue sarcoma, high cyclin A expression has shown an unfavorable effect

on the outcome (Aaltomaa et al. 1999; Huuhtanen et al. 1999; Santala et al. 2014).

2.9.1. CYCLIN-DEPENDENT KINASE 4/6 INHIBITORS

The first-generation of non-specific cdk-inhibitors exhibited a high rate of adverse effects, which limited the dose and efficacy. Finally, the invention of cdk-specific inhibitors reduced toxicity and led to successful clinical trials. The first-in-class oral inhibitor of cdk4 and 6 was palbociclib, which, when combined with endocrine therapy, was perceived as a breakthrough in the management of advanced estrogen-receptor-positive human epidermal growth factor receptor 2 (HER2) -negative breast cancer (Cristofanilli et al. 2016). Currently, two other cdk4/6 inhibitors—ribociclib and abemaciclib—are approved by the US Food and Drug Administration and European Medicines Agency with the indication for advanced hormone receptor-positive breast cancer (O'Shaughnessy et al. 2018; Goetz et al. 2017). The improved progression-free survival and RRs are comparable to all three cdk4/6 inhibitors, whereas slight differences in adverse effects exist. Cdk 4/6 inhibitors arrest the cell cycle in the mid G1 phase, although they do not have a cytotoxic effect (Figure 5). The most frequent dose-limiting adverse event of all these drugs is myelotoxicity; however, neutropenic infections are rare. Today all these agents are investigated in numerous clinical trials as a monotherapy and combined with other medication or radiotherapy in different malignancies. Cdk 4/6 inhibitors have demonstrated efficacy for example in melanoma, non-small cell lung carcinoma, head and neck squamous cell carcinoma, ovarian cancer and liposarcoma (Schettini et al. 2018; Adkins et al. 2019). In a phase II trial of advanced well-differentiated/dedifferentiated liposarcoma treatment with single-agent palbociclib showed a favorable 12-week PFS rate of 57% when compared with an expected 12-week PFS of 40% for an active second-line medical agent in historical series. The majority of these tumors (>90%) display *CDK4* amplification and they commonly express Rb. (Dickson et al. 2013; Dickson et al. 2016)

2.10.ESTROGEN RECEPTORS AND CANCER

Estrogen receptors belong to the superfamily of nuclear receptors, which regulate gene expression in target tissues both in physiological and pathological processes. The two main isoforms are estrogen receptor α (ERα) and ERβ. The DNA-binding domains differ only slightly as they have 96% in common, whereas in the ligand-binding domain there is only 60% homology. In addition to natural estrogen receptor ligand 17β-estradiol, SERM, such as tamoxifen, can serve as ligands for both receptor types with tissue-specific agonist/antagonist action (Kuiper et al. 1997). Once the ligand binds to the receptor, ERα and ERβ dimerize either as homodimers (αα or ββ) or heterodimers (αβ) (Cowley et al. 1997). The conformational changes of the dimers enable their binding with estrogen-responsive elements of DNA promoters in the target genes followed by interaction with co-regulators. In addition to the classical genomic pathway, the effects of estrogens are conveyed by the non-genomic pathway. Estrogen signaling triggers protein-kinase cascades via the third estrogen receptor, G-protein coupled estrogen reseptor 1, and it is connected with epigenetic regulation mechanisms (DNA methylation, micro RNA:s, histone modifications) both upstream and downstream (Filardo et al. 2007; Vrtacnik et al. 2014).

ERα expression predicts beneficial response for hormonal therapy in breast cancer, and in hormonally treated patients, it predicts favorable prognosis. ERβ has shown anti-proliferative properties, and it has been associated with both increased disease-free survival and overall survival in breast carcinoma (Tan et al. 2016; Honma et al. 2008; Nakopoulou et al. 2004). It has been proposed that ERα and ERβ heterodimers decrease binding of coregulators and thus the transcriptional activity of ERα (Haldosen, Zhao, and Dahlman-Wright 2014).-However, the studies of ERβ impact on breast cancer outcome have reported inconsistent results, which may be because of several ERβ receptor subtypes and differences in ERβ function, depending on the presence of ERα and disease stage. In other cancer types, the function of estrogen receptors is less investigated. In colorectal and prostate carcinoma, ERβ acts as a tumor suppressor (Niv 2015; Weihua et al. 2002). On the contrary, in

pancreatic carcinoma, ERβ expression correlates with adverse prognosis, and in bladder cancer, ERβ promotes growth and metastasis (Ou et al. 2018; Seeliger et al. 2018).

Endometrial carcinomas are estrogen-dependent tumors. In contrast to breast cancer, signaling via progesterone receptor (PgR) opposes the actions of estrogen and inhibits proliferation in the uterus and ovaries. The most common gynecological sarcomas, uterine leiomyosarcoma and endometrial stromal sarcoma, both express ERα and PgR, whereas little is known about significance of ERβ. Endometrial stromal sarcoma is an often indolent hormone-sensitive malignancy, managed at early-stage operatively and with adjuvant progestin and in metastatic phase with progestins, aromatase inhibitors or gonadotropin-releasing analogs (Amant et al. 2014). The predictive role of steroid receptor expression, however, has not been confirmed in small-scale studies of this rare entity. In endometrial stromal sarcoma ERα, as well as PgR and androgen receptor positivity, have all been connected with improved survival (Park et al. 2018). Uterine leiomyosarcoma is a more aggressive mesenchymal tumor treated primarily with surgery and in metastatic stage with chemotherapy and targeted drugs. Case reports and small series have implied activity for endocrine therapy. Moreover, in a phase II study aromatase inhibitors demonstrated beneficial outcome for uterine leiomyosarcoma patients with high levels of ERα and PR expression (George et al. 2014). Similarly with endometrial stromal sarcoma, in leiomyosarcoma high ERα and PR expression have shown to be prognostic for favorable outcome (Akhan et al. 2005; Raspollini et al. 2003).

In extrauterine soft tissue sarcomas the role of estrogen signaling is more uncertain, although ERα and ERβ are expressed in different types of healthy mesenchymal tissues including for example vascular smooth muscle, adipose tissue and peripheral nerves. In ERα negative and ERβ positive rhabdomyosarcoma cell lines estrogen stimulated proliferation indicating ERβ-mediated signal transduction (Greenberg et al. 2008). In contrast in a series of variable soft tissue sarcomas either ERα or ERβ immunoexpression correlated with proliferation (Li, Hisaoka, and Hashimoto 2003).

Furthermore, the prognostic significance of hormone receptors in unclear. In a soft tissue sarcoma study ERα negativity and PgR positivity were together prognostic for poor survival whereas a large liposarcoma study failed to show a connection between PgR expression and disease-free survival (Valkov et al. 2011; Ingram et al. 2014). With over 50 subtypes, the expression of estrogen receptors varies widely in soft tissue sarcomas.

3. Aims of the study

This thesis aimed to evaluate the outcome of oncological treatments in desmoid tumors in a single-institution series. Furthermore, the goal was to identify novel biomarkers to predict recurrence and understand the underlying pathogenesis better.

The specific objectives of this study were as follows:

I To study the outcome of radiotherapy and the patterns of recurrence after radiotherapy in desmoid tumors,

II To investigate the activity of cyclin-dependent kinase 4/6 inhibitor ribociclib combined with hormonal therapy in desmoid tumor, and

III–IV To evaluate the predictive impact of critical cell cycle regulators such as cyclin A, D1, Ki67, and ERβ as markers of disease recurrence and their possible association with other clinicopathological variables.

4. Patients and methods

4.1. PATIENTS

Data on clinical parameters were obtained from medical records, and time of death was acquired from the Population Register Center. In all the studies, histologic slides were reviewed, and an experienced sarcoma pathologist confirmed the diagnosis.

4.1.1. STUDY I

Our radiotherapy study consisted of 41 eligible patients and 44 tumors treated with 49 courses of radiotherapy at Helsinki University Hospital between 1987 and 2010. Due to missing imaging for response evaluation, two patients were evaluated only in the dose distribution analysis. Six radiotherapies in four patients were not included in the final analysis due to inadequate follow-up, imaging, or concurrent medical therapy.

The median age at diagnosis was 45, and the majority of patients were female (68%, 28/41). FAP had affected 10% (4/41) of the patients. Margins were classified as positive in 43% (21/49) and negative in 5% (5/49), and 47% (23/49) of radiotherapies were sole treatments. The demographics and the tumor characteristics at initial presentation are listed in Table 4.

Only adult patients received radiotherapy. Photons were used in 39, electrons in four and both in four radiotherapy courses. Radiotherapy was carried out either with static fields (41) or using intensity-modulated radiotherapy (IMRT) technique (4). Two treatments did not include the details of the used radiation technique.

Table 4. *Clinical variables in studies I, III, IV, and in the complete tissue microarray (TMA) series (modified from studies I, III, and IV).*

	I	TMA	III	IV
n	41	90	76	83
Median age at diagnosis	45 (5–79)	35 (17–78)	35 (17–72)	35 (17–72)
Gender				
Female	28	63	53	59
Male	13	27	23	24
Pregnancy associated (%)	0	13 (15)	11 (15)	13 (16)
FAP	4 (10)	11 (12)	9 (12)	11 (13)
Median tumor diameter, cm	8 (2–26)	6 (1–40)	6 (1–40)	6 (2–40)
Site				
Trunk	14 (34)	57 (63)	50 (66)	54 (65)
Extremities	21 (51)	17 (19)	15 (20)	15 (18)
Head and neck	2 (5)	6	4 (5)	4 (5)
Intra-abdominal	2 (5)[a]	9 (10)	6 (8)	9 (11)
Multicentric	4 (10)	1 (1)	1 (1)	1 (1)
Surgery and margins				
Margin negative	5 (10)	45 (50)	35 (46)	39 (47)
Margin positive	21 (43)	27 (30)	24 (32)	26 (31)
Unknown or no surgery	23 (47)[b]	18 (20)	17 (22)	18 (22)
Median follow-up time, years	7 (0.8–17)	11 (0.1–29)	11 (0.1–29)	11 (0.1–29)

[a]Two multifocal diseases included an intra-abdominal tumor.

[b]In study I 44 tumors were treated with 49 radiotherapy courses of which radiotherapy alone was carried out in 23 treatments.

TMA = tissue microarray, FAP = familial adenomatous polyposis

4.1.2. STUDY II

Study II describes a case report of a young female with FAP and multiple desmoid tumors located in the abdominal wall, upper arms, chest wall, and

back and suprapubic regions. To prevent the development of colorectal cancer, she underwent a prophylactic colectomy. Due to desmoid tumors, she was treated with eight resections, four radiotherapies, and several lines on systemic therapy before experimental treatment with ribociclib, letrozole, and goserelin. Radiotherapy was delivered in 2 Gy fractions with total doses of 50 Gy, 60 Gy, 40 Gy, and 60 Gy.

4.1.3. STUDIES III AND IV

Study III included 73 and study IV 83 surgically treated desmoid tumor patients. Patients were handled at Helsinki University Hospital between years 1987 and 2011. In both studies, females predominated by 69.7% and 71.1%, respectively. Table 4 summarizes demographic and baseline characteristics in studies III and IV. All the patients included in the survival analysis underwent R0 or R1 resection.

4.2. CLINICAL AND RADIOLOGICAL DATA (I, II)

The responses after radiotherapy and systemic therapy in studies I and II were reassessed according to RECIST 1.1. criteria. Additionally, in study II, the treatment with ribociclib, letrozole, and goserelin was evaluated according to WHO criteria. In study II, only non-irradiated tumors and growing tumors after previous irradiation were included.

In study I, the recurrences were delineated in CT/MR images using treatment planning software (Eclipse® 11.0, Varian Medical Systems Inc., Helsinki, Finland). The recurrence images were then rigidly co-registered with the initial dose plan. Anatomical landmarks were used to achieve optimal co-registration. The locations of treatment failures were grouped as in-target, marginal, and out-of-target. In-target recurrence situated ≥ 95% within the volume receiving ≥ 95% of the prescribed radiation dose. A marginal relapse crossed the 95% isodose, and less than 95% of the relapse volume was located inside the volume receiving ≥ 95% of the radiation dose. Out-of-target failure was outside the 95% isodose of the target volume.

4.3. IMMUNOHISTOCHEMISTRY (III, IV)

A TMA was created of paraffin-embedded formalin-fixed desmoid tumor tissue blocks. The pathologist defined and marked the representative tumor areas to the donor block, and three or more core biopsies of each block were punched using a 1-mm-diameter punch with a manual microarrayer (BeecherInstruments Inc, Silver Spring, MD, USA). Three parallel recipient tissue array blocks were formed from the cylindrical tissue cores. Human colon, stomach, and pancreas tissue specimen were implanted in the block corner as a reference for staining intensity and orientation.

After the tissue blocks were sectioned into 4-µm slides, they were deparaffinized in xylene and hydrated multiple times through graded alcohols. Heat-mediated antigen retrieval with Tris-EDTA buffer solution (pH9) was used. After that, the specimen was stained with cyclin A (Ventana, Arizona, USA), cyclin D1 (Ventana, Arizona, USA), Ki67 (Novocastra, Newcastle, UK), and estrogen receptor-β (Ventana, Arizona, USA) antibodies (see Table 5 for antibodies and dilutions). The section was subjected to pretreatment and staining with the Autostainer 480 (LabVision, Fremont, CA, USA) using Dako REAL EnVision Detection System. Counterstaining was conducted with hematoxylin and 3,3-diaminobenzidine (DAB+) solution. Finally, the slides were mounted in mounting medium (Pertex®, Histolab, Sweden).

Table 5. Antibody characteristics in studies III and IV.

Antibody	Clone	Source	Dilution
Anti-human Ki67 Antigen Clone MIB-1	M7240	Novocastra (Newcastle, UK)	1:100
Cyclin D1	SP4-R	Ventana Medical Systems (Arizona, USA)	Prediluted
Cyclin A	6E6	Novocastra (Newcastle, UK)	1:50
Estrogen receptor-β	EMR02	Ventana Medical Systems (Arizona, USA)	1:100

The TMA sections were scanned with Pannoramic 250 digital scanner (3DHistech, Budapest, Hungary). Evaluable tumor areas in each of the cores were contoured using Pannoramic Viewer software (3DHistech, Budapest, Hungary). Furthermore, the percentage of the positive nuclei was determined with NuclearQuant software (3DHistech, Budapest, Hungary), with each core individually evaluated.

The specimen with under 100 or 300 tumor cells for ERβ and proliferation markers were excluded from the analysis, respectively. Regarding Ki67, cyclin A, and cyclin D1, the cores with over 500 detected cells were primarily selected for further analysis; in other cases, the uppermost staining rate was chosen. Cyclin A, cyclin D1, Ki67, and ERβ expressions were tiered from 10th to 90th percentiles to find the optimal cut-off value. To compare our results with those of other investigators, we tested cyclin D1 cut-offs at 5% and 10% and ERβ cut-off at 1% (Table 7).

4.4. STATISTICAL METHODS

IBM SPSS Statistics for Windows versions 22-25 (SPSS, Chicago, IL, USA) was used for statistical analysis. Time to recurrence and time to progression (TTP) were calculated from the beginning of the therapy (e.g., radiotherapy, medical therapy) or from the first operation with curative intent until documented recurrence, progression, or last follow-up date. Cox regression analysis was performed to analyze the connection between clinicopathological parameters, cyclin A, cyclin D1, Ki67, ERβ, and TTR in univariate and multivariate analysis. Analysis of cyclin D1 was stratified for the use of adjuvant radiotherapy. Kaplan-Meier model was applied to compare TTR in different groups using the log-rank test.

For testing associations, Pearson's analysis was utilized for continuous data, and Student's *t*-test was used for categorical data, as appropriate. Mixed general ANOVA model tested the association between cyclin D1 expression and Ki67 separately in ERβ-positive and -negative groups. Two-tailed *p*-value was set significant when under 0.05.

4.5. ETHICAL CONSIDERATIONS

The study obtained approval from the ethics committee of Helsinki University Hospital (270/13/03/00/2011 and 2449/2017) and the National Supervisory Authority for Welfare and Health. For study II, the patient provided informed consent.

5. Results

The median follow-up time was seven years in study I and 11 years in studies III and IV. The clinical and pathological parameters are summarized in Table 4.

5.1. STUDY I

The median prescribed radiotherapy dose for both postoperative and radiotherapy alone was 50 Gy (ranging from 20 to 63 Gy) with a median fraction size of 2 Gy. After definitive radiotherapy, a complete response (CR) was recorded in three tumors (14%), a partial response (PR) in nine (41%), and stable disease (SD) in ten tumors (45%) with an objective RR of 55% (12/22). The median time to response was 14 months and to progression four years in only five tumors. None of the tumors progressed within six months from the start of definitive radiotherapy.

After definitive or postoperative radiotherapy, the dose was the only factor that was significantly associated with TTP in multivariate analysis with a *p*-value of 0.02 (HR 0.7, 95%CI 0.5–0.9). Except for radiation dose in univariate analysis, none of the examined factors (age, gender, FAP, tumor diameter, location, recurrence status, surgical margin) was significantly associated with TTP (p=0.2-0.6). The analyses of radiotherapy dose were repeated, including only the first treated desmoid tumors; 2 Gy fraction equivalent doses were also used, where radiation dose retained the significant impact on TTP. Local control rate was 67% (4/7) with doses under 50 Gy, 75% (24/32) from doses 50 to 59.9 Gy, and 100% (8/8) with doses over or equal to 60 Gy. In Figure 6, the Kaplan-Meier curve shows local control divided into groups by total radiation dose into 2 Gy fractions equivalent doses.

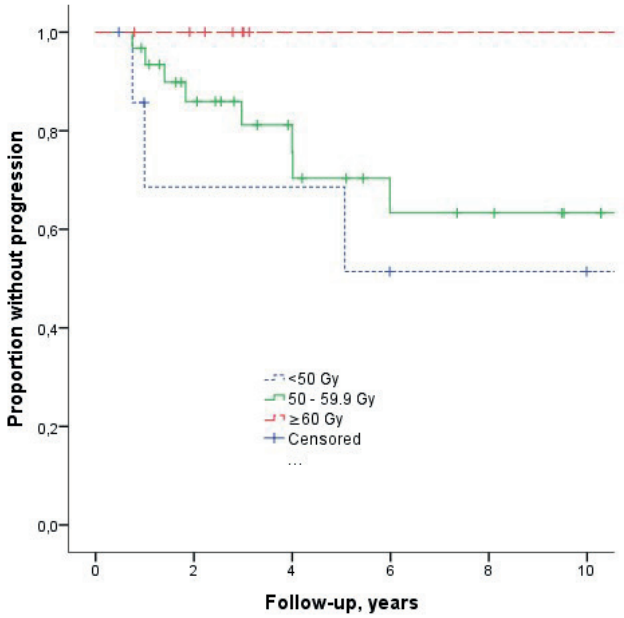

Figure 6. Time to progression in 43 patients with desmoid tumor and treated with 47 postoperative or definitive radiotherapies. Equivalent total doses to 2 Gy fractions were calculated with an α/β ratio of 3 Gy.

Relapses after postoperative or definitive radiotherapy were studied in 10 patients with 10 desmoid tumors, of which one tumor was irradiated twice. In the local relapse analysis, two of the recurrences were located in-target, and nine were marginal (Table 6). None of the relapses was situated completely out-of-target. Two of these patients were examined only in the local relapse analysis due to insufficient imaging.

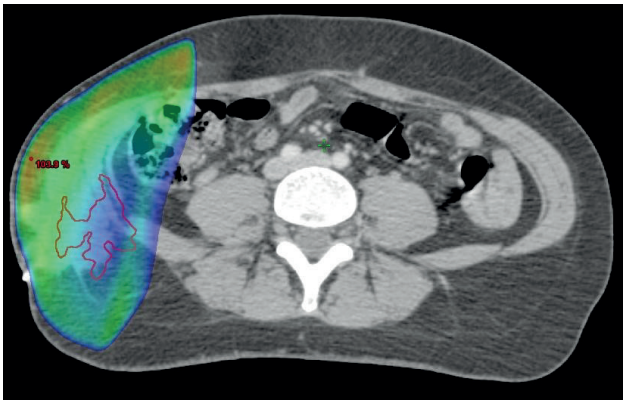

Figure 7. The contoured local recurrence volume in the diagnostic magnetic resonance imaging (MRI) was co-registered with the radiotherapy planning computed tomography (CT) image. The local failure after 50 Gy in 2 Gy fractions definitive radiotherapy was classified as in-target.

One radiation-induced pleomorphic undifferentiated sarcoma was observed nine years after desmoid tumor radiotherapy. The malignancy was situated in the irradiated volume. The tumor was successfully resected, and no recurrence was detected within six years of follow-up.

Table 6. *Analysis of local recurrences after 11 definitive or postoperative radiotherapies. Adapted from (Santti et al. 2017)*

	Radiotherapy type	Prescription dose (Gy)	LR median (Gy)	LR min (Gy)	Location of LR	LR in relation to boost
1	Definitive	50	42.4	7.6	Marginal	Marginal
2	Postoperative	60	58.6	4.1	Marginal	Marginal
3	Postoperative	50	0.5	0.2	Marginal	
4	Definitive	50	50.3	19.8	Marginal	
5	Definitive	30.6	5.8	0.7	Marginal	
6	Postoperative	60	3	0.7	Marginal	Out-of-target
7	Postoperative	45	44.7	44	In-target	In-target
8	Postoperative	50	35.6	2.6	Marginal	
9	Definitive	46	37.9	0.8	Marginal	
10	Definitive	50	48.7	26.4	Marginal	
11	Definitive	50	49	24.6	In-target	

LR = local recurrence

5.2. STUDY II

Ribociclib 600 mg was combined with monthly goserelin 3.6 mg s.c. and daily letrozole 2.5 mg p.o with three weeks on and one week off dosing schedule. After the initiation of treatment, the patient could reduce the dose of opioids as the pain eased. The treatment with ribociclib, letrozole, and goserelin stabilized the growth of multiple desmoid tumors. The sum of perpendicular measures decreased by 18% according to WHO criteria. Adverse events included grade 1 fatigue, neck pustules, vesicular hand rash, and tumor itching. Due to grade 3 to 4 neutropenia, ribociclib was first reduced to 400 mg and subsequently to 200 mg. Ten months after the initiation of the ribociclib-based therapy, it was discontinued due to grade 4 neutropenia despite several dose reductions. A pleural lesion started to grow quickly and was irradiated with 30 Gy total dose in 10 daily fractions.

A month after the discontinuation of the therapy, deep leucopenia 0.8 10^9/l and thrombocytopenia 64 g 10^9/l was detected. Acute promyelocytic leukemia was diagnosed from a bone marrow aspirate and verified with fluorescent in situ hybridization. The patient was hospitalized for leukemia therapy. With all-trans retinoic acid and arsenic trioxide therapy, morphological and molecular remission was achieved.

Responses and TTP for all pharmacological agents are displayed in Figure 8. For this patient, partial response was achieved with toremifene, and the disease was stabilized with the combination of goserelin and toremifene as well as with goserelin and letrozole.

○ = partial response, ◊ = stable disease, Δ = progressive disease

Figure 8. Outcome of systemic therapies in one patient in study II assessed by TTP and RECIST criteria 1.1. Modified from (Santti, Beule, et al. 2019)

5.3. STUDIES III AND IV

The median immunopositivity of Ki67 was 3.9%, cyclin A 1.5%, cyclin D1 15.6%, and ERβ 10.8%. Table 7 summarizes the expressions of these biomarkers in studies III and IV. β-catenin was expressed in all tumors, when positive expression was defined as detectable staining.

Table 7. *Expression of estrogen receptor β and proliferation biomarkers Ki67, cyclin A, and cyclin D1.*

	n	Median positivity %	Cut-off % (≥positive)	High expression (%)
Ki67	70	3.9 (0.3–13.8)	5.99	30
Cyclin A	74	1.5 (0–9.9)	2.98	19
Cyclin D1	77	15.6 (0.7–90.7)	5	82
			10	64
Estrogen receptor β	83	10.8 (0–74)	1	82
			28.9	29

In Cox multivariate analysis, high expression of cyclin A (HR 1.9, 95%CI = 1.1–3.2, p = 0.02), positive surgical margin (HR 6.0, 95%CI = 1.6–22.5, p = 0.008), and extremity location (HR 5.3, 95%CI = 1.7–16.8, p = 0.005) were associated with faster recurrence. When cyclin A expression was divided into percentiles, cut-off value 2.98% at 80th decile was associated with worse TTR with HR of 3.5 (95% CI = 1.4–6.6, p = 0.006).

High Ki67 or cyclin D1 immunopositivity did not significantly predict for recurrence either in univariate analysis or when divided into two groups. Ki67 showed the highest HR of 2.3 with the cut-off value of 5.99% at 70% percentile (p = 0.07) approaching the limit of significance. In univariate analysis, the high expression of ERβ appeared to be linked with short TTR with a hazard ratio of 1.02 (95% CI = 1.0–1.04, p = 0.06). The association was significant (HR 2.6, 95% CI 1,2–6.0, p = 0.02) when ERβ expression was divided into groups at 70% percentile with 28.9% cut-off value.

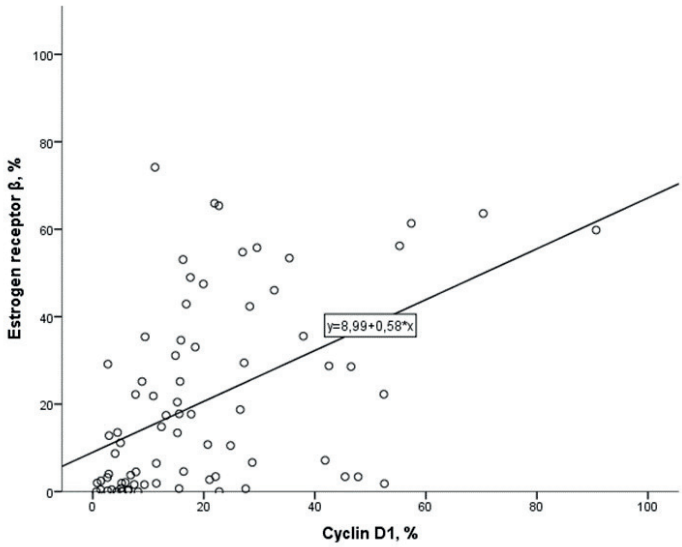

Figure 9. Scatter diagram demonstrates a significant positive linear relationship between estrogen receptor β and cyclin D1 immunoexpression rates (r_p=0.34, p=0.004).

Cyclin D1, cyclin A and Ki67 immunoexpression levels showed significant positive correlations with one another (p≤0.001-0.004), with correspondingly increasing rates. A positive correlation was observed between proliferation markers and ERβ immunoactivity (p=0.001-0.004), Figure 9 illustrates the connection between ERβ and cyclin D1 rates. Correlations between all analyzed biomarkers and clinicopathological parameters are listed in Table 8.

Table 8. *Correlations between clinicopathological features, estrogen receptor β and proliferation biomarkers. Adapted from (Santti et al. 2018; Santti, Ihalainen, et al. 2019)*

	ERβ		**Cyclin D1**		**Cyclin A**		**Ki67**	
	co	*p-value*	*co*	*p-value*	*co*	*p-value*	*co*	***p-value***
Age at diagnosis	0.04	0.7	-0.08	0.5	-0.17	0.2	-0.26	**0.03**
Gender[a]		0.4		**0.047**		**0.04**		0.2
Tumor diameter	-0.02	1	0	0	0	**0.03**	0	**0.01**
Tumor location[b]		0.3		0.1		0.8		0.7
Surgical margins[c]		0.9		0.5		1		0.4
FAP-associated[d]		0.3		0.2		0.1		**0.001**
Pregnancy-associated desmoid		1		1		0		**0.04**
Postoperative radiotherapy		0.7		**0.006**		0.5		0.3
Ki67 (%)	0.35	**0.003**	0.4	**0.001**	0.73	**<0.001**		
Cyclin A (%)	0.4	**0.001**	0.34	**0.004**			0.73	**<0.001**
Cyclin D1	0.34	**0.004**			0.34	**0.004**	0.4	**0.001**
ERβ			0.34	**0.004**	0.4	**0.001**	0.35	**0.003**

[a]1=female, 2=male [b]0 = other, 1= limbs [c]1 = R0, 0 = R1 or R2 [d] 1=no, 2=yes

6. Discussion

6.1. RADIOTHERAPY IS EFFECTIVE IN DESMOID TUMORS (I)

Studies comparing outcome after radiotherapy alone and surgery combined with adjuvant radiotherapy have yielded similar results with no statistically meaningful difference in local control between the groups (Nuyttens et al. 2000; Yao et al. 2014). This finding is in accordance with our results as surgery before radiotherapy had no significant impact on TTP. In case achieving adequate surgical marginals is not possible or would cause significant morbidity, radiotherapy alone should be considered. However, long-term radiation-related toxicity should be noted, and systematic therapy should be considered as another option especially in young patients.

In our series, the objective RR was 55% after radiotherapy alone. The European Organisation for Research and Treatment of Cancer (EORTC) conducted a prospective phase II study including 44 patients with an inoperable primary, recurrent, or incompletely operated desmoid tumor and treated with radiotherapy as the sole treatment (Keus et al. 2013). Echoing our results, the EORTC study showed that the objective RR was 50%. Furthermore, the local control rate was 81.5% at three years using moderate dose radiation of 56 Gy in 28 fractions (Keus et al. 2013). In our study, the five-year local control rate after definitive or postoperative radiotherapy was comparable, 77%, with a median radiation dose of 50 Gy. The reported long-term local control after radiotherapy alone has varied in different series from 70% to 93% (Spear et al. 1998; Guadagnolo, Zagars, and Ballo 2008; Nuyttens et al. 2000). This evidence illustrates that radiotherapy is a powerful treatment modality in desmoid tumors with a favorable long-term outcome.

The optimal radiation dose has been a subject of controversy in desmoid tumor management. Our study demonstrated a significant dose-response relationship in multivariate analysis in patients treated with radiotherapy. Previously dose-response has been reported in studies with superior local

control, using doses over 45 to 54 Gy (Nuyttens et al. 2000; Baumert et al. 2007; Ballo, Zagars, and Pollack 1998; Choi et al. 2018; Ergen et al. 2016), while other authors barely succeeded in detecting a dose-response relationship (Zlotecki et al. 2002; Gluck et al. 2011). However, recurrences can occur even with doses ≥ 60 Gy, and radiation-related morbidity exceeds with higher doses (Ballo et al. 1999; Spear et al. 1998). A large portion of desmoid patient population has decades of life left. Therefore, for radiotherapy alone, 56 Gy and postoperative radiotherapy 50 Gy in once-daily fractions of 2 Gy have been recommended (Kasper, Baumgarten, et al. 2017). In our analysis, no other factor (age, tumor size or location, recurrence status, surgical margin) alongside radiation dose influenced progression-free time, which may be due to the small patient cohort and selection of patients treated only with radiotherapy.

We investigated radiological failure patterns following radiotherapy of desmoid tumors. In literature, the majority of local recurrences have emerged in-field, contrasting our analysis with 82% (9/11) of recurrences classified as marginal and only 18% (2/11) as in-target (Nuyttens et al. 2000; Guadagnolo, Zagars, and Ballo 2008). One reason for the difference might be diverse or absence of definitions of local failures. Notably, in our study, local failure was classified marginal if only a portion of the relapse received a total radiation dose. However, CT/MR images of recurrences were not implemented in the treatment position and while using immobilization. These facts and the use of rigid image coregistration might have led to small inaccuracies in the determination of patterns of failure. In line with our results, a study using dose-distribution analysis reported 61% (11/18) of relapses situated at the margin of target volume or in areas receiving less than 50 Gy (Baumert et al. 2007). In our analysis, both in-target recurrences received suboptimal median doses of 44.7 Gy (postoperative radiotherapy) and 49 Gy (radiotherapy alone). The characteristic infiltrative growth of desmoid tumors may explain the high occurrence of marginal recurrences. Our findings emphasize the significance of adequate radiation dose for the target volume, precise target delineation, and sufficient margins. We found no out-of-target recurrences, which is in concordance with literature (Guadagnolo, Zagars, and Ballo 2008). Of note,

most studies analyzing recurrences were retrospective and included an extensive period. During the last decades, the generalized use of MRI and modern radiation techniques (e.g., IMRT) may change the patterns of local failures after irradiation.

6.2. RIBOCICLIB MAY HAVE ACTIVITY IN DESMOID TUMORS (II)

In hormone-sensitive breast cancer cdk 4/6 inhibitors palpociclib, abemaciclib and ribociclib have shown eminent efficacy. The median PFS combined with ribociclib and letrozole was 25 months in postmenopausal women with ER-positive HER2 negative advanced or metastatic breast cancer (HR 0.57). With letrozole alone, the median PFS was 16 months in the phase III trial (Spazzapan et al. 2017). Furthermore, in the management of metastatic or locally advanced soft tissue sarcoma palbociclib alone and ribociclib combined with doxorubicin are being investigated in ongoing phase II studies (NCT03242382 and NCT03009201). In this study, treatment with ribociclib, goserelin, and letrozole relieved pain and stabilized multiple desmoid tumors for ten months. Tumor size was reduced by 18% according to WHO criteria. Like estrogen-receptor-positive breast cancers, desmoid tumors are hormone-sensitive: Endocrine treatments have yielded 51% RR with a 9-month median length of therapy (Bocale et al. 2011). Both in breast cancer and desmoid tumors, high cyclin D1 expression has been detected. In desmoid tumors, excessive β-catenin is involved in the regulation of cyclin D1 gene *CCND1* transcription, and in breast cancer, estrogen receptors target *CCND1*, leading to cyclin D1 overexpression (Saito et al. 2001). In breast cancer, hormonal therapy and cdk 4/6 inhibitors have shown synergistic effect by cotargeting the interacting signaling pathways (Abraham et al. 2018). Our study suggests an activity for ribociclib combined with hormonal therapy in desmoid tumors, although findings of an individual case report cannot be generalized. Further research is required and if further case reports or series affirm the favourable effect of ribociclib in desmoid tumor patients a controlled trial should be conducted to validate the results.

RECIST 1.1. criteria have been the standard method for response evaluation in desmoid tumors. However, unidimensional measurements may not capture the response optimally as desmoid tumors show irregular and infiltrative growth. To optimate response evaluation in our study, we measured response using both WHO and RECIST criteria. Different response endpoints were also tested in the follow-up study of γ-secretase inhibitor PF-03084014, including RECIST and WHO criteria, CT density, and MRI enhancement ratio (Villalobos et al. 2018). The mean time to response was similar in comparison with RECIST and WHO criteria; however, an early decrease in MRI enhancement ratio correlated with a subsequent response with RECIST and WHO. Furthermore, automated volumetric tumor evaluation via CT or MRI has been suggested (Kummar et al. 2017).

The patient developed acute promyelocytic leukemia, possibly secondary to a previous treatment. The effect of cdk 4/6 inhibitors on hematopoiesis is reversible; therefore, in agreement with the literature, we believe it is unlikely that ribociclib had induced the secondary malignancy (Kassem et al. 2018). The patient was previously irradiated four times at a relatively young age and coped with three courses of doxorubicin, both of which are known to be associated with therapy-related acute promyelocytic leukemia. Radiotherapy may be considered as the most likely contributing factor. Radiation is generally used with careful consideration in young desmoid patients due to occasional indolent biology of the disease. However, for our patient, radiotherapy could not be avoided due to inoperable, enlarging, and symptomatic tumors. The patient benefited from the radiations she received.

6.3. IMMUNOEXPRESSION OF ESTROGEN RECEPTOR B AND PROLIFERATION BIOMARKERS IN DESMOID TUMORS (III AND IV)

Consistent with desmoid tumors frequently indolent clinical course, in this study, the expression level of different cell cycle markers was generally low. The mean immunopositivity rate for Ki67 was 4.7%, which echo earlier publications with mean Ki67 expression rates from undetectable to 8.7%

(Jilong et al. 2007; Stalinska et al. 2009; Dubova et al. 2012). Likewise, cyclin D1 and A positivities were low with median rates of 15.6% and 1.5%, respectively. Cyclin A has not been investigated previously in desmoid tumors whereas cyclin D1 protein with a 5% cut-off has been present in up to 71% of desmoid tumors (Saito et al. 2001; Jilong et al. 2007). With a similar cut-off, a higher proportion of tumors, 82%, showed positive cyclin D1 expression in our study, with the percentage of positive tumor cells ranging widely from 0.7% to 90.7%. The extensive cyclin D1 expression could be explained by its relationship with the β-catenin pathway in desmoid tumors. The vast majority of desmoid tumors exhibit active Wnt/β-catenin signaling, which induces the transcription of cyclin D1 gene *CCND1*.

In desmoid tumors, the expression of ERβ has appeared diverse with positive expression from 7% to 100% with cut-offs from detectable to 10% (Ishizuka et al. 2006; Deyrup, Tretiakova, and Montag 2006). In this report with a 1% cut-off, ERβ overexpression was detected in 82% of desmoid tumors with 10.8% median percentage of positive cells. Validating our results, the more recent publications reported positive ERβ expression from 72% to 100% of tumors (Mignemi et al. 2012; Santos et al. 2010; Colombo et al. 2012). The variance in results may be due to multiple cut-points used, non-standardized immunohistochemical methods, scoring, and differences with patient populations. The other primary estrogen receptor α expression has remained negative in desmoid tumors (Santos et al. 2010; Leithner et al. 2005). Therefore, desmoid tumor hormonal sensitivity could be mediated via ERβ.

In desmoid-type fibromatosis large tumor diameter, young age, extremity location and in some studies positive surgical margin have predicted faster recurrence after operative treatment (Crago et al. 2013; Janssen et al. 2017; Yao et al. 2014). In study III positive surgical margin (HR 6.0, p=0.008) and extremity location (HR 5.3, p=0.005) were associated with poorer outcome when adjusted for confounding factors. These results contrast with study I, where either surgical margin or tumor location predicted for recurrence. The explanation for the discrepancy may be the smaller number of patients in

study I and the differing patient cohorts because the more complicated patients were assigned for radiotherapy.

High cyclin A expression predicted faster recurrence after resection in multivariate analysis ($p = 0.02$, HR 0.7) whereas Ki67 and cyclin D1 had no impact on TTP in desmoid tumors. In an explorative analysis, Ki67 showed a trend inclining toward the prediction of the worse outcome, although it did not reach statistical significance. There is an ample body of research on the prognostic significance of Ki67 in various malignancies. However, only in one desmoid tumors study, Ki67 showed positive predictive value for TTR in univariate analysis (Brueckl et al. 2001). In agreement with our report, other desmoid tumor studies failed to show an association between Ki67 and TTP (Machado et al. 2017; Mueller et al. 2016). In our study, proliferation markers cyclin A, cyclin D1, and Ki67 correlated with each other. The reason why cyclin A was the strongest predictor for recurrence could be related to its core role in the cell cycle. During S-phase, cyclin A/cdk1 complex triggers DNA replication. Cyclin A has not been extensively investigated; however, cyclin A overexpression has been linked to a poor outcome in breast cancer, soft tissue sarcomas, and laryngeal carcinoma, for example (Saarilahti et al. 2003; Huuhtanen et al. 1999; Ahlin et al. 2009).

In our work, the positive relationship found between proliferation markers Ki67, cyclin D1, and cyclin A varied from lesser degree to strong. The reason for the variable correlation may be related to other factors influencing the cell cycle regulation. ERβ expression correlated positively with cell cycle markers Ki67, cyclin D1, and cyclin A. When divided into two groups, very high expression of ERβ predicted unfavourable clinical outcome in operated desmoid tumors, as did cyclin A. A series of 120 soft tissue sarcoma patients found no association between expression of ERα or β and proliferation measured by Ki67 (Li, Hisaoka, and Hashimoto 2003). These findings cohere with the absence of hormonal responsiveness in extrauterine soft tissue sarcomas and the known hormonal dependency in desmoid tumors. The exact biological role of ERβ in different tissues is not well understood. In breast cancer, ERβ mediates antiproliferative activity, especially in the absence or

low expression of ERα, whereas some studies have suggested ERβ a role in myofibroblastic differentiation in stromal breast cells (Tan et al. 2016; Sapino et al. 2006). Our results suggest that in desmoid tumors, ERβ signaling may be connected with both proliferation and poor outcome.

6.4. LIMITATIONS AND STRENGTHS

This study comprised patients treated at Helsinki University Hospital. This selection of patients may cause referral bias because the more challenging patients are usually admitted to a tertiary center. Availability of a multidisciplinary sarcoma team and the consistent use of treatment guidelines are positive aspects of this single-institution study. Diagnosis of this rare disease entity can be demanding, and in this study for all patients, the diagnoses were reviewed and verified by an experienced sarcoma pathologist.

The retrospective study design in studies I, III, and IV may have caused selection bias. This bias could favor surgery combined with adjuvant radiotherapy because larger tumors situated adjacent to vulnerable structures are more often addressed to radiotherapy alone. Additionally, the long period may have affected the outcome of the studies. Radiotherapy and surgical techniques have evolved during the past decades, and for all the patients, the exact surgical margins or the tumor diameters could not be verified. The evaluation of treatment-related toxicity was inaccurate, and mild side effects were seldom recorded initially. However, the extended follow-up was the strength of the study.

The TMA-based technique allows evaluation of large tumor materials efficiently saving time, reagents, and tumoral tissue. Compared with full tissue sections, using TMA may cause inaccuracy due to intratumoral heterogeneity. However, desmoid tumors consist of relatively homogenous tissue. TMA has been validated in fibroblastic tumors with an excellent 96% concordance with triplicate cores when Ki67 was examined in two categories (Hoos et al. 2001). We used at least three cores per specimen in three parallel TMA slides to ensure the accuracy of the results. Simultaneous processing of several tumor

specimens in one array may decrease the variability related to immunohistochemical staining. A number of factors can influence the outcome in immunohistochemistry: fixation, sectioning, slide storage time, inadequate deparaffination, antigen retrieval technique, selection of antibody clones and reagents, counterstaining and interpretation procedure. We used digital image analysis for quantitative assessment of nuclear biomarker expression. The analysis was not fully automated, allowing monitoring and adjustments in each sample if needed. Visual screening may be challenging, especially when assessing low proliferation (Kwon et al. 2019). In breast cancer, digital analysis of Ki67 has shown very high inter-operator reproducibility (Acs et al. 2019). Due to increased efficacy, accuracy, and technological advances, the use of digital pathology is emerging both in research and clinic in the near future.

The patient cohort was relatively small, as was the number of events, which reduced the statistical power in multivariate analysis. However, taking into account the extremely low incidence of desmoid tumors, our patient cohort size was above average and could be considered a valuable contribution in this field.

7. Conclusions

Our results indicate that radiotherapy is an effective treatment in desmoid tumors. Indeed, surgery before radiotherapy did not improve the outcome. We also found a positive dose-response relationship for patients treated with radiotherapy. Most local failures after radiotherapy seem to be due to inadequate coverage of the target volume and extremely low dose level. In combination with endocrine treatment, our case presentation shows that ribociclib seems to have clinical activity in desmoid tumors.

High cyclin A expression independently predicts faster recurrence in operated desmoid tumors. More precise risk stratification might aid decision-making about additional therapy after surgery. Furthermore, our study illuminates the connection between ERβ signaling and proliferation in desmoid tumors.

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