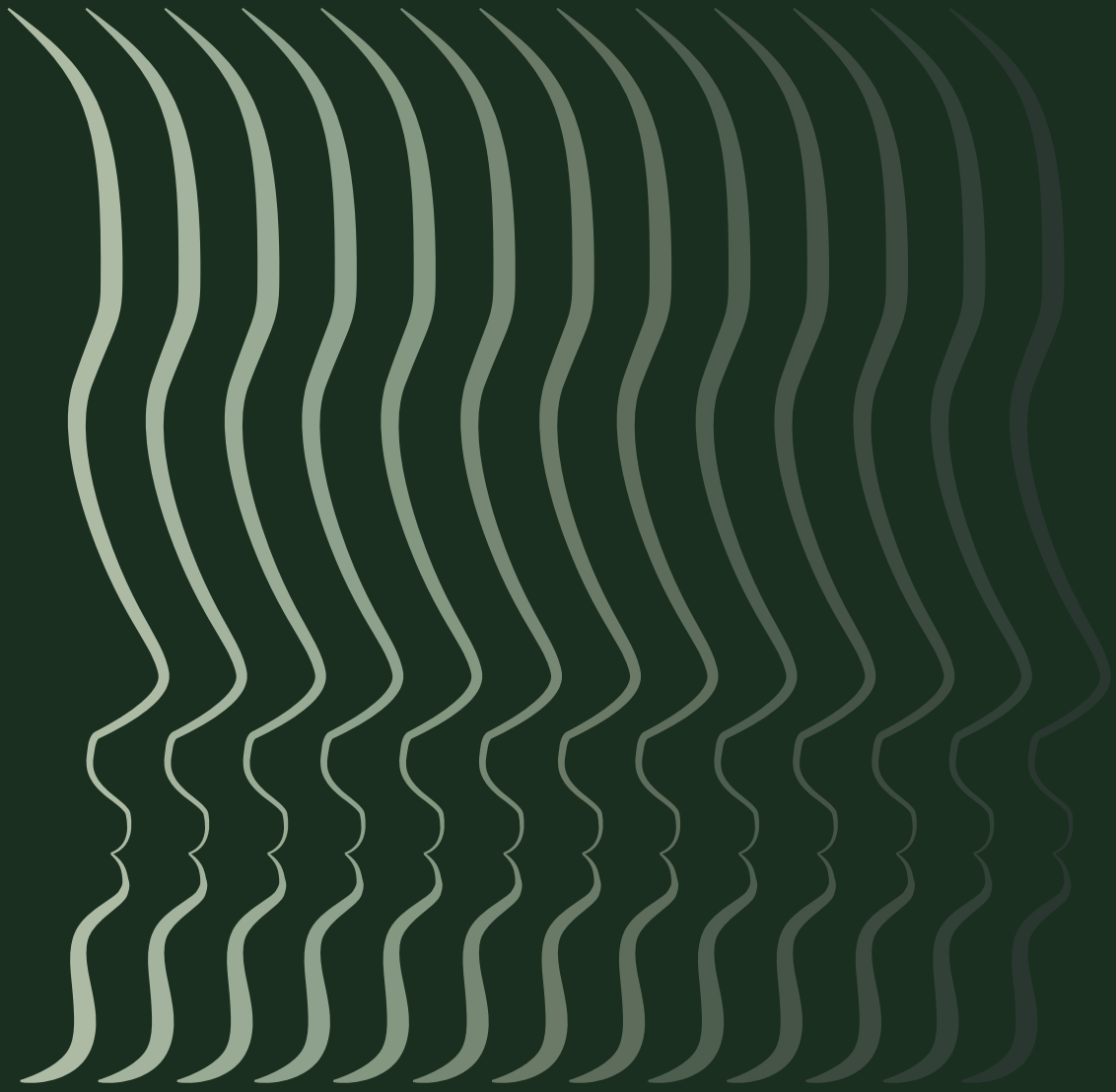
THE MANY FACES OF DESMOID-TYPE FIBROMATOSIS



MILEA J.M. TIMBERGEN

The Many Faces of Desmoid-type Fibromatosis

De vele gezichten van desmoïd-type fibromatose

Milea Janne Mea Timbergen

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Milea J.M. Timbergen
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	Prof. dr. S. Sleijfer
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The Many Faces of Desmoid-type Fibromatosis

1

General Introduction and Aims of this Thesis

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Desmoid-type fibromatosis (DTF) is a rare, soft tissue tumour with an incidence in the Dutch population of 5 patients per million people per year [1]. The likelihood that a doctor encounters a desmoid patient in his professional career is low, but early recognition, referral to a specialized centre, and accurate treatment are crucial. This thesis describes desmoid-type fibromatosis in the broadest sense and aims to contribute to the knowledge of this rare disease.

Desmoid-type fibromatosis has been given a variety of names since its discovery about 185 years ago [2, 3]. These include: aggressive fibromatosis, desmoid tumour, deep fibromatosis, fibromatosis, and desmoid fibromatosis. Just like the variety of names, DTF displays a wide range of clinical presentations and outcomes. DTF has no metastatic potential and cannot undergo malignant transformation. However, it can display aggressive and invasive growth, and has a tendency towards local recurrence. For this reason DTF is classified as a 'locally aggressive but non-metastasizing' tumour by the World Health Organization (D48.1) [4]. It mostly affects females aged between 20 and 40 years [1]. The clinical presentation can vary between a small lump without significant symptoms, to a large infiltrating and debilitating tumour, having a significant impact on the patients' life.

Genetics and Molecular Biology

DTF tumours express cell surface markers and genes that are characteristic of mesenchymal stem cells and seem to have a mesenchymal origin [5]. It is a clonal fibroblastic proliferation that arises in connective tissue. Since connective tissues comprises a large part of the body's musculoskeletal system, DTF can develop anywhere in the body [6].

Roughly two types can be distinguished: hereditary (familiar) and sporadic DTF. The hereditary type occurs more frequent in patients with familial adenomatous polyposis (FAP), and causes intra-abdominal DTF tumours. FAP-related DTF is an autosomal dominant disorder caused by germline mutation of the *adenomatous polyposis coli* (APC) gene. This hereditary condition predisposes to the development of extra- and intra-intestinal neoplasms, including malignant colorectal carcinoma and DTF tumours [7]. The cumulative rate of DTF in FAP patients is 20.6% at 60 years of age [8]. About 10% of FAP patients die from intra-abdominal DTF tumours. After colorectal carcinoma, DTF is the second most common cause of death in FAP [9].

The second type, "sporadic-DTF", derives from a single progenitor cell [10] and is the focus of this thesis. In contrast to hereditary DTF, sporadic DTF is commonly localized extra-abdominally (trunk or extremities) or in the abdominal wall [6]. As a result, sporadic DTF is rarely fatal but can cause substantial morbidity and thereby sincerely impairing quality of life. The precise aetiology remains tenuous. Several studies report correlations with (spontaneous or iatrogenic) trauma and hormones, although translational studies, investigating the biological rational of these correlations, are lacking [11]. The majority of sporadic DTF tumours, about 85%, have mutations in the *CTNNB1* (β-catenin) gene [12-14]. These mutually exclusive mutations are located in exon 3 and most commonly cause the following changes: a replacement of threonine to alanine at codon 41 (T41A), a replacement of serine for phenylalanine (S45F), or a replacement of serine for proline (S45P) at codon 45. These mutations block the phosphorylation and subsequent degradation of β-catenin, which consequently leads to its stabilization and an increased level of β-catenin in the cytoplasm and in the nucleus [15]. This aberrant level of β-catenin is useful for the diagnosis of DTF as immunopositivity for β-catenin can distinguish DTF from other myofibroblastic proliferations [16]. The group without a mutation in the *CTNNB1* gene, about 5-15%, has been designated as "wild-type" in the past [13]. However, this group decreases over time as more sensitive sequencing tools, such as Next Generation Sequencing, become more widely available and reveal other rare mutations in *CTNNB1* or other genes such as *APC* [13, 17, 18].

Diagnosis and Treatment

During the diagnostic work-up, imaging (i.e., magnetic resonance imaging (MRI), ultrasound, or computed tomography) and a histologic tissue biopsy are obtained. The differential diagnosis of DTF is broad and includes scar tissue, keloid, nodular fasciitis, low-grade fibromyxoid sarcoma, and low-grade myofibroblastic sarcoma amongst other soft tissue tumours [16]. Once the diagnosis of DTF is confirmed, using β-catenin immunopositivity and sequencing of the *CTNNB1* gene, treatment in a sarcoma-specialised centre is a necessity [19].

The treatment of DTF has dramatically changed over the past decade. Despite a doctors' natural urge to start any form of treatment immediately after diagnosis, active surveillance (i.e., "wait and see") has become the first treatment strategy for asymptomatic DTF tumours [19]. Various retrospective studies show that "active surveillance" is safe [20], and that a minority of patients need to switch to an "active form" of treatment [21]. Furthermore,

spontaneous regression is observed in up to 20% of patients [21]. Especially for DTF tumours located in the chest wall, head, neck and upper limb, this active surveillance approach is beneficial since these "unfavourable locations" are generally considered challenging to treat, yielding severe morbidity [22].

Forms of 'active treatment' like surgery, radiotherapy and systemic therapy, are indicated in case of a symptomatic and/or progressive DTF tumour [19]. Surgery is first in line in case of failure of the active surveillance approach [22, 23]. Radiotherapy as a single treatment modality has not shown any improvement of the risk of progression compared to surgery with adjuvant radiotherapy [24, 25]. It can decrease symptoms and cause disease stabilization or even a partial or a complete response in patients with inoperable DTF [26]. Adjuvant radiotherapy is only given in cases with a high chance of recurrence, which would be difficult to treat due to the unfavourable tumour location.

Several systemic treatment options are available to treat symptomatic and progressive DTF, for which surgery and/or radiotherapy is not a suitable option. Systemic options include non-steroidal anti-inflammatory drugs (NSAID's) alone or in combination with anti-hormonal agents such as tamoxifen [27-29], low dose chemotherapy such as a doxorubicin [30, 31], or a combination of methotrexate low dose with either vinblastine or vinorelbine [32-36], gamma-secretase inhibitors such as Nirogacestat [37], and tyrosine kinase inhibitors such as imatinib [38-40], sorafenib [41, 42], pazopanib [43, 44], or nilotinib [19]. There is no consensus about the sequence of systemic treatments and the exact working mechanisms of these systemic agents in DTF remains unclear. Randomized data is currently only available for tyrosin kinase inhibitors (sorafenib vs. placebo (phase 3) [42] and pazopanib vs. methotrexate-vinblastin (phase 2) [43] and gamma-secretase inhibitors (Nirogacestat vs. placebo) [45]. The first trial reported an advantage for sorafenib in the 2-year progression-free survival (PFS) over placebo (81% (95% confidence interval [CI], 69-96) versus 36% (95% CI, 22-57). The second trial reported an advantage for pazopanib over methotrexate-vinblastin in progression-free proportion of patients measured at 6 months (83.7% (95% CI 69.3–93.2) vs 45% (95% CI 23.1–68.5). Gamma-secretase inhibitors, such as Nirogacestat, form an attractive therapeutic option as they inhibit the final step in the Notch signalling pathway, a pathway which is also known for its cross talk with the Wnt signalling pathway [46]. Clinical phase 1 and 2 trials of the gamma-secretase inhibitor PF-03084014 (later named Nirogacestat) showed promising results in which a substantial part of patients experienced partial response or disease stabilisation [47, 48]. These results led to a phase 3 trials of which the inclusion is already closed and the results

are currently awaited [45]. If positive, these results will lead to the first officially approved drug for DTF tumours.

Despite all these positive scientific advances, determination of the true therapeutic value of the most above-mentioned treatments remains challenging, as many trials only included progressive, inoperable, refractory DTF tumours.

In this thesis, we focus on three aspects of DTF. Several studies identified a prognostic role for the *CTNNB1* mutation but so far, no biological differences have been found. The first part therefor focusses on the molecular origin and biology of DTF. In this part, we aim to explain the observed clinical behaviour of the different mutations types by identification of biological differences. Due to the rarity of DTF, uniform imaging protocols are lacking, and diagnosis can be challenging. Treatment decisions are depending on symptoms and tumour site and up till now, the *CTNNB1* mutation is not incorporated in the clinical decision-making. The second part, deals with simplifying the diagnostic process by the use of radiomics. Furthermore, it focusses on two commonly used treatments: active surveillance and surgery. With low mortality rates and irrelevance of traditional oncology endpoints, the search for novel endpoints in clinical trials continues. The third part encompasses health-related quality of life (HRQoL) describing the disease from a patients' perspective. Insight into common DTF-related HRQoL-problems will lead to the development of a DTF-specific HRQoL-tool and HRQoL can be used as an endpoint in clinical trials.

Part I – Genetics and Molecular Biology

Mutations in the *CTNNB1* gene cause accumulation of β-catenin in the nucleus and consequently aberrant Wingless (Wnt)/β-catenin signalling [15]. Knowledge about the influence of other signalling pathways in the pathogenesis of DTF is limited. In **Chapter 2**, we reviewed the current available literature regarding DTF and common cell signalling pathways such as JAK/STAT, Notch, PI3 kinase/AKT, mTOR, Hedgehog, the oestrogen pathway, and the growth regulatory pathway. Additionally, we described the current evidence for the use of therapies targeting the aforementioned signalling pathways. As the Wnt/β-catenin signalling pathway is one of the most important oncogenic pathways, we aimed to gain more insight into the Wnt/β-catenin signalling pathway in the setting of DTF. Several studies indicate that there is a difference in the clinical behaviour between the different *CTNNB1* mutation types (T41A, S45F, and S45P) and wild-type DTF [12, 49-51].

Therefore, we investigated whether we could potentially explain this difference by studying mRNA expression data of known Wnt target genes in **Chapter 3**.

DNA methylation is an epigenetic modification that influences gene expression and hence, gene activity [52]. Abnormal DNA methylation have been described in various solid tumours and sarcomas [53, 54]. In **Chapter 4** we examined and compared genome-wide DNA methylation patterns of DTF tumours containing a T41A or an S45F *CTNNB1* mutation.

Part II - Diagnosis and Treatment

Radiomics, makes use of computational computer algorithms designed to link imaging features to molecular, pathological and clinical features [55]. By the annotation of tumours on conventional imaging, already obtained during the routine diagnostic work-up, radiomics can be used to contribute to diagnosis, prognosis and treatment decision making [56, 57]. In **Chapter 5** we investigated whether radiomics can be used to differentiate extremity DTF from other extremity tumours such as fibromyxosarcomas, myxoid liposarcomas and leiomyosarcomas on pre-treatments MRI. Additionally, we investigated whether our radiomics model could be used to differentiate the various *CTNNB1* mutation types.

In recent years, active surveillance has obtained a more prominent role in the treatment of asymptomatic DTF. The recommendation that active surveillance should be the front-line approach for treating DTF published in the first European consensus guideline (2015) [58], was based on the results of five retrospective studies [20, 59-62]. As the results of the three prospective studies [63-65] are still awaited, we performed a systematic literature review described in **Chapter 6**. This review systematically evaluates the results of the active surveillance approach in published retrospective series.

Since DTF is a rare disease, randomized controlled trials to investigate the efficacy of certain treatments are scarce. For a long time, surgery remained the gold standard for treating DTF; however, the risk of local recurrence after surgery was high, between 20% and 68% [66, 67]. Several studies [12, 49-51] found a significant correlation between risk of recurrence and mutation status and claimed that the S45F mutated DTF tumours have the highest chance of recurrence. Often, these studies included both primary and recurrent DTF tumours, and

several patients received adjuvant therapy after surgery, distorting the true prognostic value of the *CTNNB1* mutation. In **Chapter 7**, we investigated the association of the different *CTNNB1* mutations with the risk of recurrence, in a large international cohort of primary DTF patients treated with surgery solely. Studies were included based on an extensive literature search and individual patient data was used to create a large homogenous cohort of DTF patients.

Part III - Health-related Quality of Life

The rarity, the high morbidity, and the increasing use of active surveillance as a first line management are reasons to better study the patients' perspective. In **Chapter 8**, we performed a systematic literature search to investigate which HRQoL-tools are being used for DTF in clinical practice and in research setting. Additionally, we organized focus groups to gain insight into the HRQoL-problems experienced by DTF patients. In **Chapter 9**, we presented the retrieved HRQoL-issues to a new, international cohort of DTF patient and to healthcare professionals involved in the care of DTF patients, to identify the most important HRQoL-issues. In **Chapter 10**, we describe the study protocol of the QUALIFIED study (The evaluation of health-related quality of life issues experienced by patients with desmoid-type fibromatosis): an international, multicentre, cross-sectional, observational cohort study which evaluates HRQoL-problems in the adult DTF population.

Part IV - General Discussion and Future Perspectives

This thesis describes the many faces of DTF. We provide insights into the molecular biology contributing to DTF pathogenesis, we incorporate new techniques such as radiomics to change the diagnostic pathway, we assess the role of the *CTNNB1* mutation in risk of recurrence, and we evaluate how DTF impacts a patients' life. In **Chapter 11**, the results from the previous chapters are discussed. **Chapter 12** outlines future perspectives for DTF research.

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Genetics and Molecular Biology

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Activated signalling pathways and targeted therapies in desmoid-type fibromatosis: A literature review

Milea J.M. Timbergen, Ron Smits, Dirk J. Grünhagen, Cornelis Verhoef, Stefan Sleijfer, Erik A.C. Wiemer

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Abstract

Desmoid-type fibromatosis (DTF) is a rare, soft tissue tumour of mesenchymal origin, which is characterized by local infiltrative growth behaviour. Besides "wait and see", surgery and radiotherapy, several systemic treatments are available for symptomatic patients. Recently, targeted therapies are being explored in DTF. Unfortunately, effective treatment is still hampered by the limited knowledge of the molecular mechanisms that prompt DTF tumorigenesis.

Many studies focus on Wnt/β-catenin signalling, since the vast majority of DTF tumours harbour a mutation in the *CTNNB1* gene or the *APC* gene. The established role of the Wnt/β-catenin pathway in DTF forms an attractive therapeutic target, however, drugs targeting this pathway are still in an experimental stage and not yet available in the clinic.

Only few studies address other signalling pathways that can drive uncontrolled growth in DTF such as JAK/STAT, Notch, PI3 kinase/AKT, mTOR, Hedgehog, and the oestrogen growth regulatory pathways. Evidence for involvement of these pathways in DTF tumorigenesis is limited and predominantly based on the expression levels of key pathway genes or on observed clinical responses after targeted treatment. No clear driver role for these pathways in DTF has been identified, and a rationale for clinical studies is often lacking. In this review, we highlight common signalling pathways active in DTF and provide an up-to-date overview of their therapeutic potential.

Introduction

Desmoid-type fibromatosis (DTF) is a clonal fibroblastic proliferation of the soft tissues that arises in musculoaponeurotic structures [1]. It has a mesenchymal origin since DTF tumours express cell surface markers and genes that are characteristic of mesenchymal stem cells [2]. The incidence in the Dutch population is 5 patients per million people per year [3]. Unfortunately, worldwide epidemiological data is lacking. The abdominal wall and the trunk are the most common localisations and symptoms can vary, depending on tumour location and size [4, 5]. Roughly, two types can be distinguished: sporadic and hereditary DTF. The first type is considered to be a monoclonal disorder, since it derives from a single progenitor cell [6]. This "sporadic" type is commonly localized extra-abdominally or in the abdominal wall [5]. The precise aetiology of sporadic DTF remains tenuous. Several studies report correlations with (spontaneous or iatrogenic) trauma and hormonal status [7-10]. The hereditary type occurs more frequent in patients with familial adenomatous polyposis (FAP), and causes intra-abdominal DTF tumours. This DTF type is an autosomal dominant disorder caused by germline mutation of the *adenomatous polyposis coli* (*APC*) gene, and is associated with the formation of hundreds of colon polyps which can transform into malignant colorectal tumours in time (reviewed by De Marchis et al. [11] and Lips et al. [12]). The cumulative rate of DTF in FAP patients is 20.6% at 60 years of age [13].

Desmoid-type fibromatosis is considered to be a borderline tumour because of its incapability to metastasize [1]. The mortality of this disease is low and seldom described in literature. However, local aggressive growth can cause significant morbidity by infiltrating surrounding structures, causing pain or functional loss. Currently, "wait and see" is the first line therapy in case of asymptomatic DTF. Several retrospective studies report that a minority of patients on a "wait and see" protocol experience progression and that progression usually occurs within two years after tumour development [14]. Additionally, up to one third of patients experience disease regression without any form of treatment [15-17]. Three prospective studies investigating a "wait and see" approach (NCT02547831, Italy; NTR 4714, the Netherlands; NCT01801176, France) examine the natural growth behaviour of DTF and their relationship with *CTNNB1* mutations [18-20]. Surgery is the treatment of choice in case of failure of the "wait and see" management [21]. Radiotherapy is mainly used as an adjuvant treatment in case of incomplete surgical resection. Radiotherapy as a single treatment modality may be considered for patients in whom local control is the primary treatment goal [21]. When both surgery and radiotherapy are not an option due to tumour localization (e.g., near vital structures), or because of comorbidities, several other treatment options are available like local cryoablation and partial systemic chemotherapy via

isolated limb perfusion [21]. Although not widely used, as the evidence for their effect in DTF is only based on small patient series, some patients benefit from these local therapies for example when limb salvage is the treatment goal [22-25]. Besides targeted drugs, other systemic options include more classic chemotherapeutic compounds like vinblastine, vinorelbine, methotrexate, doxorubicin, dacarbazin, either as a single agent or as combination therapy [21]. Although most studies describe small retrospective case series and include patients who received other treatments prior to their cytotoxic treatment, multiple studies indicate a potential effect of these drug regimens [26-29].

The aggressive growth behaviour, in combination with the high recurrence rate, creates the need for effective drugs targeting the molecular mechanisms that drive tumorigenesis [30, 31]. This is especially true for large, symptomatic tumours, which cannot be treated surgically, or with radiotherapy. As stated above, several systemic options are available with variable efficacy in different patients, but no consensus about the nature and the sequence of systemic treatments has been established [21]. As of yet, the exact working mechanisms of these systemic agents in DTF remain unclear.

A better understanding of the molecular mechanisms that prompt tumorigenesis and influence DTF progression will contribute to the development and implementation of new targeted therapies. This review comprehensively screened the available literature regarding active cell signalling and biochemical pathways and reviews pathway-specific targeted drugs investigated in DTF. Additionally, the challenges of DTF research, as well as the future perspectives, are discussed. The abbreviations used in the text, tables and figures are explained in Supplemental Material 1.

The Wnt/ß-catenin signalling pathway in desmoid-type fibromatosis

The Wnt/ß-catenin signalling pathway

The canonical Wnt/ß-catenin pathway coordinates cell fate decisions during the developmental process and in adult homeostasis. Target genes of this signalling pathway are involved in regulating the balance between self-renewal, differentiation, apoptosis, and in stem cell maintenance (reviewed by Nusse and Clevers [32] and Steinhart and Angers [33]). Activation of the Wnt/β-catenin pathway involves a Wnt ligand binding to the transmembrane receptor Frizzled, forming a complex with a co-receptor that is the LDL receptor-related protein 5 or 6 (LRP5 and LRP6). The ß-catenin protein is a key mediator in the Wnt/β-

catenin signalling pathway, and its stability is normally regulated by a degradation complex consisting of the tumour suppressor APC, a scaffolding protein axin, and two constitutively active serine-threonine kinases i.e., casein kinase 1α (CK1α/δ), and glycogen synthase kinase 3 (GSK3). Within this complex β-catenin is sequentially phosphorylated by CK1 and GSK3 on serine/threonine residues (Ser45, Thr41, Ser37, Ser33), thus forming a docking site for the E3 ubiquitin ligase; β-TrCP. This ubiquitinylates β-catenin, which is subsequently degraded by the proteasome. Activation of the Wnt/β-catenin pathway by binding of the Wnt ligand to the frizzled/LRP heterodimer recruits the degradation complex to the membrane via the dishevelled protein (DVL) disrupting the degradation complex and consequently the phosphorylation of β-catenin, leading to its stabilization and translocation into the nucleus. In the nucleus it operates as a transcriptional activator, bound to members of the T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factor family, and possibly to other co-activators of Wnt target genes (reviewed by Nusse and Clevers [32]).

The Wnt/β-catenin signalling pathway in cancer

The Wnt/β-catenin signalling pathway contributes to cancer by promoting progression of cells through the cell cycle, by inhibiting apoptosis via the expression of anti-apoptotic genes, by affecting cell proliferation via the expression of growth factors and their corresponding receptors, by influencing cell motility through the expression of cell adhesion and extracellular matrix proteins and via stem cell maintenance (reviewed by Nusse and Clevers [32]). Aberrant signalling of the Wnt/ß-catenin pathway has been implicated in several epithelial tumours (e.g., colorectal carcinoma [34] and endometrial carcinoma [35]) and in mesenchymal tumours (e.g., osteosarcomas [36, 37], malignant fibrous histiocytomas and liposarcomas [38]).

The Wnt/ß-catenin signalling pathway in desmoid-type fibromatosis

The relationship between the Wnt/β-catenin signalling pathway and DTF has been extensively studied. It is believed that this pathway is crucial to DTF pathogenesis because of the fact that the vast majority (about 85%) of DTF tumours harbour a mutation in exon 3 of the *CTNNB1* (ß-catenin) gene, making the protein more resistant to proteolytic degradation [39-41]. Less frequently, loss-of-function mutations in the *APC* tumour suppressor gene are observed, most commonly in the context of FAP [12]. In both cases, β-catenin translocates into the nucleus aberrantly activating target genes. This nuclear accumulation can be determined by immunohistochemistry (IHC), and serves as a diagnostic tool differentiating DTF from other bone-, soft tissue and fibrous tumours [42]. The group of wild-type (WT) ß-catenin DTF, comprises about 15% of all DTF tumours, and is defined as "having no *CTNNB1* mutations

in exon 3". The number of DTF patients assigned to this group decreases over time since next generation sequencing is able to detect ß-catenin mutations located on exon 3, in tumours where the traditional Sanger sequencing method is not sensitive enough [43, 44].

Interestingly, the β-catenin mutations observed in DTF are almost exclusively confined to residues T41 and S45, while alterations at other N-terminal phosphorylation residues, that is D32-S37, are rarely observed. Recently, Rebouissou et al. showed in liver cancers that the T41 and S45 mutants activate the pathway only weakly compared to others [45]. Apparently, this weak activation is ideal for DTF outgrowth in line with the "just-right" signalling hypothesis that postulates that each tumour type selects for an optimal level of β-catenin signalling that is ideal for tumour initiation and progression [46]. In accordance, the APC mutant proteins observed in DTF retain some functionality in regulating β-catenin levels. The specific β-catenin mutation may be of clinical relevance since several groups reported a higher recurrence rate in *CTNNB1* S45F mutated DTF tumours compared to other *CTNNB1* (T41A) mutated tumours and WT DTF [30, 47-49]. This issue is however still under debate as others have reported contradictory results [41, 50].

Using a β-catenin reporter assay in primary DTF cultures, Tejpar et al. validated the enhanced β-catenin signalling present in DTF. They also showed that in the nucleus, β-catenin is mainly associated with TCF7L1 (also known as TCF3) to regulate target genes. Expression of TCF7 (TCF1) and LEF1 could not be identified, while solely a minority of DTF samples expressed TCF7L2 (TCF4) [51]. Others found that several matrix metalloproteinases (MMP-3, MMP-7 and MMP-9) are expressed in DTF implying a role for MMP's in DTF invasiveness [52, 53]. In fact, Kong et al. showed that MMP inhibition decreases tumour invasion and motility [52]. Matono et al. showed that MMP7 is more abundantly expressed in *CTNNB1* mutated DTF compared to *CTNNB1* WT, and hypothesized a correlation between MMP7 and prognosis as previously was demonstrated in pancreatic cancer [54, 55]. The MMP-inhibitor ilomastat (galardin /GM6001) was investigated in two studies, showing a decrease in DTF-cell (human and murine) migration and invasion capability [52, 53]. In $Apc^{+/}Apc^{1638N}$ mutant mice, DTF tumour volume was decreased (Table 1) [52].

Table 1. Overview of drugs used *in vitro* /*vivo* studies targeting a signalling pathway in DTF

Drug	Ref.	Setting	Effect
Wnt/ß-catenin signalling pathway			
MMP inhibitor Ilomastat /Galardin (GM6001)	[52]	*Apc*$^{+}$/*Apc*^{1638N} mice Murine PCC from DTF and NF	↓ DTF cell invasion and motility ↓ tumour volume in mice
	[53]	Human PCC from DTF and normal fascia (n=7)	↔cell growth ↓ DTF cell invasion
		Human PCC from DTF and normal marginal tissues *Apc*$^{+}$/*Apc*^{1638N}-Cox2$^{-/-}$ and *Apc*$^{+}$/*Apc*^{1638N} -Cox2$^{+/+}$ mice	↓ DTF cell and NF proliferation ↔ apoptosis in DTF cells and NF ↔ tumour number in mice (sulindac) ↓ tumour volume in mice (sulindac)
NSAID Sulindac / Indomethacin / DFU	[56]	Human PCC from DTF and normal marginal tissues *Apc*$^{+}$/*Apc*^{1638N}-Cox2$^{-/-}$ and *Apc*$^{+}$/*Apc*^{1638N} -Cox2$^{+/+}$ mice	↓ DTF cell and NF proliferation ↔ apoptosis in DTF cells and NF ↔ tumour number in mice (sulindac) ↓ tumour volume in mice (sulindac)
NSAID Sulindac	[57]	Human PCC from DTF,CRC	↓ DTF cell growth ↔ cell morphology
NSAID Piroxicam (+DFMO)	[58]	*Apc*$^{-/+}$*p53*$^{+/-}$, *Apc*$^{+/+}$*p53*$^{+/-}$, *Apc*$^{-/+}$*p53*$^{+/+}$ mice	↓ DTF tumour number
Angiostatic factor Endostatin	[59]	Human PCC from FAP-related DTF and CRC	↑ apoptosis (CRC cultures) ↑ cell death (DTF cultures)
Benzoxazocine Nefopam	[60]	Human PCC from DTF and NF *Apc*^{1638N} mice	↓ cell proliferation, modest change in apoptosis ↓ ß-catenin protein level ↓ tumour number and volume (mice)
Hedgehog signalling pathway			
Hedgehog inhibitor Triparanol	[61]	Human PCC from DTF *Apc*$^{+/1638N}$; Gli2$^{+/-}$ and *Apc*$^{+/1638N}$; Gli2$^{+/+}$ mice	↓ tumour volume (*Apc*$^{+/1638N}$ mice) ↓ number of tumours (*Apc*$^{+/1638N}$; *Gli2*$^{+/-}$) ↓ number of tumour cells, viability, proliferation rate (DTF cells) ↔ apoptosis (DTF cells)
Notch signalling pathway			
ɣ-secretase inhibitor PF-03084014	[62]	Human PCC from DTF	↓ Notch signalling (↓ NICD and Hes1 expression) ↑ cell cycle arrest ↓ cell growth, migration and invasion
JAK/STAT signalling pathway			
Cytokines Interferon-ß	[63]	Human PCC from DTF and NF *Apc/Apc*^{1638N}; A*pc*^{1638N}; *Ifnar1*$^{-/-}$ and *Apc/Apc*^{1638N}; *Ifnar1*$^{+/+}$	↔ apoptosis (human vs. murine DTF and NF) ↓ cell proliferation (human/ murine DTF and NF)

Table 1. *(continued)*

Drug	Ref.	Setting	Effect
PI3K /AKT/ mTOR signalling pathway			
Tyrosin kinase inhibitor Sorafenib (± Everolimus)	[64]	Human PCC from DTF	↓ DTF cell proliferation and invasion (sorafenib) ↓ mTOR signalling (↓ phospho-S6K levels (everolimus))
Growth regulatory signalling pathway			
Cytokines TGF-ß1	[65]	Human PCC from DTF, fibroma, NF	↔ cell proliferation in DTF, fibroma and NF cell culture ↑ GAG accumulation in extra-cellular matrix ↑ collagen synthesis
	[66]	Human PCC from DTF and NF	↑ active unphosphorylated fraction of ß-catenin
Cytokines rhEGF /rhTGF-α	[67]	Human PCC from DTF	Up- and down regulation of genes in response to stimulation with rhEGF /rhTGF-α
Cytokines rhEGF/AG1478 /SB431542	[68]	Human PCC from DTF	↑ DTF cell motility (rhEGF)
Oestrogen driven pathway			
Anti-oestrogen Tamoxifen/ Toremifene	[69]	Human PCC from DTF	↓ cell growth (tamoxifen ± oestrogen) ↔ cell growth (toremifene ± oestrogen)
Anti-oestrogen Toremifene	[70]	Human PCC from DTF, fibroma and NF	↔ cell proliferation (^{3}H-tymidine incorporation) ↓ GAG (DTF, fibroma and NF cultures) ↓ collagen production (^{3}H-proline incorporation) ↓ TGF-ß1 levels in culture medium ↓ TGF-ß1 mRNA expression levels ↓ TGF-ß1 receptor affinity
	[71]	Human PCC from DTF and NF	↑cell death (DTF and NF culture) ↓ collagen production (^{3}H-proline incorporation) ↓ procollagen α_1 mRNA expression (DTF culture) ↓ type I and III collagen ↑ collagenase activity ↔ MMP-1, ↑ MMP-2, ↓ TIMP-1
	[72]	Human PCC from DTF, Gardner-syndrome related fibroblast and NF	↓ GAG synthesis and secretion ↓ active TGF-ß1, ↔ total (active + latent) TGF-ß1 ↓ number TGF-ß1 receptors (DTF cells) ↓ TNF-α production

↓, decrease; ↑, increase; ↔, no effect; CRC, colorectal cancer; DFMO, Difluoromethylornithine; DFU, selective COX-2 blocker (5,5-dimethyl-3-(3-¯uorophenyl)4-(4-methylsulphonyl)phenyl-2(5H)-furon one); DTF, desmoid-type fibromatosis; FAP, familial adenomatous polyposis; GAG, glycosaminoglycan; PCC, primary cell culture; NF, normal fibroblast; NSAID, Non-steroidal anti-inflammatory drug

Pharmacological options targeting the Wnt/ß-catenin signalling pathway

Although many studies implicated aberrant Wnt/β-catenin signalling in DTF tumorigenesis, therapeutic targeting of this pathway remains challenging. Wnt/β-catenin target-genes that do form attractive therapeutic targets in DTF are *cyclooxygenase* (COX), a member of the COX enzyme family (COX1 and COX2) and the *vascular endothelial growth factor* (VEGF), a protein that regulates angiogenesis. A role of COX in DTF has been indicated by the expression of COX2 and by the expression of phosphorylated, and thus activated, associated growth factors receptors such as the platelet derived growth factor receptor α and ß (PDGF-α and PDGF-ß) [56, 73]. Activation of their receptors (PDGFR-α /PDGFR-ß) takes place by an autocrine/paracrine loop and is initiated by COX2 overexpression due to Wnt/ß-catenin deregulation [56, 73]. Inhibition of COX with sulindac decreased cell proliferation in DTF cell culture and therefore forms an attractive therapeutic target in DTF, especially because COX inhibitors are already widely used in the clinic [56, 57]. Halberg et al. reported decreased DTF tumour numbers in $Apc^{Min/+}p53^{-/-}$ mice treated with piroxicam, a drug which is a non-steroidal anti-inflammatory drug (NSAID) which works by inhibiting both prostaglandins and the COX enzyme (Table 1) [58].

A preclinical study by Poon et al. used the non-opioid analgesic drug nefopam (benzoxazocine class), and reported a decrease in ß-catenin levels and cellular proliferation rate, as well as a reduction in tumour number and volume in $Apc^{+/}Apc^{1638N}$ mice (Table 1) [60]. The working mechanism of this drug in DTF has not been entirely clarified yet, but is presumably due to an inhibition of serine-9-phosphorylation of GSK3-β (Figure 1) [60].

Overexpression of VEGF has been correlated with ß-catenin nuclear staining in DTF [74]. Additionally, microvessel density, a phenomenon correlated to angiogenesis, was shown to be higher in samples with VEGF overexpression. This high vascularity potentially increases the growth potential of DTF tumours [74]. These findings reveal a possible new treatment strategy for DTF by interfering with angiogenesis. Endostatin, an anti-angiogenic protein with the ability to inhibit the Wnt/β-catenin signalling pathway in colorectal cancer cells, directed the induction of cell death in primary FAP-associated DTF-cells in culture [59]. Endostatin has been proven to be well tolerated in a Phase 1 study, with minimal toxicities in patients with solid tumours other than DTF; however, no studies report the use of endostatin for DTF in the clinic [75].

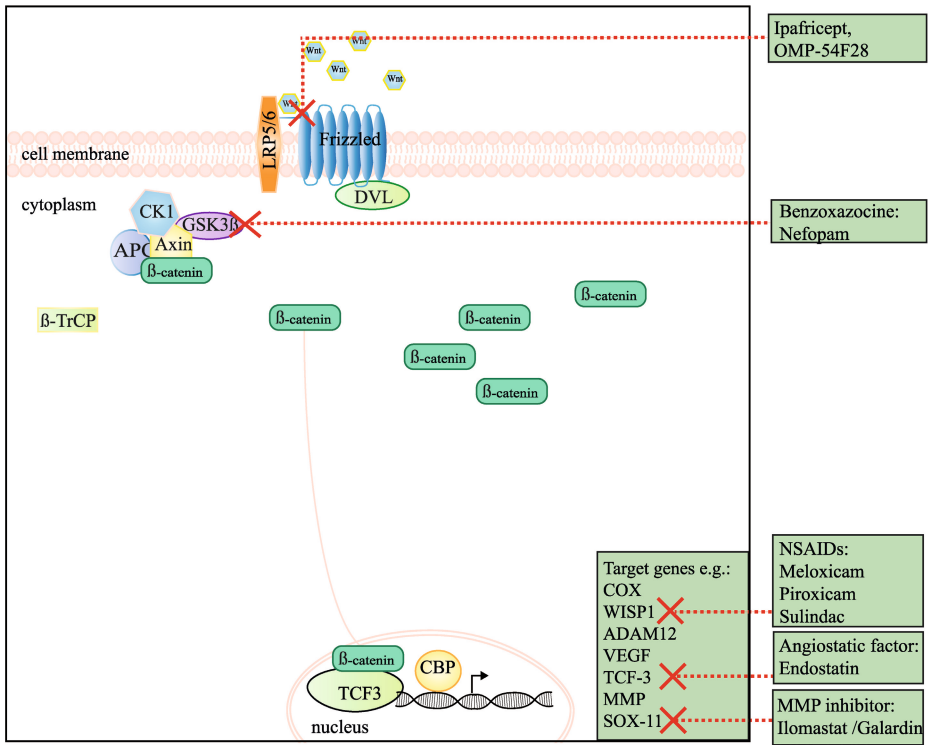

Figure 1. A schematic presentation of the Wnt/β-catenin signalling pathway and the drugs that target this pathway in DTF. The graph shows that ipafricept (OMP-54F28), inhibits Wnt signalling by acting as a decoy receptor inhibiting Wnt signalling through the Frizzled 9 receptor. NSAIDs, like meloxicam, the angiogenesis inhibitor endostatin and MMP inhibitors act on target genes of the Wnt signalling pathway. The drug Nefopam, a non-opioid analgesic drug of the benzoxazocine class suppresses the effect of high levels of β-catenin.

The blockage of Wnt/β-catenin signalling with the truncated Frizzled 9 receptor fused to the IgG1 Fc region (ipafricept, OMP-54F28), was recently tested in a Phase 1 study for solid tumours (Table 2). In this study, two patients with DTF were included that both exhibited stable disease, although it is unclear if this can be directly attributed to the treatment [76].

While the above-mentioned treatments, targeting Wnt/β-catenin targets constitute attractive therapeutic possibilities, no prospective clinical trials using these treatment strategies in sporadic DTF have been designed. Experimental inhibitors of Wnt/β-catenin signalling have been developed, however, systemic abolition of Wnt secretion is not preferable since this will result in defects in gut homeostasis, affects the immune system and affects both ß-catenin-dependent and independent Wnt signalling (reviewed by Zimmerli et al. [90] and Enzo et al. [91]). Figure 1 displays the Wnt/β-catenin signalling pathway and putative drug targets in the context of DTF.

Table 2. Overview of drugs used clinical trials targeting signalling pathways in DTF

Drug	Ref.	Setting	Tumour type	N of DTF patients	Efficacy in DTF
Wnt/ ß-catenin signalling pathway					
Frizzled 9 receptor blocker Ipafricept (OMP-54F28)	76	Phase 1	Advanced solid tumours (n = 26)	2	n = 2 SD (> 6 months)
Notch signalling pathway					
PF-03084014	77	Phase 1	DTF	7	ORR 71.4% (95%CI 29-96.3%) n = 5 PR, median TTR 9.9 months n = 1 PD
	78	Phase 2	DTF	17	n = 5 PR (after a median of 32 cycles, 95 weeks), n=11 SD
PI3K /AKT/ mTOR signalling pathway					
Receptor Tyrosin Kinase inhibitor Imatinib	79	Phase 2	DTF	40	n = 2 TS (<1 year) at 3 months: n=1 CR, n=3 PR, n=28 SD, n = 5 PD 3-months NPRR 91% (95% CI 77-96), 6-months NPRR 80%, 12-months NPRR 67%
	80	Phase 2	DTF	51	At 2-months: n=48 SD 2-months PFS 94%, 4-months PFS 88%, 1 year PFS 66%
	81	Phase 2	DTF	19	n = 3 PR, n = 4 SD (> 1 year), 1-year disease control rate 36.8%, TTF 325 days
	82	Phase 2	Imatinib-sensitive tumours (n = 186)	20	DTF patients: n = 2 PR, n = 8 SD, n = 7 PD, n = 3 unknown. Median TTP 9.1 months (95%CI 2.9-17.0 months).
Imatinib (+ nilotinib)	83	Phase 2	DTF	39	OS 100%, PAR at 6 months: 65% At 21 months: n = 7 PR, ORS 19% n = 8 imatinib + nilotinib due to PD under imatinib 3-months PAR 88%
Imatinib + gemcitabine (I+G) or Imatinib+ doxorubicin (I+D)	84	Phase 1	solid tumours (n = 16)	1 (I+G)	DTF patients: ceased treatment due to dose-limiting toxicity (grade 2 neutropenia)

Table 2. *(continued)*

Drug	Ref.	Setting	Tumour type	N of DTF patients	Efficacy in DTF
Sunitinib	85	Phase 2	Non-GIST sarcomas (n = 53)	1	DTF patients: n = 1 NR
	86	Phase 2	advanced DTF	19	Median FU 20.3 months (1.8-50.7 months), 2-year PFS 74.7%, OS 94.4%. n = 5 PR, n = 8 SD, n = 3 PD, n = 3 not evaluable. Median duration of the response 8.2 months (range 2.0-17.3 months)
Sorafenib (+ topotecan)	87	Phase 1	Paediatric solid malignancies (n = 13)	2	DTF patients: n = 1 PR
	88	Phase 3	Advanced and refractory DTF	87	2-year PFS of sorafenib 81% (95%CI 69-96%) vs. placebo 36% (95%CI 22-57%) ORR of sorafenib 33% (95% CI 20-48%) vs. placebo 20% (95% CI 8-38%)
Oestrogen driven pathway					
Anti-oestrogen + NSAID Tamoxifen + sulindac	89	Phase 2	Paediatric DTF	59	n = 4 PR, n = 1 CR 2-year PFS 36%, OS 96%

CI, confidence interval; CR, complete response; DTF, desmoid-type fibromatosis; GIST, gastro-intestinal stromal tumour; N, number of patients; NPRR, non-progressive response rate; NR, no response; ORR, objective response rate; ORS, overall response rate, OS, overall survival; PAR, progression arrest rate; PD, progressive disease; PFS, progression free survival; PR, partial response; SD, stable disease; TS, treatment stop; TTF, time to treatment failure; TTP, Time to progression; TTR, time to recurrence

The Hedgehog signalling pathway in desmoid-type fibromatosis

The Hedgehog signalling pathway

The Hedgehog (Hh) signalling pathway plays an essential role in embryonic development, in adult tissue homeostasis, tissue renewal and tissue regeneration. Precursor proteins of Hh ligands, including Sonic (Shh), Indian (Ihh) and Desert (Dhh), undergo autocatalytic cleavage and cholesterol alterations at the carboxy terminal end, and palmitoylation at their amino terminal end. This process results in a dually-lipidated protein, which is released from the secreting cell surface. Subsequently, the Hh ligands interact with cell surface proteins like Glypican and the proteins of the of heparin sulfate proteoglycan family

enhancing their stability and promoting internalization when bound to Patched (PTCH1). Binding of Hh proteins to the canonical receptor PTCH1 and to co-receptors GAS1, BOC and CDON initiates Hh signalling. This results in the release of PTCH1 mediated repression of the transmembrane protein Smoothened (SMO), a G-protein coupled receptor (GPCR)-like protein, which consequently leads to an accumulation of SMO in the cilia and phosphorylation of its cytoplasmic tail. Smoothened, regulates the downstream signal transduction which dissociates glioma associated oncogene (GLI) proteins, from kinesin-family protein, KIF7 and SUFU. GLI proteins serve as bifunctional transcription factors, capable of activating and repressing transcription, and form a key intracellular component of the Hh pathway (reviewed by Wu et al. [92] and Briscoe and Thérond [93]).

The Hedgehog signalling pathway in cancer

Aberrant Hh signalling in cancer is attributed to an increased endogenous Hh ligand expression, or to activating mutations of Hh pathway components (reviewed by Wu et al. [92]). Aberrant uncontrolled activation of Hh has been described in numerous tumour types including: rhabdomyosarcoma [94], colorectal cancer [95], basal cell carcinoma [96] and medulloblastoma [97].

The Hedgehog signalling pathway and its role and therapeutic potential in desmoid-type fibromatosis

As the Hh pathway has the ability to maintain mesenchymal progenitor cells in a less differentiated state with greater proliferative capacity, it is possible that it influences proliferation of DTF cells in a similar manner because of the mesenchymal origin of these cells [61]. Ghanbari et al. showed that Hh signalling is active in DTF by identifying a significant upregulation of Hh target genes *GLI1*, *PTCH1* and *Hedgehog interacting protein* (HHIP) in human DTF samples compared to adjacent normal tissues. Additionally it was demonstrated that expression of *Gli1*, *Gli2*, and *Ptch1* in mouse (*Apc*$^{+/1638N}$) tumours was upregulated compared to normal tissue. *In vivo*, pharmacological inhibition of Hh with triparanol, which works by interference with the posttranslational modification of Hh signalling molecules and with the sterol-sensing domain of the receptor PTCH1, led to a reduction in tumour volume in *Apc* $^{+/1638N}$ mice. Genetic approaches to reduce Hh signalling in DTF using *Apc*$^{+/1638N}$;*Gli2*$^{+/-}$ mouse models, gave rise to the development of fewer and smaller tumours (Table 1) [61, 98]. Current inhibition of the Hh signalling pathway in the clinic acts via the pharmacological inhibition of SMO, however no clinical trials studying Hh inhibitors in DTF have been carried out. Figure 2 displays the Hh pathway and proposed working mechanism of target drugs in DTF.

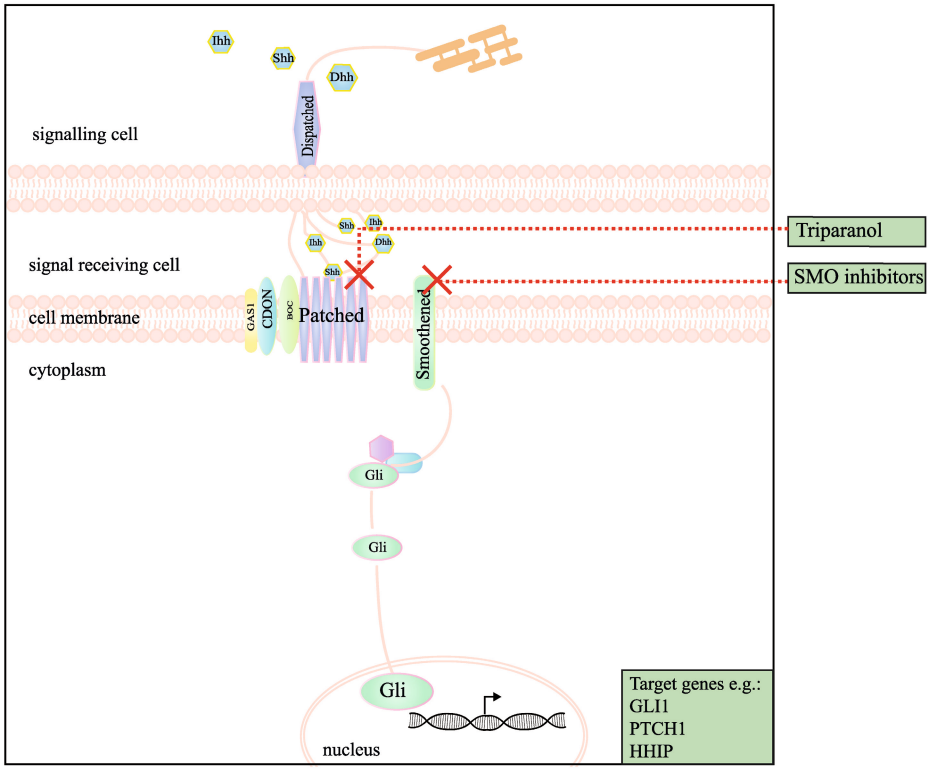

Figure 2. A schematic presentation of the Hedgehog signalling pathway and the drugs that interfere with this pathway in DTF. The graph depicts that inhibition of the Hedgehog pathway, by SMO inhibitors, works by blockage of Smoothened (SMO), a key regulator of downstream signaling by GLI transcription factors. The compound triparanol is known for inhibition of the cholesterol biosynthesis but can also interfere with Hedgehog signalling molecules including the Hedgehog ligand receptor Patched 1.

The Notch signalling pathway in desmoid-type fibromatosis

The Notch signalling pathway

Notch signalling is essential for regulating cell-fate during tissue development and for managing cell proliferation, differentiation and survival, neurogenesis and homeostasis in adult tissues (reviewed by Artavanis-Tsakonas et al. [99]). There are four mammalian transmembrane Notch receptors (Notch receptor family type 1-4; NOTCH 1-4). Each receptor is a Ca^{2+}-stabilized heterodimer containing three domains: an extracellular (NECD), a transmembrane (NTMD) and an intracellular domain (NICD) (reviewed by Takebe et al. [100]). These receptors can interact with ligands; members of the Delta-like (DLL1, DLL3 and DLL4), and the Jagged (JAG1 and JAG2) families. In case of ligand

binding, the receptor undergoes two processing steps. The first cleavage is mediated by a member of the disintegrin and metalloproteinase family (ADAM10 or ADAM17) and releases the NECD which remains bound to its ligand and is internalized by endocytosis in the cell that sends the signal. Subsequently in the receiving cell, a presenilin-dependent ɣ-secretase complex, removes the NICD from the NTMD. This NICD is translocated into the nucleus where it interacts with the CSL (CBF1/Suppressor of hairless/Lag-1) repressor complex, converting it into an activation complex that interacts with a co-activator protein mastermind-like 1 (MAML1). These interactions results in the transcriptional activation of several Notch target genes such as *MYC*, *p21*, *HRT*, Notch receptors, Notch ligands, *cyclin D1*, and *HES*-family members (reviewed by Takebe et al. [100]. and Ranganathan et al. [101]).

The Notch signalling pathway in cancer

Deregulation of the Notch signalling pathway is described in hematologic malignancies, notably T-cell acute lymphoblastic leukaemia which harbours an activating mutation in NOTCH 1 that result in a constitutive Notch signalling pathway activity [102]. Although activating mutations in members of the Notch family are uncommon in solid tumours, Notch signalling may play a role in tumorigenesis (reviewed by Egloff and Grandis [103]). For example, NOTCH3 transcript and protein levels are upregulated in a subset of colorectal cancers promoting tumour growth [104].

The Notch signalling pathway and its role and therapeutic potential in desmoid-type fibromatosis

Inhibition of Notch signalling forms an appealing therapeutic approach. Small molecular inhibitors, including ɣ-secretase inhibitors (GSI), siRNAs and monoclonal antibodies against Notch receptors and ligands have been developed (reviewed by Yuan et al. [105]). Particularly GSI's are of interest as these drugs inhibit the final Notch processing step by which NICD is released to act in the nucleus, consequently blocking Notch signalling. A number of GSI's (e.g., MK-0752 and RO4929097) have already been studied in solid cancers other than DTF in early phase clinical trials [106, 107].

Few studies investigated the role of the Notch signalling in DTF, however, DTF tumours have been shown to express NOTCH1 and its downstream target HES1 [108]. Preliminary evidence, from a phase 1 clinical trial indicated a partial response in five out of seven DTF patients to the oral GSI PF-03084014 (Table 2) [109]. This prompted an *in vitro* study performed by Shang et al., which demonstrated a significant higher expression of nuclear HES1 in DTF tissues compared to scar tissue by IHC and reported expression of NOTCH1, JAGGED1

and HES1 in DTF cells by Western Blot analysis. Additionally, it was demonstrated that PF-03084014, decreased NICD and HES1 expression in a dose dependent manner in DTF cells, and that Notch signalling inhibition contributed to impaired DTF cell proliferation by inducing a cell cycle G1 arrest and decreasing migration and invasion (Table 1) [62]. Two other clinical trials (a phase 1 trial with seven DTF patients and phase 2 trial with seventeen DTF patients) showed promising results with a significant part of patients experiencing partial response or stable disease (Table 2) [77, 78]. Figure 3 displays the Notch pathway and putative drug targets in the context of DTF.

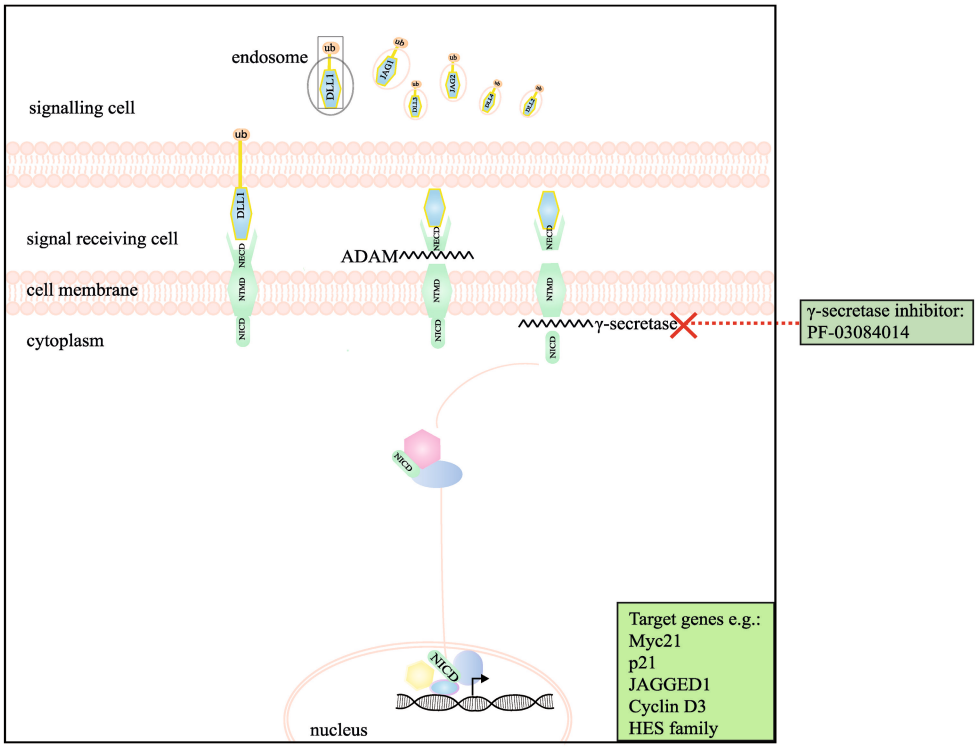

Figure 3. A schematic presentation of the Notch signalling pathway and the drugs that interfere with this pathway in DTF. The graph depicts that the Notch signalling pathway can be targeted by the use of γ-secretase inhibitors e.g., PF-03084014.

The JAK/ STAT signalling pathway in desmoid-type fibromatosis

The JAK/STAT signalling pathway

The Janus-activated kinase (JAK) and signal transducer and activator of transcription (STAT) signalling pathway regulates cell proliferation, survival, differentiation, migration and apoptosis, and has a role in pathogen resistance. The JAK/STAT pathway is the main signalling mechanism for many cytokines and growth factors. A variety of ligands e.g., interferon-α /interferon-ß (IFN), secreted by leukocytes (IFN-α), fibroblasts (IFN-ß) and various other cells involved in immune responses, and interleukins (IL) together with their cognate receptors, stimulate the pathway. Ligand binding causes receptor dimerisation and subsequent activation of the JAK tyrosine kinases associated with the cytoplasmic domains of the receptor. JAKs in close proximity are trans-phosphorylated and phosphorylate the receptors. The phosphorylated receptor sites can then serve as docking sites for cytoplasmic transcriptions factors (STATs). These STATs become phosphorylated by JAKs, dimerise and are translocated into the nucleus where they activate or repress the transcription of target genes (reviewed by O'Shea et al. [110]).

The JAK/STAT signalling pathway in cancer

Dysregulation of the JAK/STAT signalling pathway has been observed in several cancers including haematological and solid malignancies such as breast (reviewed by Banerjee and Resat [111]) and prostate cancer (reviewed by Bishop et al. [112]). Hallmarks of JAK/STAT dysregulation are aberrant cytokine production, the occurrence of activating *JAK* mutations or mutations in other upstream oncogenes and activating mutations in STAT (reviewed by O'Shea et al. [110]).

The JAK/STAT signalling pathway and its role and therapeutic potential in desmoid-type fibromatosis

In both human DTF samples and murine DTF models (*Apc/Apc*^{I638N}), an increased expression of type 1 IFN response genes (e.g., *MxA, MxB, IFITI1,* and *IFNAR1*) have been identified suggesting an activated JAK/STAT signalling. Genes activated by this signalling pathway, have been shown to have an anti-proliferative effect on DTF cells and normal fibroblasts (Table 1) [63]. Regression of DTF after treatment with IFN has been described in several case reports [113-116]. A retrospective study by Leithner et al. examined thirteen DTF patients receiving IFN-α ± tretinoin (a natural metabolite of vitamin A [retinol]). This study indicated no evidence of disease in seven out of nine patients (adjuvant group) and a mean disease-free interval of 22 months (±18 months), and progressive disease in two patients.

Stabilization of DTF occurred in four patients which received IFN-α± tretinoin [117]. Despite these encouraging results, no prospective clinical trials in DTF have been carried out yet.

The PI3 kinase/AKT and mTOR signalling pathways in desmoid-type fibromatosis

The PI3 kinase/ AKT and mTOR signalling pathways

The phosphoinositide 3 (PI3) kinase signalling pathway plays a critical role in various cellular processes like cell growth, survival, proliferation, metabolism and differentiation (reviewed by Engelmann et al. [118]). The pathway is activated by plasma membrane proteins including receptor tyrosine kinases, integrins, B- and T-cell receptors, cytokine receptors and GPCRs and entails the formation of membrane-bound phosphatidylinositol-3,3,5-triphosphate (PIP3) by the enzyme PI3 kinase. Proteins that harbour a pleckstrin-homology (PH) domain like AKT (protein kinase B or PKB) and PDK1 bind to the 3-phosphoinositides on the membrane. Subsequent phosphorylation of AKT at the Thr308 and Ser473 residues, by PDK1 and mTORC2, respectively, fully activates its serine/threonine kinase potential. AKT consequently phosphorylates many downstream substrates, thereby regulating various cellular functions (reviewed by Hers et al. [119]). Importantly, AKT also leads to downstream activation of the mTOR complex 1 (mTORC1) pathway by phosphorylation of its negative regulators TSC2 and PRAS40. Activation of this complex provides a growth advantage for cells, as mTORC1 is critical for cell maintenance by sensing nutritional and environmental cues and responding by inhibiting autophagy and regulating translation thereby stimulating cell growth and proliferation (reviewed by Hers et al. [119]. and Dowling et al. [120]).

The PI3 kinase/AKT and mTOR signalling pathways in cancer

Dysregulation of the PI3 kinase/AKT signalling pathway is frequently encountered in cancers and facilitates tumorigenesis. Over-activation of AKT may be caused by the presence of gain-of-function mutations in PI3K subunits, or loss-of- function mutations in Phosphatase and Tensin homolog deleted from chromosome ten (PTEN) or PTEN expression loss. PTEN, a tumour suppressor is a lipid phosphatase, negatively regulating AKT by dephosphorylation of PIP3. Alternatively, overexpression or activating mutations in tyrosine kinase receptors and their ligands, as well as the interaction of Ras with PI3K, can excite AKT activity (reviewed by Brugge et al. [121]and Keniry and Parsons [122]).

The PI3 kinase /AKT and mTOR signalling pathways and their role and therapeutic potential in desmoid-type fibromatosis

The relationship between DTF and the PI3 kinase/AKT and the mTOR signalling pathways has not been extensively studied. Immunohistochemical analysis of twenty-nine DTF tumour samples indicated the expression of ß-catenin and PDGFR-ß in all samples. No expression could be detected of PDGFR-α and phospho-Ser-473, suggesting inactive AKT signalling [123]. Meazza et al. showed that a substantial part of paediatric DTF cases had an E17K mutation in either AKT1 (eight out of twenty-eight 28; 31%), however no AKT1 mutations were observed in adult DTF cases (n = 33) [124]. Interestingly, DTF patients with an E17K AKT1 mutation had a longer recurrence free survival rate in agreement with the mutation-induced stimulation of downstream AKT signalling.

Recently, Rosenberg et al. reported the anti-tumour effect of sorafenib, a multi-kinase inhibitor that targets multiple tyrosin kinases (e.g., VEGFR, c-Kit and PDGFR) expressed on patient derived DTF cell lines (Table 1). It was found that sorafenib decreased proliferation and invasion in a dose dependent manner and that the Ras/MEK/ERK and the PI3kinase/AKT/mTOR signalling pathways were affected. Additionally, they investigated the efficacy of everolimus, an mTOR inhibitor, as monotherapy and in combination with sorafenib, and found no synergistic or additive inhibitory effect on cellular proliferation (Table 1) [64]. Cates et al. compared the expression profiles of selected receptor- and non-receptor tyrosine kinases and downstream effectors of signalling activity in DTF (n = 27), reactive scars (n = 14) and fibrous tissue (n = 6) (Table 1). PDGFR-β, FAK 1 and MET were detected by IHC in almost all DTF and scar tissues and in at least half (PDGFR-β, FAK1) or none (MET) of the fibrous control tissue. Of note, AKT was phosphorylated in 56% of DTF samples, but significantly higher levels were observed in scar tissues and only low levels were observed in a subset of fibrous tissues [125].

Inhibition of various tyrosine kinases including PDGFR, with imatinib is a promising treatment strategy for DTF. Imatinib is effective in other solid tumours of mesenchymal origin like gastro-intestinal stromal tumours (GISTs) and dermatofibrosarcoma protuberans, in which it targets KIT or PDGFR-α /PDGFR-ß (reviewed by Kosela-Paterczy et al. [126, 127]). The efficacy of imatinib (either alone or combined with other treatment) was observed in several early phase clinical studies with DTF patients (Table 2) [79-84]. Chugh et al. and Penel et al. reported a 1-year progression free survival (PFS) of 66% and 67% respectively. However, the results of these studies should be interpreted with caution, since the majority of patients included in these trials received other treatments prior to treatment with imatinib (Table 2). Additionally, the relevant targets of imatinib in DTF remain unclear [81, 123]. Two phase 2 clinical trials including

DTF patients studied the effect of sunitinib (Table 2). George et al. did not detect a response to sunitinib in the single DTF patient that was included in their study [85]. In contrast, a study by Jo et al. showed a 2-year PFS of 75% in nineteen patients with advanced DTF [86]. A phase 1 study, investigating the use of sorafenib in paediatric solid malignancies, of which two patients had DTF, showed a partial response on sorafenib in one patient [87]. Recently published results from the phase 3 clinical trial (NCT02066181; sorafenib versus placebo for advanced and refractory DTF) showed a 2-year PFS rate of 81% (95% confidence interval [CI] 69-96) with an objective response rate (ORR) of 33% (95% CI, 20-48) in the sorafenib group versus 2-year PFS of 36% (95% CI, 22-57) and an ORR of 20% (95% CI, 8-38) in the placebo group [88]. The biological mechanisms underlying the activity of sorafenib in DTF remain unclear because sorafenib targets multiple tyrosine kinases thereby affecting multiple pathways.

The use of imatinib, sunitinib and sorafenib, led to study the tyrosine kinase inhibitor (TKI) pazopanib in the setting of DTF. Pazopanib targets the VEGFR1-3, PDGFR-α and PDGFR-ß, amongst others. A retrospective study by Szucs et al., described a partial response in three out of eight patients with DTF and stable disease in five out of eight patients with a median PFS of 13.5 months [128]. Another retrospective study in adolescents and young adults with DTF by Agresta et al. reported tumour reduction after pazopanib use with only mild toxicities (Table 2) [129]. One clinical trial is currently ongoing to investigate TKIs in the setting of DTF: pazopanib (NCT01876082, phase 2 study) (Table 3) [130].

Table 3. Overview of ongoing clinical trials with targeted drugs in DTF

Drug	Ref.	NCT number	Setting	Current status
PI3 kinase/AKT/ mTOR signalling pathway				
Receptor Tyrosin Kinase inhibitor Imatinib + vactosertib	[131]	NCT03802084	Phase 1/2 clinical trial	Not yet recruiting
mTOR inhibitor Sirolimus	[132]	NCT01265030	Phase 1 clinical trial Phase 2 clinical trial (children and young adults)	Recruiting
Notch signalling pathway				
Notch inhibitor PF-03084014	[133]	NCT01981551	Phase 2 clinical trial	Active, not recruiting
	[134]	NCT03785964	Phase 3 clinical trial	Not yet recruiting
Growth regulatory signalling pathway				
PDGF-a /PDGF-β /VEGFR Pazopanib	[130]	NCT01876082	Phase 2 clinical trial	Recruiting
NCT, national clinical trial				

The growth factor regulatory signalling pathways in desmoid-type fibromatosis

The growth regulatory signalling pathways

The superfamily of transforming growth factor-ß (TGF-ß) regulates cell proliferation, differentiation, apoptosis and development. Two ligand subfamilies are recognized; the TGF-β nodal subfamily, and bone morphogenetic protein (BMP) subfamily. Ligand binding of either TGF-β ligands or BMP ligands facilitates the oligomerisation of type I and type II serine/threonine receptor kinases. In case of signalling, intracellular effectors, R-SMAD's, are phosphorylated in the cytoplasm whereupon they partner with SMAD4 and translocate to the nucleus. In the nucleus they regulate, in conjunction with transcription factors/corepressors or co-activators, the transcription of TGF-ß target genes. Growth factor signalling pathways are initiated by various growth factors (e.g., insulin-like growth factors, platelet-derived growth factor, and hepatocyte growth factor) and induce phosphorylation of downstream targets via activation of their associated receptor tyrosine kinases. The signal is transduced through various intracellular intermediate molecules, frequently including PI3 kinase/AKT and Ras/Raf/MAPK pathways to ultimately affect gene expression (reviewed by Massague et al. [135]).

The growth factor regulatory signalling pathways and their role and therapeutic potential in desmoid-type fibromatosis

The role of TGF-ß in DTF has been established by the expression of TGF-ß target genes (e.g., several collagen types and metalloproteinases) and by the upregulation of TGF-ß signalling pathway components (phospho-SMAD2 and phospho-SMAD3, α-SMA and PAI1) in comparison to normal fascia [66]. Mignemi et al. investigated TGF-ß signalling comparing DTF tissue with hypertrophic scars and fibrous tissue in human samples. It was discovered that the levels of TGF-ß receptor type 1 were similar in DTF and scar tissue, but that this receptor could not be detected in fibrous tissue. Phosphorylated SMAD2/3 could be detected in the majority of DTF samples (74%) but only in a minority of scar tissues (29%) and not at all in fibrous tissue [136]. Additionally, TGF-ß stimulates β-catenin transcriptional activity, which indicates that this growth factor might play an important role in the development of DTF [66]. Multiple studies reported overexpression of platelet derived growth factors and their associated receptors (PDGFα, PDGFRα, PDGFß and PDGFRß) in neoplastic fibrous proliferations including DTF and myofibromatoses [137, 138].

Various studies investigated the effect of TFG-ß and epidermal growth factor (EGF) on DTF cell lines and stimulation with these cytokines caused up- and down regulation of various target genes (e.g., SMAD4), changes in β-catenin levels, and increased production of glycosaminoglycan and collagen. Additionally, treatment with EGF increased DTF cell motility (Table 1) [65-68].

Cross-talk between the insulin-like growth factor (IGF) and the oestrogen receptor (ER) mediated signalling has been demonstrated in breast-cancer cells. Activation of MAPK, which is located downstream of IGF-1, enhances ER induced transcription via ER phosphorylation. Therefore, it is presumed that growth factor signalling pathways and the oestrogen pathways complement and overlap each other (reviewed by Dhingra et al. [139]). Toremifene, a drug which also inhibits collagen synthesis and protein kinase C, works on both the growth factor regulatory signalling pathway and the oestrogen pathway and will be discussed in the next section.

The role of the oestrogen driven pathway in desmoid-type fibromatosis

The oestrogen driven pathway

Oestrogens affect various physiological processes that concern the development of the reproductive system and several reproductive functions. The role of oestrogens in cancer has been described in breast cancer and uterine tumours (reviewed by Dhingra et al. [139]). Two subtypes of oestrogen receptors (ER) haven been identified: ER-α (ESR1) and ER-ß (ESR2) to which oestrogens can bind. Without the ligand, the intracellular ER is considered to be in an inactive state, forming a complex with two heat shock proteins (Hsp90 and Hsp56) and various other proteins. Upon binding of the ligand oestrogen, Hsp90 is detached from the complex which leads to ER phosphorylation. Next, the ER dimerises and translocates to the nucleus where it can interact with specific DNA sequences, oestrogen response elements (ERE), causing transcriptional activation (reviewed by Dhingra et al. [139]. and Picariello et al. [140]).

The oestrogen driven pathway and its role and therapeutic potential in desmoid-type fibromatosis

The role of sex hormones, particularly oestrogen, in DTF tumorigenesis is primarily based on clinical observations, and prompted the use of anti-hormonal agents. DTF arises frequently in females at the reproductive age [4]. Moreover, tumour growth during pregnancy,

accelerated tumour growth by using oral contraceptives and tumour regression in menopause are reported [10, 141]. Although DTF does not express ER-α, Deyrup et al. discovered that DTF tumours express ER-ß [142]. The involvement of oestrogens in DTF offers a variety of interesting hormone-related drugs which can be a potential treatment for DTF. Tamoxifen is most extensively studied in the setting of DTF and is often used in various dosages in combination with other drugs, frequently NSAIDs, like sulindac (reviewed by Bocale et al. [143]). A meta-analysis by Bocale and Rotelli et al. verified that tamoxifen, administered as a single agent, gave an overall response rate (partial or complete response) of 58% (22 out of 38 patients). In combination with NSAIDs, this response rate decreased to 35% [143]. Likewise, toremifene has been shown to have an anti-tumour effect in DTF [71, 144]. Although it does not influence cell proliferation, toremifene decreased the total amount of glycosaminoglycan's (GAG), TGFß1, collagen and fibronectin levels and it diminished the affinity of type I and II TGFβ1 receptors for ^{125}I-TGF-ß1 (Table 1) [65, 70]. Toremifene in retrospective clinical studies, administered alone or in combination with other drugs like melatonin, sulindac or IFN-α, yielded an overall complete and partial response rate of 56%. When comparing tamoxifen and toremifene, used as a single agent, no differences in overall response rate were found (reviewed by Bocale et al. [143]). Raloxifene, a drug initially developed for treating chronic osteoporosis, was administered to thirteen patients with FAP-related DTF which were refractory to other treatments. Eight patients displayed a complete remission and a partial response was seen in five cases [145]. Despite a clear involvement of oestrogens in DTF, response rates to anti-oestrogen agents vary and the number of prospective clinical studies is still limited. One phase 2 trial combined sulindac (NSAID) with tamoxifen in pediatric DTF patients and showed a 1-year PFS of 36% (Table 2) [89].

Future directions and conclusion

This review aims to provide a summary of the current knowledge of important, cancer-related signalling pathways in the setting of DTF. The role of Wnt/β-catenin signalling in DTF has been firmly established in numerous studies, showing the presence of β-catenin signalling enhancing mutations in the vast majority of tumours. Therapeutic options targeting the Wnt/β-catenin signalling pathway remain scarce and are not yet widely tested in the clinical setting for DTF. Several clinical trials, targeting other signalling pathways, like Notch and Hedgehog, are currently ongoing, but few study the contribution of these pathways to DTF tumorigenesis.

A major challenge remains to study DTF in the preclinical setting. This is partly due to the rarity of the tumour type, but also to the limited availability of pure DTF cell lines and other cell and animal models [3]. Culturing a fresh DTF resection specimen, inevitably leads to an overgrowth of WT fibroblasts and concomitant loss of DTF tumour cells. Separating tumour cells from their surrounding stromal cells remains challenging and time consuming. Even if a "pure" DTF cell line is obtained, DTF tumour cells often reach senescence after several passages. To our knowledge, no studies investigating the effect of immortalization protocols on primary DTF cells have been published yet. Experimenting with primary cultures consisting of both stromal cells and tumour cells is an alternative but has its drawbacks. Additionally, representative cellular and animal models of DTF (e.g., organoids or mice expressing mutated *CTNNB1*) in relevant tissues are difficult to generate and expensive to maintain. The existing *Apc*$^{+}$*/Apc* ^{1638N} mouse model has already been proven as a useful model for FAP-associated DTF and is often used as a tumour model for non-FAP related DTF (Table 1) [61, 146]. A mouse DTF model based on specific β-catenin mutations is, to our knowledge, currently not available. A recently developed genetically engineered *Xenopus tropicalis* model harbouring a mutated APC, may yield another DTF tumour model that can be exploited as a platform to define novel therapeutic targets and preclinical validation studies [147]. Well-defined preclinical models are as necessary as well-annotated large series of DTF tumour samples, to better understand DTF biology and to provide experimental support and rationale for translational research investigating the inhibition of signalling pathways in DTF.

Additionally, signalling pathways are often seen as separate entities, however, in reality cross-talk occurs between different pathways. The precise interactions between different signalling- and biochemical pathways is complex and still poorly understood. Aberrant signalling of one pathway can often be corrected via compensatory mechanisms in another pathway [148]. In DTF tumours and cell lines, the Wnt, Notch and Hh pathways have been shown to be involved in cross-talk, implicating optimal therapeutic efficacy, is reached when all interacting pathways are inhibited in a combinatorial approach [149]. Future studies should not focus on individual signalling cascades but rather on the simultaneous inhibition of multiple pathways.

Furthermore, clinical studies to evaluate the efficacy of systemic and targeted treatments without any randomization procedures remain challenging in current clinical practice as they are often difficult to interpret. Due to the unpredictable growth behaviour of DTF with reports of spontaneous regression without treatment and stable disease, it is difficult to

distinguish the true treatment effects from natural growth behaviour. Moreover, the design of randomized controlled trials might be restricted by the rarity of the disease and the small number of DTF patients with an indication for systemic treatment. Despite these challenges, future studies should include signalling pathways other than Wnt/β-catenin signalling to uncover additional driver genes and pathways in DTF and to clarify the potential working mechanisms of target drugs in the setting of DTF.

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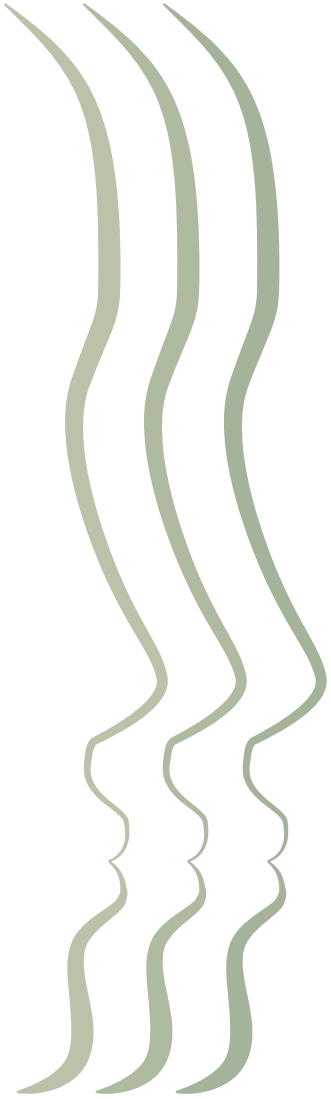
Supplemental Materials 1

List of abbreviations used in this manuscript

ADAM	A disintegrin and metalloproteinase
AKT	AKT8 virus oncogene cellular homolog
APC	Adenomatous polyposis coli
BOC	Brother of CDON
BMP	Bone morphogenetic protein
CDON	Cell adhesion associated, oncogene regulated
	Casein kinase
CK	Cyclooxygenase
COX	CBF1/ Suppressor of hairless/Lag-1
CSL	Complete response
CR	Colorectal cancer
CRC	Difluoromethylornithine
DFMO	Selective COX-2 blocker (5,5-dimethyl-3-(3-
DFU	uorophenyl)4-(4-methylsulphonyl)phenyl-
	2(5H)-furon one)
Dhh	Desert hedgehog
DLL	Delta-Like Canonical Notch Ligand
DVL	Dishevelled protein
DTF	Desmoid-type fibromatosis
EGF	Epidermal growth facto
ER	Estrogen receptors
ERE	Estrogen response elements
ERK	extracellular signal-regulated kinase
FAP	Familial adenomatous polyposis
GAG	Glycosaminoglycans
GAS	Growth arrest specific
GIST	Gastro-intestinal stromal tumours
GLI	Glioma-associated oncogene
GPCR	G-protein coupled receptor
GSI	Gamma-Secretase Inhibitors
GSK	Glycogen synthase kinase
HES	Hairy enhancer of split /HES Family BHLH
	Transcription Factor
Hh	Hedgehog
HHIP	Hedgehog interacting protein
Hsp	Heat shock proteins
IGF	Insulin growth factor
IFN	Interferon
Ihh	Indian hedgehog
IL	Interleukins
Jag	Jagged
JAK	Janus-activated kinase
KIF7	Kinesin family protein
KIT	v-KIT Hardy-Zuckerman 4 Feline Sarcoma
	viral oncogene homolog/ KIT oncogene
LEF	Lymphoid enhancer factor
LRP	Lipoprotein receptor-related protein
MAML1	Mastermind-like 1
MAPK	Mitogen-activated protein kinase
MEK	MAPK/Erk kinase
MET	Hepatocyte growth factor receptor
MMP	Matrix Metalloproteinase
mTOR	Mechanistic Target Of Rapamycin
NECD	Notch extra-cellular domain
NF	Normal fibroblast
NICD	Notch intra-cellular domain
NPRR	Non-progressive response rate
NR	No response
NTMD	Notch transmembrane domain
NSAID	Non-steroidal anti-inflammatory drug
ORR	Objective response rate
OS	Overall survival
PAR	Progression arrest rate
PCC	Primary cell culture
PD	Progressive disease
PDK	Phosphoinositide-dependent kinase
PDGF(R)	Platelet Derived Growth Factor (Receptor)
α/ß	α/ß
PFS	Progression free survival
PH	Pleckstrin-homolog
PI3	Phosphatidylinositol 3
PI3K	Phosphatidylinositol 3-kinase
PIP3	Phosphatidylinositol (3,4,5)-triphosphate
PR	Partial response
PTCH1	Patched 1
PTEN	Phosphatase and tensin homolog deleted on
	chromosome ten (10)
RTK	Receptor tyrosine kinase
SD	Stable disease
Shh	Sonic hedgehog
SMAD	SMA- and MAD-related protein / SMAD
	Family member
SMO	Smoothened
STAT	Signal Transducer and Activator of
	Transcription
SUFU	Suppressor of fused homolog
TCF	T-cell factor
TGF-ß	Transforming growth factor-ß
TKI	Tyrosin kinase inhibitor
TS	Treatment stop
TTF	Time to treatment failure
TTP	Time to progress
VEGF	Vascular Endothelial Growth Factor
WT	Wild-type
WISP	Wnt-1 Inducible Signaling pathway Protein 1
Wnt	Wingless



3

Wnt target genes are not differentially expressed in desmoid tumours bearing different activating β-catenin mutations

Milea J.M. Timbergen, Matthijs L. Janssen, Cornelis Verhoef, Dirk J. Grünhagen, Frederic Chibon, Marcel Smid, Stefan Sleijfer, Erik A.C. Wiemer

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Abstract

Introduction

Sporadic desmoid-type fibromatosis (DTF) is a rare soft tissue tumour of mesenchymal origin. It is characterized by local invasive growth and unpredictable growth behaviour. Three distinct mutations involving the *CTNNB1* (β-catenin) gene have been identified in the vast majority of DTF tumours, which cause activation of the Wnt signalling pathway and impacts prognosis. This study examines whether the different *CTNNB1* mutants (T41A, S45F) occurring in DTF tumours differentially affect Wnt signalling activity, which might explain the different disease course between DTF patients harbouring different *CTNNB1* mutations.

Materials and methods

Real-time polymerase chain reaction (RT-PCR) on 61 formalin fixed paraffin embedded DTF samples with known *CTNNB1* status was used to measure the relative mRNA expression level of Wnt target genes *AXIN2, DKK1* and *CCND1*. Additionally, publicly available mRNA expression data retrieved from the Gene Expression Omnibus of 128 DTF samples were used for an unsupervised cluster analyses based on the expression of a selection of Wnt targets.

Results

No statistically significant difference in relative expression levels of Wnt target genes *AXIN2, DKK1* and *CCND1* was identified between either *CTNNB1* wild-type, S45F or T41A mutated DTF samples. Moreover, the hierarchical cluster analyses using selected Wnt targets did not discriminate between different *CTNNB1* mutation types.

Conclusions

No differences in the expression levels of Wnt target genes were observed between the different *CTNNB1* mutation types in DTF tumours. Further studies are needed to decipher the mechanism accounting for the diverse disease courses between DTF patients with different *CTNNB1* variants.

Introduction

Sporadic desmoid-type fibromatosis (DTF) is a soft tissue tumour characterized by local invasive growth and unpredictable growth behaviour with phases of progression, stable disease and even spontaneous regression [1-4]. The incidence in the Dutch population is approximately five cases per million people per year [5]. Affected patients are mostly females, with a peak incidence between the second and fourth decade of life [6-9]. Sporadic DTF has a mesenchymal origin, arising in musculoaponeurotic structures and can develop at practically any location in the body [10]. The most common site is the abdominal wall whereas the predominant extra-abdominal sites are the trunk and the proximal part of the extremities [7, 11]. Treatment includes conservative management via active surveillance, surgical resection, radiotherapy or systemic therapy. The latter option comprises non-steroidal anti-inflammatory drugs (NSAIDs), anti-hormonal agents such as tamoxifen, low dose chemotherapy such as a combination of methotrexate with either vinblastine or vinorelbine, and tyrosine kinase inhibitors such as imatinib, sorafenib, or nilotinib [12]. No recommendations about the sequence of existing systemic treatment options can be given yet, although the recent results of a randomized phase 3 trial suggest a possible role of sorafenib in symptomatic patients [13]. Recurrence rates after treatment are high with a 5-year local recurrence rate of 49% after surgery [9].

The *CTNNB1* (β-catenin) gene is mutated in the vast majority of sporadic DTF tumours [9, 14-22]. Normally, β-catenin acts as a key mediator in the Wingless (Wnt) signalling pathway by operating as a transcriptional activator through binding in the nucleus to members of the TCF/LEF transcription factor family. The *CTNNB1* mutations, found in DTF, mainly affect two codons in exon 3; substituting threonine at position 41 with alanine (T41A) and replacing serine at position 45 with either phenylalanine (S45F) or proline (S45P) [8, 9]. These mutations prevent its phosphorylation, poly-ubiquitination and subsequent proteasomal degradation. Instead, β-catenin is stabilized and translocated into the nucleus where it drives transcription of Wnt target genes. The Wnt signalling cascade is involved in several biological processes like embryonic development and maintenance and regeneration of adult cells [23, 24]. The aberrant activation of the Wnt signalling pathway, as in DTF, is observed in various malignancies like colorectal cancer, breast cancer and non-small cell lung cancer, and is considered to be a driver of tumorigenesis [14, 25-28]. The *CTNNB1* genotype-phenotype relation has been extensively investigated in liver cancer, providing evidence that different *CTNNB1* mutations are linked to different levels of β-catenin activation. The S45 mutation leads to a weak activation and T41 mutations are associated with a moderate activation which may yield differences in clinical behaviour [29]. Desmoid tumours that harbor a S45F

mutation exhibit a higher recurrence rate after primary resection than wild-type (WT) and T41A mutant tumours [9, 20, 30, 31] and were shown to be more resistant for the NSAID meloxicam [32]. However, others report conflicting results and could not reveal an impact of *CTNNB1* mutations on outcome [18, 22]. The molecular mechanisms, underlying a *CTNNB1* genotype-phenotype relationship, are not known, although Hamada et al. reported that S45F desmoid cells have a stronger nuclear β-catenin staining in a preclinical model and observed an upregulation of Wnt target genes *AXIN2* and *CCND1* compared to WT and T41A cells [33].

Here we examined whether the *CTNNB1* mutants, encountered in DTF, differentially affect Wnt signalling activity in human desmoids and whether this may explain the variable clinical behaviour between the different *CTNNB1* mutants.

Materials and methods

Dutch and French dataset

A Dutch, formalin fixed paraffin embedded (FFPE) DTF cohort (n=64), with *CTNNB1* mutation status determined with Sanger sequencing, previously described by van Broekhoven et al. was available for this study [20]. Samples were derived from patients with sporadic, aggressive fibromatosis who underwent surgical excision of their tumour. Clinical characteristics included: sex, tumour localization (extra-abdominal, abdominal wall or intra-abdominal), age at diagnosis and tumour size in millimetres.

Additionally, the clinical and molecular data from a French, fresh frozen DTF sample set (n = 128), containing a total of 54613 probe sets, previously described by Salas et al. were accessed through the Gene Expression Omnibus (series matrix file, accession number GSE58697) [34]. This file contains data normalized using the GCRMA algorithm. The data on the *CTNNB1* mutations status of these tumours were kindly provided by dr. Frederic Chibon, Cancer Research Center of Toulouse, France. Clinical data included: sex, age at the time of diagnosis, tumour size in millimetres, tumour localization, and tumour status (primary or recurrent). The patient and tumour characteristics of both sample sets are listed in Table 1.

Table 1. Patients and tumour characteristics of the Dutch and French cohort. The subgroups includes tumours harbouring a T41A, S45F or a wild-type *CTNNB1* mutation

		Dutch cohort				French cohort			
		Total group (n = 64)		Subgroup (n = 61)		Total group (n = 128)		Subgroup (n = 93)	
Clinicopathological characteristics		Number of patients (%)				Number of patients (%)			
Sex	Male	23	(35.9%)	22	(36.1%)	45	35.2%	36	38.7%
	Female	41	(64.1%)	39	(63.9%)	79	61.7%	56	60.2%
	Unknown	-	-	-	-	4	3.1%	1	1.1%
Median age in years at diagnosis (minimum age – maximum age)		35	(4-79 years)	34	(4-79 years)	38	(1-74 years)[a]	37	(1-73 years) [b]
Mutation type	T41A	38	59.4%	38	(62.3%)	45	35.2%	45	(48.4%)
	S45F	12	18.8%	12	(19.7%)	34	26.6%	34	(36.6%)
	WT	11	17.2%	11	(18%)	14	10.9%	14	(15.1%)
	Other	3	4.7%*	-	-	12	9.4%**	-	
	Unknown	-	-	-	-	23	18%	-	
Tumour site	Extra-abdominal	38	59.4%	38	(62.3%)	86	67.2%	66	(71%)
	Abdominal wall	24	37.5%	21	(34.4%)	24	18.8%	20	(21.5%)
	Intra-abdominal	2	3.1%	2	(3.3%)	12	9.4%	6	(6.5%)
	Unknown	-	-	-	-	6	4.7%	1	(1.1%)
Median tumour size on radiology in mm (minimum size – maximum size		45	(10-135 mm) [c]	45	(10-135 mm)[d]	70	(5-300 mm)[e]	70	(5-300 mm)[f]

[a] n = 117, n = 11 missing value; [b] n = 90, n = 3 missing value; [c] n=47, n = 17 missing value; [d] n = 44, n = 17 missing value; [e] n = 107, n = 21 missing value; [f] n = 84, n = 9 missing value
** n = 3 133T>C (S45P); ** n = 6 133T>C (S45P), n = 1 c.113_149delCTAC; n=1 c.132_191del; n = 1 c.133delinsAAGG; n = 1 c.97_102del; n = 1 c.105_107del; n = 1 c.[98C>T(+)99T>G]*

RNA Isolation and RT-qPCR

Total RNA was extracted from two to four tissue sections (20 µm) of DTF FFPE tumour samples using the RecoverAll™ total nucleic acid isolation kit (Ambion/ Life Technologies) according to the manufacturer's recommendations. Total RNA quality and concentration were determined using a Nanodrop-2000 (Isogen Life Science). The 260nm/280nm ratio was ≥1.80 for all RNA preparations. The mRNA expression of *AXIN2*, *CCND1* and *DKK1* was assessed by RT-qPCR. In brief: 50 ng RNA was reverse transcribed with RevertAid™ H Minus (Thermo Fischer Scientific, EP0452) to generate cDNA. The resulting 4 µL cDNA was subsequently pre-amplified for 15 cycles in a final volume of 8 µL using Taqman PreAmp mastermix (Thermo Fisher Scientific) in combination with 100-fold diluted primer-probe combinations for *AXIN2*, *CCND1, Dickkopf 1* (*DKK1*) and Peptidylprolyl Isomerase A (*PPIA),* (Thermo Fisher Scientific; Assay Ids Hs00610344_m1, Hs00765553_m1, Hs00183740_m1 and Cat # 4333763F, respectively). Next, for each sample, four individual - gene transcript specific - real time PCRs were carried out in a Mx3000P (Agilent) for 40 cycles using SensiFast Probe Lo-ROX master mix (BioLine) according the manufacturer's recommendation and the same four primer-probe combinations. A 10 ng/µL down to 0.04 ng/µL serially diluted RNA sample isolated from MCF7 cells and expressing all targets was used in each experiment to monitor the efficiencies of the RT-qPCRs and a minus RT sample as a negative control. All targets were amplified with an equal efficiency (98%-110%). Finally, levels of the target genes were normalized on the stable expressed PPIA mRNA levels using the delta Cq normalization method.

Statistical analysis of AXIN2, CCND1 and DKK1 expression in WT and CTNNB1 DTF mutants

The relative expression values of *AXIN2*, *CCND1* and *DKK1* were measured by RT-PCR on FFPE samples, in the Dutch DTF cohort. Median values and the maximum interquartile range (IQR) were calculated. Because of non-normally distributed data, a Kruskal-Wallis test was performed to identify differences in ranked expression levels of *AXIN2, CNND1* and *DKK1* (p-value < 0.05, confidence interval 95%) between DTF with various *CTNNB1* mutation types (T41A, S45F) and DTF with no mutations in exon 3 of *CTNNB1* (WT). A Bonferroni correction was used to correct for multiple testing.

To validate the results of the RT-PCR data, the most variable probes of *AXIN2, CCND1* and *DKK1*, were selected based on the highest IQR using log2 transformed data of the total French cohort. Because of a non-normal distribution, a Kruskal-Wallis test was performed to identify differences in ranks of expression levels of *AXIN2, CNND1* and *DKK1* (p-value

< 0.05, confidence interval 95%) between DTF with various *CTNNB1* mutation types (T41A, S45F) and DTF with no mutations in exon 3 of *CTNNB1* (WT).

Selection of Wnt target genes

To rule out that the limited set of Wnt genes analysed so far caused a selection bias affecting the outcome of our analyses, the number of Wnt targets genes was increased. Wnt target genes used in a hierarchical unsupervised cluster analyses using, transcript expression data from DTF tumours, were selected using the mammalian Wnt target genes listed on the Wnt homepage (https://web.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes) as a reference [35]. Supplemental Table 1 summarizes the mammalian Wnt target genes that were selected, the corresponding Affymetrix probe sets and alias terms (https://www.ncbi.nlm.nih.gov/gene/). Since DTF tumours are of mesenchymal origin, they may express Wnt target genes partly different from those observed in epithelial cancers. Therefore, unsupervised hierarchical cluster analyses were repeated using a list of putative mesenchymal Wnt target genes in desmoids published by Denys et al [36]. Gene names, corresponding Affymetrix probe sets and alias terms are shown in Supplemental Table 2.

Hierarchical cluster analysis

For hierarchical cluster analysis, the retrieved normalized mRNA expression data of the 128 DTF samples of the French cohort [26] were log2 transformed to minimize outliers. Next, Wnt target genes were selected based on their names and alias terms as described in paragraph 2.4. In case of multiple probes representing a single gene, the probe with the highest variability, based on the maximum IQR, using the entire dataset, was selected for further analyses. An unsupervised hierarchical cluster analysis was performed using median centred expression data with Cluster 3.0 for Windows (Human Genome Center, University of Tokyo, 2002) and Java Treeview, version 1.1.6rv, for visualization. The clustering was based on the centred correlation as a distance metric using average linkage.

Ethical approval

This study was part of a protocol entitled "Translational research on soft tissue sarcomas" which was assessed by the Medical Ethics Committee of the Erasmus MC, Rotterdam, the Netherlands (MEC-2016-213).

Results

No difference in AXIN2, CCND1 and DKK1 expression between wild-type and CTNNB1 mutated desmoids

To examine whether different *CTNNB1* mutations in desmoid tumours cause differential expression of Wnt target genes, the transcript levels of *AXIN2*, *CCND1* and *DKK1* were determined by RT-PCR. In total, 64 FFPE samples from the Dutch cohort were available for the RT-PCR analysis. The cohort included 23 males (35.9%) and 41 females (64.1%). Mutational status was as follows; 11 tumours (17.2 %) were WT for exon 3, 38 (59.4%) tumours harboured a T41A mutation, 12 (18.8%) tumours harboured a S45F mutation and 3 (4.7%) tumours harboured a S45P mutation. Because of the low prevalence, the desmoids with the S45P mutation were not included in the RT-PCR analysis leaving a total of 61 patients. The tumour and patient characteristics are summarized in Table 1. The relative expression values of *AXIN2*, *CCND1* and *DDK1* transcripts were measured and the median expression levels and the maximum IQR were calculated (Table 2). A Kruskal-Wallis test shows that the mean rank of the relative expression levels was not statistically significantly different between groups for *AXIN2* and *DKK1* (Table 2). The mean rank of the relative expression level for *CCND1* showed a significant difference between groups, however failed significance after Bonferroni correction for multiple testing between mutation groups (Table 2). A dot plot depicting the relative expression levels of individual samples of AXIN2, CCND1 and DKK1 is shown in Figure 1.

Table 2. Relative expression values of Wnt target genes *AXIN2*, *CCND1* and *DKK1* for the Dutch and French subgroups (tumours harbouring a T41A, S45F or wild-type *CTNNB1* gene mutation

A. Dutch cohort, subgroup data (n = 61) [a,b]		**Median (IQR)**	**p-value c**
	AXIN2	1.48 (1.12)	0.273
	CCND1	0.61 (0.86)	0.029 [d]
	DKK1	0.02 (0.04)	0.319
B. French cohort, subgroup data (n = 93)[a]		**Median using log2 transformed data (IQR)**	**p-value [c]**
	AXIN2 (224498_x_at)	4.80 (1.48)	0.058
	*CCND1 (*208711_s_at)	5.89 (0.80)	0.486
	DKK1 (204602_at)	5.20 (1.17)	0.652

[a] T41A, S45F and wild-type CTNNB1 tumours included; [b] compared to house keeper gene Peptidylprolyl Isomerase A; [c] calculated p-values using the Kruskal-Wallis test; [d] non-significant after Bonferroni correction for multiple testing. IQR, interquartile range; AXIN2, Axin 2; CCND1, Cyclin D1; DKK1, Dickkopf1; CI, confidence interval

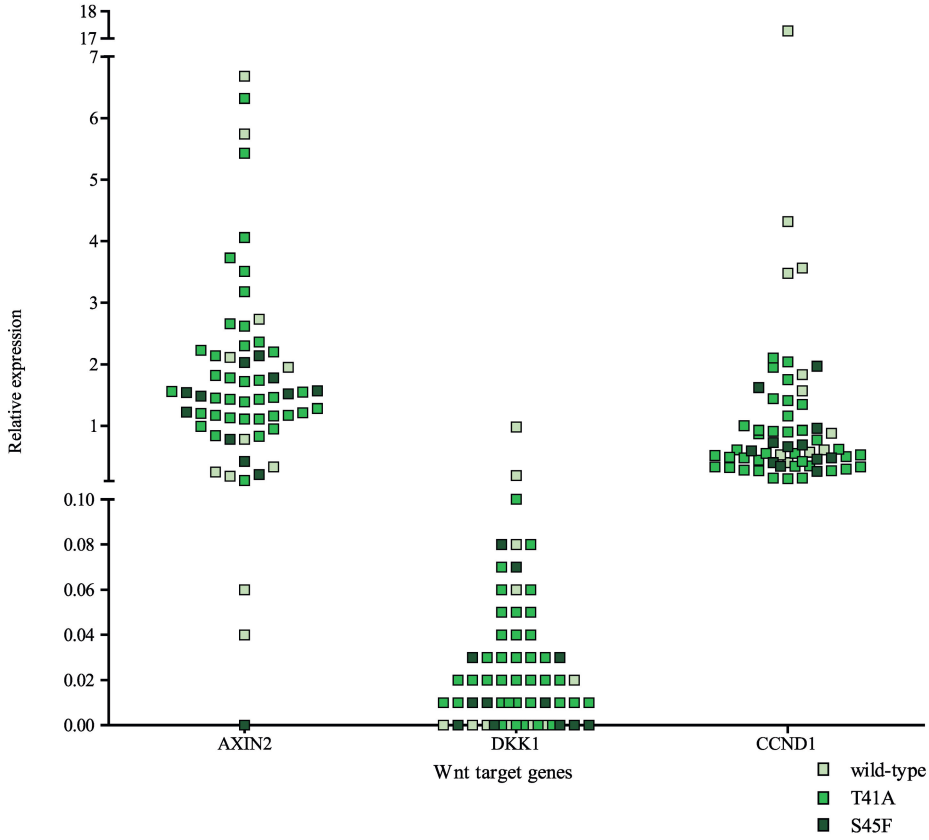

Figure 1. Dot plot depicting the individual data relative expression levels measured with realtime qPCR of Wnt target genes AXIN2, DKK1 and CCND1 in DTF harbouring either a S45F or T41A CTNNB1 mutation or wild-type DTF.

Next, mRNA expression data from the French cohort were used to validate these findings. The patient and tumour characteristics of this cohort are listed in Table 1. Ninety-three desmoid samples; *CTNNB1* WT (n = 14); T41A (n = 45) and S45F (n = 34) were selected for subsequent analysis. First, the Affymetrix probe sets for *AXIN2 (222696_at, 224176_at, 222695_s_at, 224498_x_at*) *CCND1* (208712_at, 208711_s_at) and *DKK1* (204602_at) were identified and the probes that displayed the most variable expression values after log 2 transformation, based on the highest IQR, were selected for further analysis (*224498_x_at*, 208711_s_at and 204602_at). A Kruskal-Wallis test showed no significantly differences between the mean ranks of the groups with T41A or S45F mutations and WT *CTNNB1* (Table 2).

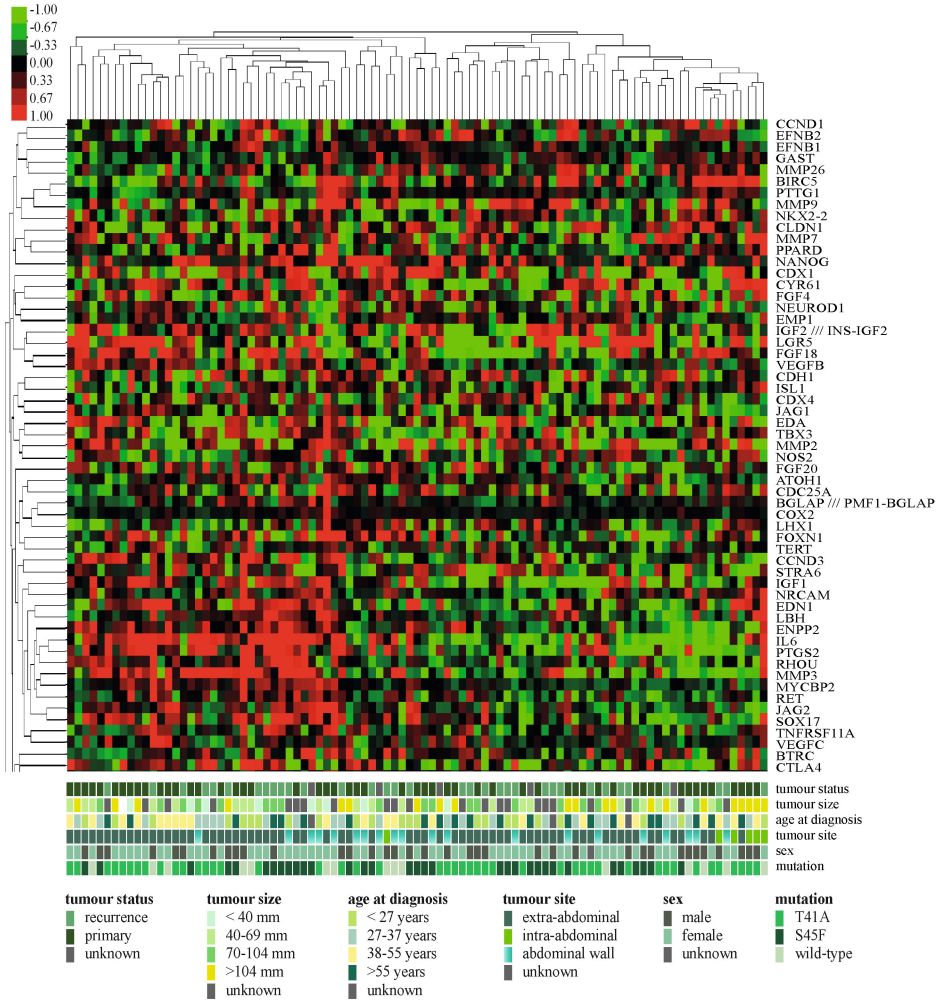

Figure 2A (part 1). Hierarchical clustering based on Wnt target mRNA expression in desmoid tumours does not discriminate wild-type and CTNNB1 S45F and T41A mutants. Heat map depicting an unsupervised hierarchical cluster analysis of 93 desmoid-type fibromatosis samples harbouring T41A or S45F CTNNB1 mutations and wild-type tumours using the mRNA expression levels of selected mammalian Wnt target genes. Red and green represent relative high and low expresion levels, respectively. Tumour status (primary or recurrent); tumour size (<40 mm, 40-69 mm, 70-104 mm, >104 mm); age at diagnosis presented in categories based on the median and the 25th and 75th percentiles (age < 27 years, 27-37 years 38-55 years and > 55 years); tumour site (intra-abdominal, extra-abdominal and abdominal wall); sex and CTNNB1 mutation status are indicated.

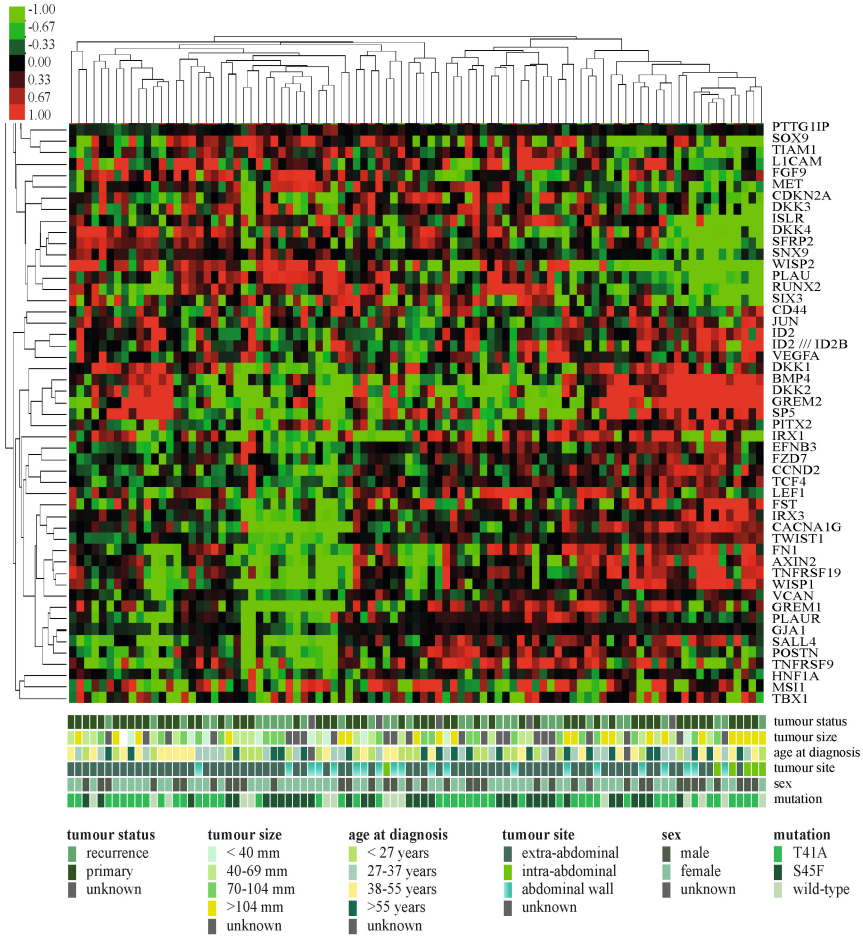

Figure 2A (part 2). Hierarchical clustering based on Wnt target mRNA expression in desmoid tumours does not discriminate wild-type and CTNNB1 S45F and T41A mutants. Heat map depicting an unsupervised hierarchical cluster analysis of 93 desmoid-type fibromatosis samples harbouring T41A or S45F CTNNB1 mutations and wild-type tumours using the mRNA expression levels of selected mammalian Wnt target genes. Red and green represent relative high and low expresion levels, respectively. Tumour status (primary or recurrent); tumour size (<40 mm, 40-69 mm, 70-104 mm, >104 mm); age at diagnosis presented in categories based on the median and the 25th and 75th percentiles (age < 27 years, 27-37 years 38-55 years and > 55 years); tumour site (intra-abdominal, extra-abdominal and abdominal wall); sex and CTNNB1 mutation status are indicated.

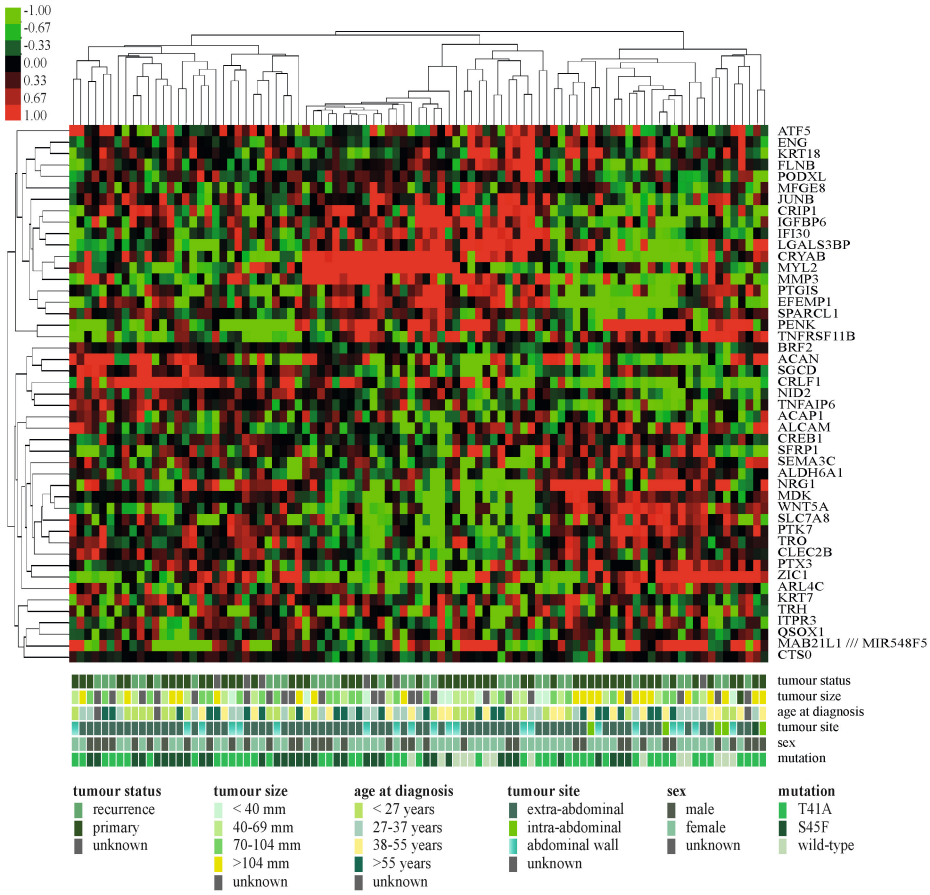

Figure 2B. Heat map depicting an unsupervised hierarchical cluster analysis of 93 desmoid-type fibromatosis samples harbouring T41A, S45F CTNNB1 mutations and wildtype tumours using selected the mRNA expression levels of a set of putative mesenchymal Wnt target genes. Red and green represent relatively high and low expression levels, respectively. Tumour status (primary or recurrent); tumours size (<40 mm, 40-69 mm, 70-104 mm, >104 mm); age at diagnosis presented in categories based on the median and the 25th and 75th percentiles (age < 27 years, 27-37 years 38-55 years and > 55 years); tumour site (intra-abdominal, extra-abdominal and abdominal wall); sex and CTNNB1 mutation status are indicated.

Hierarchical cluster analyses based on Wnt target gene expression does not discriminate between wild-type and CTNNB1 mutant desmoids

The total dataset contained 54613 probes, of which 107 probes were selected as mammalian Wnt targets and 47 were selected as mesenchymal Wnt targets. The hierarchical clustering using mammalian Wnt targets (Figure 2A) did not discriminate WT and mutated DTF (T41A and S45F) tumours, nor showed a discriminative pattern based on sex, age at the time of diagnosis, tumour size or tumour status (primary or recurrent tumour). An additional hierarchical clustering, using expression data from all 128 desmoid samples (including WT tumours as well as T41A, S45F, S45P mutated tumours, rare mutations classified as "other", and tumours with an unknown mutation type), showed similar results (Supplemental Figure 1A). Cluster analyses using putative mesenchymal Wnt targets [36] in a subgroup containing samples that are *CTNNB1* WT, T41A and S45F (Figure 2B) and the cluster analysis using all samples (Supplemental Figure 1B), also showed that the desmoid samples did not cluster according to *CTNNB1* mutational status, neither did the observed cluster appear to be driven by clinicopathological parameters like sex, age at time of diagnosis, tumour size or tumour status (primary or recurrent tumour).

Discussion

This study evaluated whether the potentially more aggressive clinical behaviour of S45F mutated DTF, compared to T41A mutants and WT desmoids, could be explained by a differential Wnt signalling activity. As aberrant Wnt/β-catenin signalling plays a pivotal role in the initiation and development of DTF, variable activity of Wnt signalling may affect cancer-related biological processes like cancer stem cell maintenance and invasiveness in a different manner [37-39]. We therefore examined and compared Wnt target expression levels using different independent DTF sample sets that included DTF designated as WT as well as T41A and S45F *CTNNB1* mutants. No evidence was found that WT tumours, T41A and S45F tumours have a differential activation of Wnt/β-catenin signalling. This finding contrasts with Hamada et al. who reported a significant increase of *AXIN2*, *CCND1* and *c-MYC* transcript levels in S45F DTF isolated single cell lines compared to human skin fibroblasts and WT and T41A cells [33].

A study conducted by Meneghello et al. showed upregulated (two to six fold) mRNA expression of *AXIN2* measured by RT-PCR but also reported that desmoid cells had downregulated mRNA expression of *CCND1* (two-four fold) when comparing DTF samples with connective tissue of non-desmoid patients [17]. In addition, Jilong et al. compared β-catenin

mutated tumours with WT tumours and found that *CCND1* was expressed more frequently in β-catenin mutated DTF [16]. Saito et al. found a statistically significant correlation between nuclear staining of β-catenin and *CCND1* overexpression [16, 40]. Although the literature on Wnt target expression in desmoids is limited and somewhat contradictory, both AXIN2 and CCND1 seem to be overexpressed in DTF. Interestingly, by studying genotype-phenotype correlations of *CTNNB1* mutations in liver cancer, others have found that T41 mutations were associated with moderate activity whereas S45 mutations led to a weak β-catenin activation which could be compensated by S45 mutant allele duplication [29].

None of our hierarchical cluster analyses, based on the expression of different Wnt target gene sets, discriminated *CTNNB1* mutated (S45 and T41) from WT DTF samples, neither were S45F and T41A clearly separated. A possible confounder in our analyses is that the WT group, now defined by the absence of mutations in exon 3 of *CTNNB1*, needs to be screened more thoroughly for *CTNNB1* mutations. It was demonstrated that a fair part of the DTF, designated as WT by Sanger sequencing, contain low frequency *CTNNB1* mutations that are only detected by whole exome sequencing or contain novel intra-genic deletions of the *CTNNB1* gene [19, 41]. Moreover, in the WT group, other genomic alterations occurred like *APC* loss, chromosome 6 loss and *BMI1* mutations which are all linked to Wnt/β-catenin activation [19]. In our analyses, we could not distinguish between WT and S45F/T41A mutant DTF corresponding to observations of Crago et al. [19]. In contrast, Colombo et al. reported that *CTNNB1*-mutated and WT DTF had different gene expression profiles [42]. In our cluster analyses, distinct clusters of samples were discerned based on the expression levels of putative Wnt target genes that were not related to *CTNNB1* mutational status. Unfortunately, we were not able to identify the origin of the cluster pattern as it could not be explained by factors like sex, age at the time of diagnosis, tumour size and tumour status (primary or recurrent tumour). Other clinicopathological factors, not included in this study, for example immune cell infiltration could potentially explain the observed cluster pattern and could be the subject of future studies. Interestingly in this respect is a recent publication from Colombo et al. describing that T41A and S45F mutated DTF displayed a different expression of inflammation related genes [42].

No indications were found in this study for a differential Wnt/β-catenin signalling activity between the T41A and S45F mutants thereby failing to explain the reported different clinical behaviour of the two DTF mutant groups. A more thorough investigation into the molecular consequences of different *CTNNB1* mutations is necessary, focusing on protein-protein interactions and identifying their genomic binding sites. Irrespective of the specific *CTNNB1*

mutation present in DTF, it is clear that Wnt/β-catenin signalling is activated which plays an essential role in carcinogenesis. Inhibition of this pathway may provide a vulnerability in DTF that can therapeutically be exploited. However, drugs should operate at the level of β-catenin, interacting with transcription co-factors or target specific key downstream factors to avoid serious adverse effects [43, 44]. Additionally, other signalling pathways like Notch1 and Hedgehog have also been implicated to play a role in DTF development and may be therapeutically targeted by small molecules [45, 46]. Future studies are needed to gain more insight into the role of these additional signalling pathways, the cross-talks between each pathway and their contribution to the DTF tumorigenesis.

Conclusions

Our study demonstrated no difference between either WT, S45F or T41A mutated DTF tumours regarding the investigated Wnt target gene expression levels. Apparently a differential Wnt/β-catenin signalling activity does not determine the observed differences in clinical behaviour between S45F and T41A DTF mutants.

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Supplemental Table 1. Selection of putative mammalian Wnt targets. Selected probes for the cluster analysis are marked by an asterisk (*)

Gene name	Gene title	Alias terms	Affymetrix Code
ATOH1	atonal bHLH transcription factor 1	ATH1; HATH1; MATH-1; bHLHa14	221336_at*
AXIN2	axin 2	AXIL; ODCRCS	224498_x_at*; 222695_s_at; 222696_at; 224176_s_at;
BGP	bone gamma-carboxyglutamate (gla) protein /// PMF1-BGLAP readthrough	OC; OCN, osteocalcin	206956_at*
BIRC5	baculoviral IAP repeat containing 5	Survivin, API4; EPR-1	202095_s_at*; 1555826_at; 202094_at; 210334_x_at;
BMP4	bone morphogenetic protein 4	ZYME; BMP2B; OFC11; BMP2B1; MCOPS6	211518_s_at*
BTRCP	beta-transducin repeat containing E3 ubiquitin protein ligase	FWD1; FBW1A; FBXW1; beta TrCP; FBXW1A; bTrCP1; betaTrCP; BETA-TRCP	1563620_at*; 204901_at 216091_s_at; 222374_at; 224471_s_at
CACNA1G	calcium channel, voltage-dependent, T type, alpha 1G subunit	NBR13; SCA42; Cav3.1; Ca(V) T.1	207869_s_at*; 210380_s_at; 211314_at; 211315_s_at; 211802_x_at
CCND	cyclin D1	BCL1; PRAD1; U21B31; D11S287E	208711_s_at*;208712_at;
	cyclin D2	No alias terms	200951_s_at*; 200952_s_at 200953_s_at; 231259_s_at
	cyclin D3	No alias terms	1562028_at*; 201700_at
CD44	CD44 molecule	IN; LHR; MC56; MDU2; MDU3; MIC4; Pgp1; CDW44; CSPG8; HCELL; HUTCH-I; ECMR-III	216056_at*; 1557905_s_at; 1565868_at; 204489_s_at; 204490_s_at; 209835_x_at; 210916_s_at; 212014_x_at; 212063_at; 217523_at; 229221_at; 234411_x_at; 234418_x_at
CDC25	cell division cycle 25A	CDC25A2, CDC25C cell division cycle 25C; PPP1R60	204696_s_at*; 1555772_a_at; 204695_at
CDH1	cadherin 1, type 1	E-cadherin; cadherin 1, UVO; CDHE; ECAD; LCAM; Arc-1; BCDS1; CD324	201130_s_at*; 201131_s_at
CDKN2A	cyclin-dependent kinase inhibitor 2A	ARF; MLM; P14; P16; P19; CMM2; INK4; MTS1; TP16; CDK4I; CDKN2; INK4A; MTS-1; P14ARF; P19ARF; P16INK4; P16INK4A; P16-INK4A	209644_x_at*; 207039_at 211156_at
CDX1	caudal type homeobox 1	No alias terms	206430_at*
CDX4	caudal type homeobox 4	No alias terms	221340_at*
CLD1	claudin 1	CLDN1; SEMP1; ILVASC	218182_s_at*; 222549_at

Supplemental Table 1. *(continued)*

Gene name	Gene title	Alias terms	Affymetrix Code
COX2	cytochrome c oxidase subunit II	No alias terms	1553569_at*; 1553570_x_at
CTLA4	cytotoxic T-lymphocyte-associated protein 4	CD; GSE; GRD4; ALPS5; CD152; CTLA-4; IDDM12; CELIAC3	221331_x_at*; 231794_at 234362_s_at; 234895_at; 236341_at;
CCN1/ Cyr61	cysteine-rich, angiogenic inducer, 61	CCN1; GIG1; IGFBP10	210764_s_at*; 201289_at
Dickkopf	dickkopf WNT signalling pathway inhibitor 1	DKK1; SK; DKK-1,	204602_at*;
	dickkopf WNT signalling pathway inhibitor 2	DKK2; DKK-2	219908_at*; 224199_at
	dickkopf WNT signalling pathway inhibitor 3	DKK3; RIG; REIC,	202196_s_at*; 214247_s_at 221126_at; 221127_s_at 230508_at;
	dickkopf WNT signalling pathway inhibitor 4	DKK4	206619_at*;
EDA	ectodysplasin A	ED1; HED; EDA1; EDA2; HED1; ODT1; XHED; ECTD1; XLHED; ED1-A1; ED1-A2; EDA-A1; EDA-A2; TNLG7C; STHAGX1	211128_at*; 206217_at; 211127_x_at; 211129_x_at; 211130_x_at; 211131_s_at
EDN1	endothelin 1	ET1; QME; PPET1; ARCND3; HDLCQ7	218995_s_at*; 1564630_at; 222802_at
EFNB	ephrin-B1	CFND; CFNS; EFB1; EFL3; EPLG2; Elk-L; LERK2	202711_at*
	ephrin-B2	EFNB2 HTKL; EPLG5; Htk-L; LERK5	202669_s_at*; 202668_at
	ephrin-B3	No alias terms	205031_at*; 210883_x_at
EMP1	epithelial membrane protein 1	No alias terms	234233_s_at*; 1564796_at; 201324_at; 201325_s_at; 213895_at; 229011_at
ENPP2	ectonucleotide pyrophosphatase/ phosphodiesterase 2	ATX; NPP2; ATX-X; PDNP2; LysoPLD; AUTOTAXIN; PD-IALPHA	210839_s_at*; 209392_at
FGF18	fibroblast growth factor 18	ZFGF5; FGF-18	206987_x_at*; 206986_at; 211029_x_at; 211485_s_at; 214284_s_at; 231382_at;
FGF20	fibroblast growth factor 20	RHDA2; FGF-20	220394_at*
FGF4	fibroblast growth factor 4	HST; KFGF; FGF-4; HST-1; HSTF1; K-FGF; HBGF-4; HSTF-1	1552982_a_at*; 206783_at

Supplemental Table 1. *(continued)*

Gene name	Gene title	Alias terms	Affymetrix Code
FGF9	fibroblast growth factor 9	GAF; FGF-9; SYNS3; HBFG-9; HBGF-9,	239178_at*; 206404_at
FN	fibronectin	fibronectin 1;FN; CIG; FNZ; MSF; ED-B; FINC; GFND; LETS; GFND2; SMDCF	214701_s_at*; 1558199_at; 210495_x_at; 211719_x_at; 212464_s_at; 214702_at; 216442_x_at;
FOXN1	forkhead box N1	WHN; RONU; FKHL20	207683_at*; 1558687_a_at;
FST	follistatin	FS	204948_s_at*; 207345_at 226847_at
FZD7	frizzled class receptor 7	FzE3	203706_s_at*; 203705_s_at
GAST	gastrin	GAS	208138_at*
GJA1	gap junction protein alpha 1	Connexin 43; CX 43, HSS; CMDR; CX43; EKVP; GJAL; ODDD; AVSD3; EKVP3; HLHS1; PPKCA	201667_at*
GREM	gremlin 1, DAN family BMP antagonist	GREM1; DRM; HMPS; MPSH; PIG2; CRAC1;	218468_s_at*; 218469_at
	gremlin 2, DAN family BMP antagonist	CRCS4; DAND2; HMPS1; IHG-2; DUP15q; C15DUPq; GREMLIN; CKTSF1B1	240509_s_at*; 220794_at 235504_at
Tcf-1	HNF1 homeobox A	HNF1A; HNF1; LFB1; TCF1; HNF4A; MODY3; TCF-1; HNF-1A; IDDM2	210515_at*; 216930_at
ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	GIG8; ID2A; ID2H; bHLHb26;	201566_x_at*; 201565_s_at
ID2 /// ID2B	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein /// inhibitor of DNA binding 2B, dominant negative helix-loop-helix protein (pseudogene)	No alias terms	213931_at*
IGF1	insulin-like growth factor 1 (somatomedin C)	MGF; IGFI; IGF-I	209540_at*; 209541_at; 209542_x_at; 211577_s_at; 241072_s_at
IGF2 /// INS-IGF2	insulin-like growth factor 2 /// INS-IGF2 readthrough	GRDF; IGF-II; PP9974; C11orf43	210881_s_at* 202409_at 202410_x_at
IL6	interleukin 6	CDF; HGF; HSF; BSF2; IL-6; BSF-2; IFNB2; IFN-beta-2	205207_at*
IRX1	iroquois homeobox 1	IRX-1; IRXB1	230472_at*

Supplemental Table 1. *(continued)*

Gene name	Gene title	Alias terms	Affymetrix Code
IRX3	iroquois homeobox 3	IRX-3	229638_at*
ISL1	ISL LIM homeobox 1	Isl-1; ISLET1	206104_at*
LHX1	LIM homeobox 1	No alias terms	206230_at*
ISLR	immunoglobulin superfamily containing leucine-rich repeat	Meflin; HsT17563	207191_s_at*
JAG1	jagged 1	AGS; AHD; AWS; HJ1; AGS1; CD339; JAGL1	229924_s_at*; 209097_s_at; 209098_s_at; 209099_x_at; 216268_s_at
JAG2	jagged 2	HJ2; SER2	32137_at*; 209784_s_at
C-JUN	jun proto-oncogene	AP-1 transcription factor subunit ,AP1; p39; AP-1;	201465_s_at*; 201464_x_at; 201466_s_at; 213281_at;
L1CAM	L1 cell adhesion molecule	S10; HSAS; MASA; MIC5; SPG1; CAML1; CD171; HSAS1; N-CAML1; NCAM-L1; N-CAM-L1	204584_at*; 204585_s_at
LBH	limb bud and heart development	No alias terms	221011_s_at*
LEF1	lymphoid enhancer-binding factor 1	LEF-1; TCF10; TCF7L3; TCF1ALPHA	221557_s_at*; 210948_s_at; 221558_s_at
LGR5/ GPR49	leucine-rich repeat containing G protein-coupled receptor 5	FEX; HG38; GPR49; GPR67; GRP49	213880_at*; 210393_at
MET	MET proto-oncogene, receptor tyrosine kinase	HGFR; AUTS9; RCCP2; c-Met; DFNB97	203510_at*; 211599_x_at; 213807_x_at; 213816_s_at
MMP2	matrix metallopeptidase 2	CLG4; MONA; CLG4A; MMP-2; TBE-1; MMP-II	1566677_at*; 1566678_at; 201069_at
MMP26	matrix metallopeptidase 26	No alias terms	220541_at*
MMP3	matrix metallopeptidase 3	Stromelysin, SL-1; STMY; STR1; CHDS6; MMP-3; STMY1	205828_at*
MMP7	matrix metallopeptidase 7	MPSL1; PUMP-1	204259_at*
MMP9	matrix metallopeptidase 9	GELB; CLG4B; MMP-9; MANDP2	203936_s_at*
MSI1	musashi RNA binding protein 1	No alias terms	206333_at*
MYCBP2	MYC binding protein 2, E3 ubiquitin protein ligase	No alias terms	201959_s_at; 1557370_s_at; 201960_s_at
NANOG	Nanog homeobox	No alias terms	231079_at*; 220184_at
NEUROD1	neuronal differentiation 1	BETA2; BHF-1; MODY6; NEUROD; bHLHa3	206282_at*; 1556057_s_at

Supplemental Table 1. *(continued)*

Gene name	Gene title	Alias terms	Affymetrix Code
NEUROG1	neurogenin 1	AKA; ngn1; Math4C; bHLHa6; NEUROD3	208497_x_at*
NKX2-2	NK2 homeobox 2	NKX2B; NKX2.2	206915_at*
NOS2	nitric oxide synthase 2, inducible	NOS2, NOS; INOS; NOS2A; HEP-NOS	210037_s_at*
NRCAM	neuronal cell adhesion molecule	Nr-CAM	204105_s_at*; 216959_x_at
PITX2	paired-like homeodomain 2	RS; RGS; ARP1; Brx1; IDG2; IGDS; IHG2; PTX2; RIEG; ASGD4; IGDS2; IRID2; Otlx2; RIEG1	207558_s_at*
PLAU	plasminogen activator, urokinase	urokinase receptor , CD87; UPAR; URKR; U-PAR	211668_s_at*; 205479_s_at
PLAUR	plasminogen activator, urokinase receptor	urokinase receptor , CD87; UPAR; URKR; U-PAR	211924_s_at*; 210845_s_at; 214866_at
POSTN	periostin, osteoblast specific factor	PN; OSF2; OSF-2; PDLPOSTN	214981_at*; 1555777_at; 1555778_a_at; 210809_s_at;
PPARD	peroxisome proliferator-activated receptor delta	FAAR; NUC1; NUCI; NR1C2; NUCII; PPARB	242218_at*; 208044_s_at; 210636_at; 37152_at
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	COX2; COX-2; PHS-2; PGG/HS; PGHS-2; hCox-2; GRIPGHS	204748_at*; 1554997_a_at
PTTG1	pituitary tumor-transforming 1	EAP1; PTTG; HPTTG; TUTR1	203554_x_at*
PTTG1IP	pituitary tumor-transforming 1 interacting protein	EAP1; PTTG; HPTTG; TUTR1	200677_at*
RET	ret proto-oncogene	PTC; MTC1; HSCR1; MEN2A; MEN2B; CDHF12; CDHR16; RET-ELE1	211421_s_at*; 205879_x_at; 215771_x_at
RHOU	ras homolog family member U	ARHU; G28K; WRCH1; hG28K; CDC42L1	223169_s_at*; 223168_at
RUNX2	runt-related transcription factor 2	CCD; AML3; CCD1; CLCD; OSF2; CBFA1; OSF-2; PEA2aA; PEBP2aA; CBF-alpha-1	236859_at*; 216994_s_at; 221282_x_at; 221283_at; 232231_at; 236858_s_at
SALL4	spalt-like transcription factor 4	DRRS; HSAL4; ZNF797	229661_at*
SFRP2	secreted frizzled-related protein 2	FRP-2; SARP1; SDF-5	223121_s_at*; 223122_s_at
SIX3	SIX homeobox 3	HPE2	242054_s_at*; 206634_at 244288_s_at

Supplemental Table 1. *(continued)*

Gene name	Gene title	Alias terms	Affymetrix Code
SNX9	sorting nexin 9	SDP1; WISP; SH3PX1; SH3PXD3A	223027_at*; 223028_s_at
SOX17	SRY box 17	VUR3; SRY-box 17 SOX 17	219993_at*; 230943_at
SOX9	SRY box 9	CMD1; SRA1; CMPD1; SRXX2; SRXY10, SRY-box 9	202936_s_at*; 202935_s_at
SP5	Sp5 transcription factor	No alias terms	235845_at*
STRA6	stimulated by retinoic acid 6	MCOPS9; MCOPCB8; PP14296	1569334_at*; 1569335_a_at; 221701_s_at
TBX1	T-box 1	Brachyury (Tbox1); TBX1 T-box 1; DGS; TGA; VCF; CAFS; CTHM; DGCR; DORV; VCFS; TBX1C; CATCH22	211274_at*; 207662_at; 211273_s_at; 236926_at; 242941_x_at
TBX3	T-box 3	TBX3; UMS; XHL; TBX3-ISO	229565_x_at*; 219682_s_at; 222917_s_at; 225544_at; 228344_s_at; 229576_s_at; 243235_at
TCF4	transcription factor 4	ITF2; PTHS; SEF2; FECD3; ITF-2; SEF-2; TCF-4; SEF2-1; SEF2-1A; SEF2-1B; SEF2-1D; bHLHb19	228837_at*; 203753_at; 212382_at; 212385_at; 212386_at; 212387_at; 213891_s_at; 222146_s_at
TERT	telomerase reverse transcriptase	TP2; TRT; CMM9; EST2; TCS1; hTRT; DKCA2; DKCB4; hEST2; PFBMFT1	1555271_a_at*; 207199_at
TIAM1	T-cell lymphoma invasion and metastasis 1	TIAM-1	213135_at*; 206409_at
TNFRSF11A	tumor necrosis factor receptor superfamily, member 11a, NFKB activator	RANK ligand; FEO; OFE; ODFR; OSTS; PDB2; RANK; CD265; OPTB7; TRANCER; LOH18CR1	207037_at*; 238846_at
TNFRSF19	tumor necrosis factor receptor superfamily, member 19	TAJ; TROY; TRADE; TAJ-alpha	224090_s_at*; 223827_at; 227812_at
TNFRSF9	tumor necrosis factor receptor superfamily, member 9	ILA; 4-1BB; CD137; CDw137; TNF family 41BB ligand	238950_at*; 207536_s_at 211786_at
TWIST1	twist family bHLH transcription factor 1	CRS; CSO; SCS; ACS3; CRS1; BPES2; BPES3; SWCOS; TWIST; bHLHa38	213943_at*
VCAN	versican	WGN; ERVR; GHAP; PG-M; WGN1; CSPG2	211571_s_at*; 204619_s_at 204620_s_at; 215646_s_at; 221731_x_at
VEGF	vascular endothelial growth factor A	VPF; MVCD1	211527_x_at*; 210512_s_at; 210513_s_at; 212171_x_at

Supplemental Table 1. *(continued)*

Gene name	Gene title	Alias terms	Affymetrix Code
	vascular endothelial growth factor B	VEGFB	203683_s_at*
	vascular endothelial growth factor C	vascular endothelial growth factor C, VRP; Flt4-L; LMPH1D	209946_at*
WISP	WNT1 inducible signaling pathway protein 1	CCN4; WISP1c; WISP1i; WISP1tc; WISP1-OT1; WISP1-UT1	211312_s_at*; 206796_at 229802_at; 235821_at
WISP	WNT1 inducible signalling pathway protein 2	CCN5; CT58; CTGF-L	205792_at*

Supplemental Table 2. Selection of putative mesenchymal Wnt targets. Selected probes for the cluster analysis are marked by an asterisk (*)

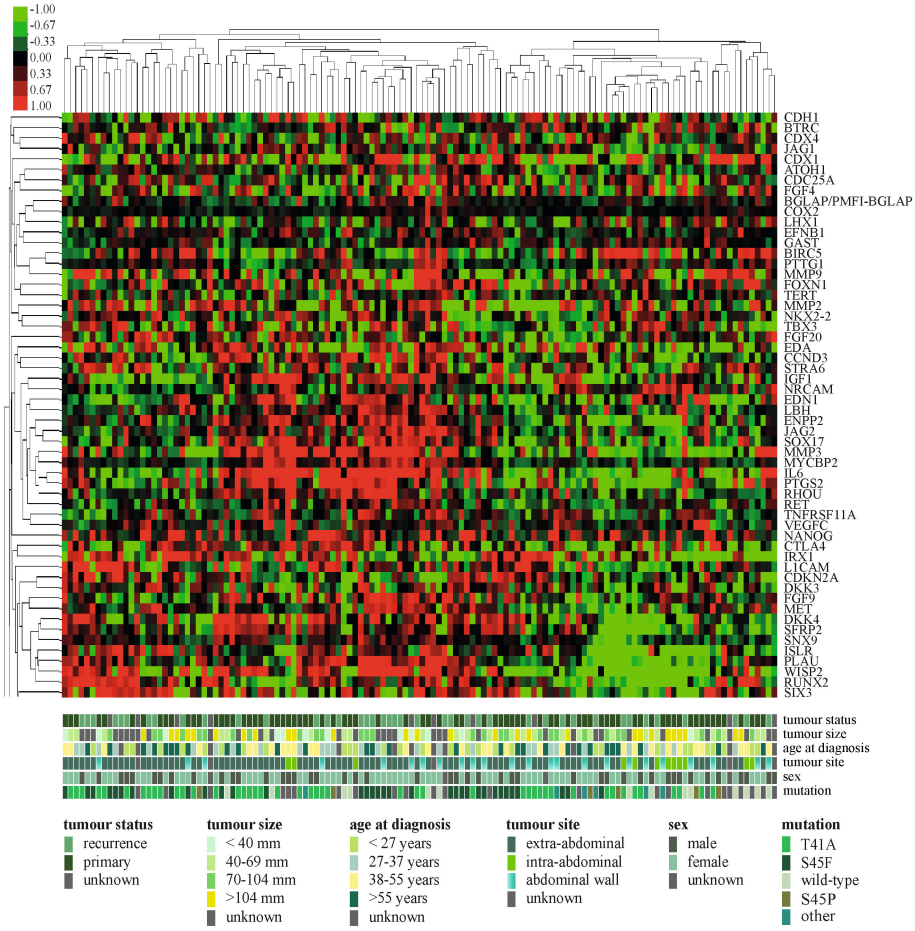
Gene name	Gene Title	Alias terms	Affymetrix Code
ACAN	aggrecan	AGC1; SEDK; AGCAN; CSPG1; MSK16; CSPGCP; SSOAOD	205679_x_at*; 1554950_at; 207692_s_at; 217161_x_at;
ACAP1	ArfGAP with coiled-coil, ankyrin repeat and PH domains 1	No alias terms	205212_s_at*
ALCAM	activated leukocyte cell adhesion molecule	MEMD; CD166	1569362_at*; 201951_at; 201952_at
ALDH6A1	aldehyde dehydrogenase 6	MMSDH; MMSADHA ALDH6A1	204289_at*; 204290_s_at; 221588_x_at; 221589_s_at; 221590_s_at
ARL4C	ADP-ribosylation factor like GTPase 4C	No alias terms	213759_at*; 202206_at; 202207_at; 202208_s_at
ATF5	activating transcription factor 5	ATFX; HMFN0395	230938_x_at*; 204998_s_at; 204999_s_at; 217389_s_at
BRF2	butyrate response factor 2	BRFU; TFIIIB50, BRF2 BRF2, RNA polymerase III transcription initiation factor subunit	218954_s_at*; 218955_at
CLEC2B	C-type lectin domain family 2, member B	CLEC2B C-type lectin domain family 2 member B; AICL; IFNRG1; CLECSF2; HP10085	209732_at*
CREB1	cAMP responsive element binding protein 1	No alias terms	225565_at*; 204312_x_at; 204313_s_at; 204314_s_at; 214513_s_at; 225572_at; 237289_at
CRIP1	cysteine-rich protein 1 (intestinal)	CRHP; CRIP; CRP1; CRP-1; CRIP1 cysteine rich protein 1	205081_at*
CRLF1	cytokine receptor-like factor 1	CLF; NR6; CISS; CISS1; CLF-1; zcytor5, CRLF1 cytokine receptor like factor 1	206315_at*
CRYAB	crystallin alpha B	MFM2; CRYA2; CTPP2; HSPB5; CMD1II; CTRCT16; HEL-S-101; CRYAB crystallin alpha B	209283_at*
CTS	cathepsin	CTSK; cathepsin K; CTSO; PKND; PYCD; CTS02; CTSO1; CTSO2	203758_at*
EFEMP1	EGF containing fibulin-like extracellular matrix protein 1	DHRD; DRAD; FBNL; MLVT; MTLV; S1-5; FBLN3; FIBL-3	201843_s_at*; 201842_s_at; 228421_s_at
ENG	endoglin	END; HHT1; ORW1	201809_s_at*; 201808_s_at

Supplemental Table 2. *(continued)*

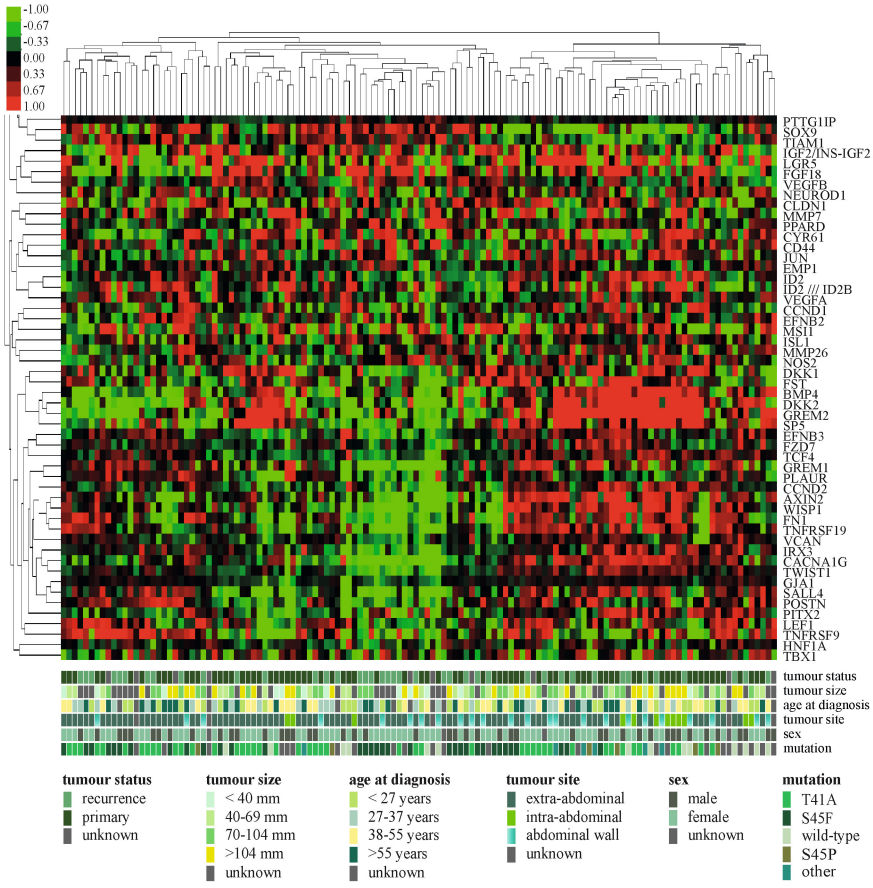
Gene name	Gene Title	Alias terms	Affymetrix Code
FLNB	filamin B, beta	AOI; FH1; SCT; TAP; LRS1; TABP; FLN-B; FLN1L; ABP-278; ABP-280 FLLNB; filamin B	208614_s_at*; 208613_s_at
IFI30	interferon, gamma-inducible protein 30	No alias terms	201422_at*
IGFBP6	insulin like growth factor binding protein 6	IBP6	203851_at*
ITPR3	inositol 1,4,5-trisphosphate receptor, type 3	IP3R; IP3R3, ITPR3 inositol 1,4,5-trisphosphate receptor type 3	239542_at*; 201187_s_at; 201188_s_at; 201189_s_at;
JUNB	jun B proto-oncogene	AP-1; JUNB ; AP-1 transcription factor subunit	201473_at*
KRT18	keratin 18, type I	K18; CK-18; CYK18	201596_x_at*
KRT7	keratin 7, type II	K7; CK7; SCL; K2C7	1558394_s_at*; 1558393_at; 209016_s_at
LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein	galectin 3 binding protein; 90K; M2BP; gp90; CyCAP; BTBD17B; MAC-2-BP; TANGO10B	200923_at*
MAB21L1 /// MIR548F5	mab-21-like 1 (C. elegans) /// microRNA 548f-5	MAB21L1 mab-21 like 1CAGR1; Nbla00126	206163_at*
MDK	midkine (neurite growth-promoting factor 2)	MK; ARAP; NEGF2 midkine	209035_at*
MFGE8	milk fat globule-EGF factor 8 protein	BA46; HMFG; MFGM; SED1; hP47; EDIL1; MFG-E8; SPAG10; OAcGD3S; HsT19888	1558960_a_at*; 210605_s_at
MMP3	matrix metallopeptidase 3	SL-1; STMY; STR1; CHDS6; MMP-3; STMY1	205828_at*
MYRL2	myosin light chain 2 myosin regulatory light chain 2, smooth muscle isoform	MLC2; CMH10; MLC-2s/v; MYL2	209742_s_at*
NID2	nidogen 2 (osteonidogen)	NID-2	204114_at*
NRG1	neuregulin 1	NRG; GGF; HGL; HRG; NDF; ARIA; GGF2; HRG1; HRGA; SMDF; MST131; MSTP131; NRG1-IT2	206343_s_at*; 206237_s_at; 208230_s_at; 208231_at 208232_x_at; 208241_at;
PENK	proenkephalin	PE; PENK-A; proenkephalin	213791_at*
PODXL	podocalyxin-like	PC; PCLP; Gp200; PCLP-1	201578_at*
PTGIS	prostaglandin I2 (prostacyclin) synthase	CYP8; PGIS; PTGI; CYP8A1	208131_s_at*; 210702_s_at; 211892_s_at;
PTK7	protein tyrosine kinase 7 (inactive)	CCK4; CCK-4	1555324_at*; 207011_s_at;

Supplemental Table 2. *(continued)*

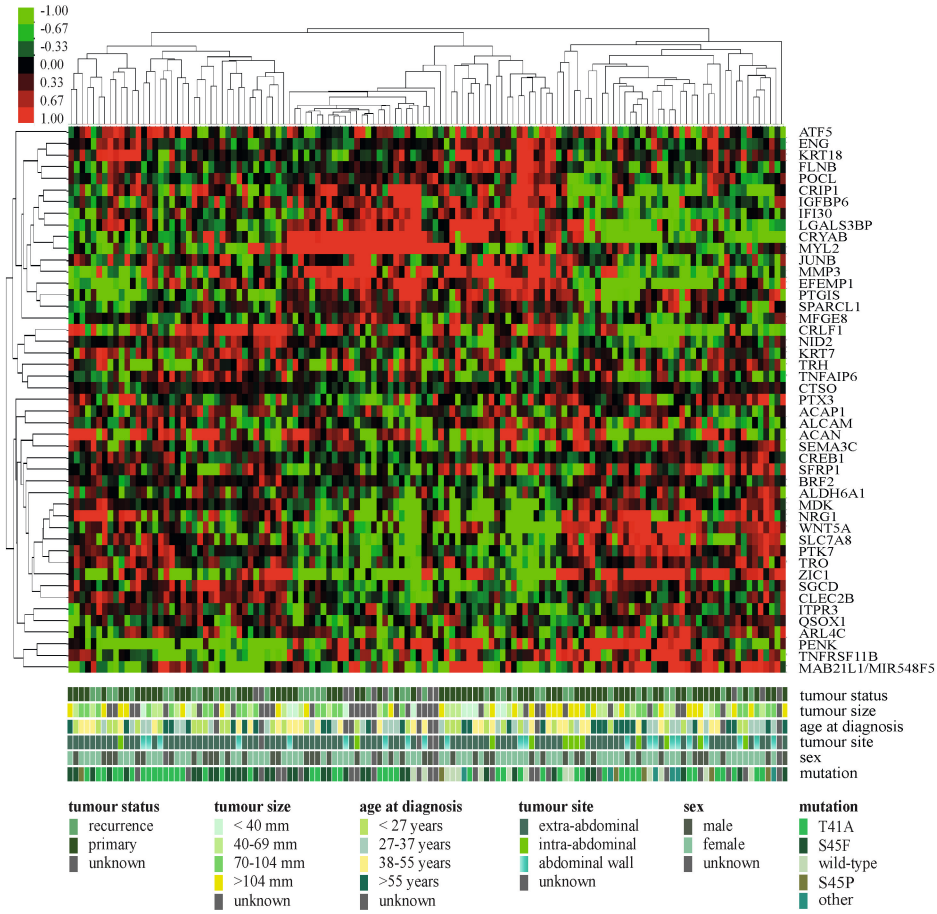
Gene name	Gene Title	Alias terms	Affymetrix Code
PTX3	pentraxin 3, long	TSG-14; TNFAIP5	206157_at*
QSCN6	Quiescin Q6	No Alias terms	230523_at*; 201482_at
SEMA3C	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	semaphorin 3C, SemE; SEMAE	203788_s_at*; 203789_s_at
SFRP1	secreted frizzled-related protein 1	No Alias terms	202037_s_at*; 202035_s_at; 202036_s_at; 228413_s_at;
SGCD	sarcoglycan delta	SGD; DAGD; 35DAG; CMD1L; SGCDP; SG-delta	210330_at*; 210329_s_at; 213543_at; 214492_at; 228602_at; 230730_at
SLC7A8	solute carrier family 7 (amino acid transporter light chain, L system), member 8	LAT2; LPI-PC1	216604_s_at*; 202752_x_at; 216092_s_at; 216603_at; 217248_s_at
SPARCL1	SPARC like 1	SC1; MAST9; PIG33; MAST 9	200795_at*
TNFAIP6	tumor necrosis factor, alpha-induced protein 6	No alias terms	206025_s_at*; 206026_s_at
TNFRSF11B	tumor necrosis factor receptor superfamily, member 11b	OPG; TR1; OCIF; PDB5	204932_at*; 204933_s_at
TRH	thyrotropin-releasing hormone	TRF; Pro-TRH	206622_at*
TRO	trophinin	MAGED3; MAGE-d3	205028_at*; 210882_s_at; 211700_s_at; 211701_s_at;
WNT5A	wingless-type MMTV integration site family, member 5A	hWNT5A;, Wnt family member 5a	205990_s_at*; 213425_at
ZIC1	Zic family member 1	ZIC; CRS6; ZNF201	206373_at*; 234716_at; 236896_at



Supplemental Figure 1A (part 1). Hierarchical clustering based on Wnt target mRNA expression in desmoid tumors does not discriminate wild-type, samples with an unknown CTNNB1 status, rare CTNNB1 mutants and CTNNB1 S45F, S45P, T41A mutants. Heat map depicting an unsupervised hierarchical cluster analysis of 128 desmoid-type fibromatosis samples harbouring T41A or S45F CTNNB1 mutations and wild-type tumours using the mRNA expression levels of selected mammalian Wnt target genes. Red and green represent relative high and low expresion levels, respectively. Tumour status (primary or recurrent); tumour size (<40 mm, 40-69 mm, 70-104 mm, >104 mm); age at diagnosis presented in categories based on the median and the 25th and 75th percentiles (age < 27 years, 27-37 years 38-55 years and > 55 years); tumour site (intra-abdominal, extra-abdominal and abdominal wall); sex and CTNNB1 mutation status are indicated.



Supplemental Figure 1A (part 2). Hierarchical clustering based on Wnt target mRNA expression in desmoid tumours does not discriminate wild-type, samples with an unknown CTNNB1 status, rare CTNNB1 mutants and CTNNB1 S45F, S45P, T41A mutants. Heat map depicting an unsupervised hierarchical cluster analysis of 128 desmoid-type fibromatosis samples harbouring T41A or S45F CTNNB1 mutations and wild-type tumours using the mRNA expression levels of selected mammalian Wnt target genes. Red and green represent relative high and low expresion levels, respectively. Tumour status (primary or recurrent); tumour size (<40 mm, 40-69 mm, 70-104 mm, >104 mm); age at diagnosis presented in categories based on the median and the 25th and 75th percentiles (age < 27 years, 27-37 years 38-55 years and > 55 years); tumour site (intra-abdominal, extra-abdominal and abdominal wall); sex and CTNNB1 mutation status are indicated.



Supplemental Figure 1B. Hierarchical clustering based on Wnt target mRNA expression in desmoid tumours does not discriminate wild-type,samples with an unknown CTNNB1 status, rare CTNNB1 mutants and CTNNB1 S45F, S45P, T41A mutants. Heat map depicting an unsupervised hierarchical cluster analysis of 128 desmoid-type fibromatosis samples harbouring wild-type, CTNNB1 samples, samples with an unknown CTNNB1 status, samples with rare CTNNB1 mutations and CTNNB1 S45F, S45P, T41A mutants, using selected the mRNA expression levels of a set of putative mesenchymal Wnt target genes. Red and green represent relatively high and low expression levels, respectively. Tumour status (primary or recurrent); tumour size (<40 mm, 40-69 mm, 70-104 mm, >104 mm); age at diagnosis presented in categories based on the median and the 25th and 75th percentiles (age < 27 years, 27-37 years 38-55 years and > 55 years); tumour site (intra-abdominal, extra-abdominal and abdominal wall); sex and CTNNB1 mutation status are indicated.

4

Differentially methylated regions in desmoid-type fibromatosis: A comparison between CTNNB1 S45F and T41A tumours

Milea J.M. Timbergen, Ruben Boers, Anne L.M. Vriends, Joachim Boers, Wilfred F.J. van Ijcken, Marla Lavrijsen, Dirk J. Grünhagen, Cornelis Verhoef, Stefan Sleijfer, Ron Smits, Joost Gribnau, Erik A.C. Wiemer

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Abstract

Introduction

The majority of desmoid-type fibromatosis (DTF) tumours harbour a β-catenin mutation, affecting specific codons in *CTNNB1* exon 3. S45F tumours are reported to have a higher chance of recurrence after surgery and more resistance to systemic treatments compared to wild-type (WT) and T41A tumours. The aim of this pilot study was to examine the genome-wide DNA methylation profiles of S45F and T41A mutated DTF, to explain the observed differences in clinical behaviour between these DTF subtypes.

Material and methods

Genome-wide analysis of DNA methylation was performed using MeD-seq on formalin fixed, paraffin embedded primary DTF samples harbouring a S45F (n = 14) or a T41A (n = 15) mutation. Differentially methylated regions (DMRs) between S45F and T41A DTF were identified and used for a supervised hierarchical cluster analysis. DMRs with a fold change ≥1.5 were considered to be differentially methylated and differences between S45F and T41A tumours were quantitatively assessed. The effect of DMRs on the expression of associated genes was assessed using an mRNA expression dataset. Protein-protein interactions between wild-type β-catenin and mutant variants and DNA methyltransferase 1 (DNMT1) were examined by immunoprecipitation experiments.

Results

MeD-seq analyses indicated 354 regions that displayed differential methylation. Cluster analysis yielded no distinct clusters based on mutation, gender, tumour site or tumour size. A supervised clustering based on DMR between small (≤34 mm) and large (>87 mm) DTF distinguished the two groups. Only ten DMRs displayed a fold change of ≥1.5 and six of them were found associated with of the following genes: *NLRP4*, *FOXK2*, *PERM1*, *CCDC6*, *NOC4L* and *DUX4L6*. The effects of DMR on gene expression yielded a significance difference ($p < 0.05$) in the expression between S45F and T41A for CCDC6 and FOXK2 but not for all Affymetrix probes sets used to detect these genes. Immunoprecipitation did not reveal an association of wild-type β-catenin or mutant variants with DNMT1.

Conclusion

This study demonstrated that S45F and T41A DTF tumours did not exhibit gross differences in DNA methylation patterns. This implies that distinct DNA methylation profiles are not the sole determinant for the divergent clinical behaviour of different DTF mutant subtypes.

Introduction

Desmoid-type fibromatosis (DTF) is a rare, non-metastasizing, invasive, mesenchymal soft tissue sarcoma [1, 2]. The presence [3]of nuclear β-catenin distinguishes DTF from other soft tissue tumours and scar tissue [4] and is caused by the fact that the majority of tumours (> 85%) harbor a mutation at specific sites in the β-catenin (*CTNNB1*) gene [5]. These mutually exclusive mutations in exon 3 of the *CTNNB1* gene result in substitution of serine at position 45 with phenylalanine (S45F), or less commonly proline (S45P), or lead to a replacement of threonine at position 41 with alanine (T41A) [5, 6]. The tumour is categorized as wild-type (WT) in case no *CTNNB1* exon 3 mutations are found. This WT group is considered heterogeneous as these tumours may contain other *CTNNB1*, outside of exon 3, or *APC* mutations [5]. Despite the shared molecular basis in the majority of DTF patients, the clinical presentation and disease course varies. Several studies indicate a prognostic role for the *CTNNB1* mutation and some claim that S45F tumours have a higher risk of recurrence after surgery in comparison to T41A tumours [6-8] or that S45F tumours are more resistant to treatment with e.g., sorafenib [9], doxorubicin [10] or meloxicam [11]. Currently, there is no biological rationale for the reported clinical differences in behaviour – particularly the risk of recurrence - of DTF with these mutation types. A recent pilot study revealed differences in the metabolomics profiles associated with T41A and S45F DTF cell lines also suggesting that - up to a certain extent - the biology of DTF with these *CTNNB1* mutations indeed differs [12].

The *CTNNB1* mutations that are predominantly observed in DTF, prevent phosphorylation and subsequent degradation of β-catenin, a key player in the Wnt/β-catenin signalling pathway. This leads to stabilisation and translocation of β-catenin into the nucleus, causing aberrant Wnt/β-catenin signalling. However, CTNNB1 has a complex role in the cell, and is involved in protein-interaction networks related to cell adhesion and transcription. Nuclear β-catenin recruits transcription factors of the TCF family and interacts with epigenetic and chromatin modifiers [13,14]. Song et al. reported a protein interaction between CTNNB1 and the DNA methyltransferase DNMT1 in cancer cells which stabilizes each protein and regulates downstream CTNNB1 and DNMT1 functions suggesting a cross-regulation between Wnt signalling and DNA methylation [15].

Changes in the DNA methylation pattern have been described in various solid tumours, including various mesenchymal neoplasms such as chondrosarcoma, Ewing sarcoma and rhabdomyosarcoma [16-21]. These distinct methylation patterns could be of diagnostic value and capable of discerning tumour subtypes, may yield clinically relevant biomarkers that can direct treatment choices, and can potentially reveal novel treatment opportunities [22-26].

The findings of Song et al. prompted us to hypothesize that the different mutations found in CTNNB1 in DTF affect interacting DNMT1 differently consequently causing altered DNA methylation patterns.

This study investigates DNA methylation patterns of the two most common mutation types of DTF (S45F and T41A), aiming to provide insight in the biological underpinnings of the different clinical behaviour of these DTF mutants.

Materials and Methods

Patient and sample selection

Patients with histologically proven, primary DTF and a S45F or T41A CTNNB1 mutation were identified in the Erasmus MC pathology database. Corresponding formalin-fixed paraffin-embedded (FFPE) tumour tissue blocks were collected from the Erasmus MC tissue bank. Similarly, clinicopathological characteristics such as sex, age at diagnosis, tumour site (extra-abdominal, intra-abdominal, or abdominal wall), and largest tumour size (in millimetres [mm]) on imaging were obtained from the patient files. The *CTNNB1* exon 3 mutations were previously determined for diagnostic purposes essentially as described by Dubbink et al. [27]. In short: Tumour DNA was extracted from FFPE tumour tissue using proteinase K and 5% Chelex®-100 chelating resin (Bio-Rad). Sequence analysis of CTNNB1 exon 3 was performed by bidirectional sequencing of PCR-amplified DNA fragments using M13-tailed forward and reverse primers. The selected patients did not receive any treatment before the specimens were obtained. An expert soft tissue sarcoma pathologist confirmed the diagnosis by examining hematoxylin-eosin stained sections of the FFPE samples.

DNA isolation

DNA was isolated from five consecutive FFPE DTF sections of 10μm using the Allprep DNA/RNA kit according to the manufacturer's recommendations (Qiagen, Hilden, Germany). The DNA quality and the concentrations were determined using a Nanodrop-2000 (Isogen Life Science, Utrecht, and the Netherlands). The 260nm/280nm ratio was ≥ 1.80 for all DNA preparations.

MeD-seq sample preparations

Methylated DNA sequencing (MeD-seq) was used to analyse genome wide DNA methylation. MeD-seqprovides single-nucleotide resolution by exploiting the properties of the DNA methylation dependent restriction enzyme *LpnPI* [28]. This enzyme generates DNA

fragments of 32 base pairs (bp) by cutting 16 bp downstream from the methylated CpG sites, which allows specific focus on the methylated regions. The MeD-seq analyses were essentially carried out as previously described [28, 29]. In brief: DNA samples were digested by *LpnPI* (New England Biolabs, Ipswich, MA, USA), resulting in snippets of 32 bp around a fully-methylated recognition site that contains a CpG. These short DNA fragments were further processed using a ThruPlex DNA–seq 96D kit (cat#R400407, Rubicon Genomics Ann Arbor, MI, USA) and a Pippin system. Stem-loop adapters were blunt-end ligated to repaired input DNA and amplified to include dual indexed barcodes using a high-fidelity polymerase to generate an indexed Illumina NGS library. The amplified end product was purified on a Pippin HT system with 3% agarose gel cassettes (Sage Science, Beverly, MA, USA). Multiplexed samples were sequenced on Illumina HiSeq2500 systems for single read of 50 bp according to the manufacturer's instructions. Dual indexed samples were demultiplexed using bcl2fastq software (Illumina, San Diego, CA, USA).

MeD-seq data analysis

Data processing was carried out using specifically created scripts in Python. The proprietary Python script is used in the context of an exclusive license from the Erasmus Medical Center with Methylomics BV. Raw fastq files were subjected to Illumina adaptor trimming and reads were filtered based on *LpnPI* restriction site occurrence between 13-17 bp from either 5' or 3' end of the read. Reads that passed the filter were mapped to hg38 using bowtie2. Genome-wide individual *LpnPI* site scores were used to generate read count scores for the following annotated regions: transcription start sites ((TSS), 1 kb before and 1 kb after), CpG-islands and gene bodies (1kb after TSS till Transcription End Site (TES)). Gene and CpG-island annotations were downloaded from ENSEMBL (www.ensembl.org). Detection of DMRs was performed between two datasets containing the regions of interest (TSS, gene body or CpG-islands) using the Chi-square test on read counts. Significance at a p-value of < 0.05 was called by either Bonferroni or FDR using the Benjamini-Hochberg procedure.

In addition, a genome-wide sliding window was used to detect sequentially differentially methylated *LpnPI* sites. Statistical significance was called between *LpnPI* sites in predetermined groups using the Chi-square test. Neighbouring significantly called *LpnPI* sites were binned and reported. Annotation of the overlap of genome-wide detected DMRs was reported for TSS, CpG-islands and gene body regions. DMR thresholds were based on *LpnPI* site count. Fold changes of read counts are mentioned in the figure legends before performing hierarchical clustering. The differentially methylated datasets generated and analysed during the current study have been deposited to the Sequence Read Archive (SRA)

data repository under accession number PRJNA604749. The DMRs with a fold change ≥1.5 were considered to be differentially methylated and were analysed separately. Non-normal distributed values were analysed using a Mann-Whitney *U* test to identify statistically significant differences in the normalized read counts between the two mutation types. A p-value of < 0.05 was considered to be statistically significant. SPSS Statistics (version 24) was used for the Mann-Whitney *U* tests (IBM, Armonk, New York, USA). The DMRs of interest were loaded in the Integrative Genomics Viewer (IGV) using the Hg 38 platform to visualize regions of interest [30].

Validation MeD-seq results using an mRNA expression dataset

Expression data, generated on an Affymetrix platform (Human Genome U133 Plus 2.0 array) of PERM1, DUX4L6, CCDC6, NOC4L, FOXK2 and NLRP4 in DTF samples (n = 128) were obtained from a publicly available dataset in the Gene Expression Omnibus (accession number GSE58697) [31] Information regarding the *CTNNB1* mutational status was kindly provided by dr. Frederic Chibon, Cancer Research Center of Toulouse, France. Only patients with an S45F or T41A mutation were selected for validation purposes. A Mann-Whitney *U* test was performed on non-normal distributed data to identify differences in mRNA expression levels of the selected genes corresponding to the identified DMRs. A p-value of < 0.05 was considered statistically significant. SPSS Statistics (version 24) was used for all statistical analyses.

Cell lines, cell transfection

The human cell lines HEK293T (embryonic kidney cells) and HCT116 (colon cancer cells) were maintained in DMEM (Gibco Life Technologies) supplemented with 10% fetal bovine serum (Greiner bio-one) and 100 IU/ml penicillin and 100 µg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO2. For transfections cells were cultured in 6-well plates to 70% confluence. Next the cells in each well were transfected with 1 µg of different N-terminal FLAG tagged β-catenin plasmids or empty pcDNA 5'UT-FLAG vector using ViaFect™ (Promega) as transfection agent. The construction of the different expression plasmids is described by Liu *et al.* [32]. The CTNNB1 plasmid variants used express FLAG tagged versions of either the wild-type (WT), T41A, S45P, exon 3 deletion or K335I β-catenin.

Cell lysates, Immunoprecipitation and Western blotting

At 48 h post-transfection cells were washed with ice-cold PBS and lysed in 500 µl of lysis buffer 25 mM Tris-HCl pH 7.5; 150 mM NaCl; 1 mM EDTA; 1% NP-40 and 5%

glycerol (Pierce IP lysis buffer) containing Halt Protease and Phosphatase inhibitor single-use cocktail (ThermoFisher Scientific). Wells were cleaned by scraping and the cell lysates collected and centrifuged at 11.000 x g for 10 minutes at 4°C to pellet insoluble cell debris. From the cleared lysates 10% was used as input control which is prepared for SDS-PAGE by adding an equal volume of 2 x Laemmli sample buffer with 0.1 M DTT (Laemmli/DTT). FLAG-tagged β-catenin is immunoprecipitated from the remainder of the lysates using prewashed ANTI-FLAG M2 Affinity Gel (Sigma-Aldrich, cat. No. A2220) for 2 h at 4°C. FLAG-beads were washed with lysis buffer for three times and resuspended in Laemmli/DTT.

Input and IP samples were heated to 95°C for 5 minutes and subjected to SDS-PAGE and electroblotted onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked in TBS/0.1% Tween'20 supplemented with 5% (w/v) BSA and incubated overnight at 4°C with rabbit monoclonal anti-DNMT1 (1:1000 DNMT1 XP®, D63A6, Cell Signaling Technology), mouse monoclonal anti-FLAG® M2 antibody (1:1000 Sigma-Aldrich, cat no. F1804) or mouse monoclonal anti-β-actin (1:10.000, Sigma-Aldrich, cat no. A5441). The primary antibodies were diluted in blocking buffer. HRP conjugated goat-anti-rabbit, goat-anti-mouse were used as secondary antibodies in TBS/0.1% Tween'20 supplemented with 5% (w/v) non-fat dried milk. Enhanced chemiluminescence (SuperSignal™ West Pico Plus Chemilumininescent Substrate, Thermo Scientific) was used to visualize the bound antibodies in a ChemiDoc MP Imager (Bio-Rad).

Ethical approval

This study was part of a protocol entitled "Translational research on soft tissue sarcomas" which was assessed by the Medical Ethics Committee of the Erasmus MC, Rotterdam, the Netherlands (MEC-2016-213).

Results

Clinical characteristics of the patients included in the MeD-Seq analysis

The vast majority of DTF tumours contain mutations in exon 3 of the *CTNNB1* (β-catenin) gene. Interestingly, the mutations are almost exclusively confined to residues T41 and S45 preventing the phosphorylation of these residues and consequently stabilizing *CTNNB1* and activating Wnt/β-catenin signalling. Although having similar effects, the T41A and S45F mutated DTF tumours were reported to display a different clinical behaviour for which the underlying biological mechanism is still unclear. Epigenetic alterations may be important in this respect,

particularly in view of the reported interaction between CTNNB1 and DNMT1 [15]. Therefore, the genome-wide DNA methylation profiles of DTF tumours were explored, comparing *CTNNB1* S45F and T41A mutated tumours. To this end, 29 FFPE samples of primary untreated DTF tumours were analysed using MeD-seq. Fifteen samples harboured a *CTNNB1* T41A mutation and 14 samples a *CTNNB1* S45F mutation, both mutations that are commonly observed. The patients had a median age of 36 years (interquartile range 26-47 years) and females were in the majority (n = 19, 66%). Most tumours were located extra-abdominally (69%) and had a median size of 55 mm (interquartile range 34-87 mm) (Table 1).

Table 1. Clinical characteristics of patients and tumour samples (n = 29) included in this study

		Total group n = 29	**T41A n (%)**	**S45F n (%)**
Sex	Female	19 (66%)	11 (73%)	8 (57%)
	Male	10 (34%)	4 (27%)	6 (43%)
Tumour location	Extra-abdominal	20 (69%)	7 (47%)	13 (93%)
	Abdominal wall	6 (21%)	5 (33%)	1 (7%)
	Intra-abdominal	3 (10%)	3 (20%)	0
Median age at diagnosis in years (IQR)		36 (26-47)	38 (33-48)	31 (20-45)
Median size in mm (IQR) [a,b]		55 (34-87)	53 (29-59)	68 (50-103)

[a]based on initial imaging; [b] n = 3 missing values; IQR, interquartile range

Differentially methylated regions between S45F and T41A DTF samples identified by MeD-seq

A genome-wide MeD-seq analysis was carried out using DNA isolated from 29 FFPE DTF samples. A total of 365 differentially methylated regions (DMRs) were found to be significantly different between S45F, and T41A DTF tumours. After excluding DMRs located on the X- and Y-chromosomes, a group of 354 DMRs remained. Of these 354 DMRs, 97 (27%) DMRs were hypomethylated in S45F (versus T41A), and 257 (83%) were hypermethylated in S45F (vs. T41A). Supplemental Materials 1 provides an overview of all 354 DMRs. A supervised hierarchical clustering of the DTF samples based on all detected DMRs, did not reveal clearly separated groups based on *CTNNB1* mutational status (Figure 1). Likewise, no distinct cluster patterns were observed on basis of sex (Figure 1), tumour site (Supplemental Figure 1) and tumour size (Supplemental Figure 2). As a meta-analysis by our group revealed tumour size to be an independent predictor for recurrence [7] a supervised cluster analysis was performed based on DMRs between small DTF (n = 6, ≤ 34 mm) and large tumours (n = 6, > 87 mm). Interestingly, the small and large DTF tumours were almost completely distinguished only one small tumour grouped together with the large tumours (Supplemental Figure 3).

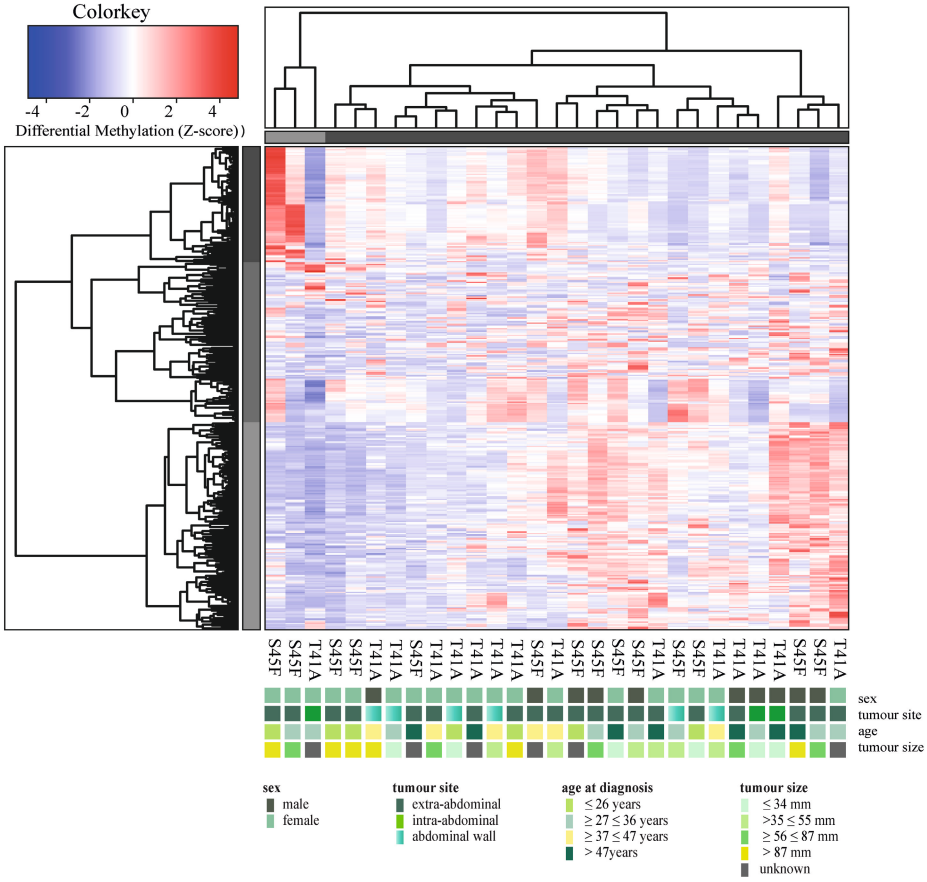

Figure 1. Supervised hierarchical clustering based on differentially methylated regions (DMRs) between S45F and T41A mutated DTF tumours together with clinicopathological features: sex, tumour site, age, and tumour size. The heat map depicts the methylation in 354 DMRs, including all fold changes and excluding DMRs present on sex chromosomal regions, in S45F and T41A DTF samples. The cluster tree on top indicates distinct subgroups of DTF samples. Grouping, however, is not based on CTNNB1 mutation type (T41A or S45F) nor on clinicopathological parameters such as sex, tumour site, age, or tumour size.

Within both CTNNB1 mutation groups, there appeared to be a considerable heterogeneity in DNA methylation between tumour samples. When considering all 354 DMRs it was noticed that the vast majority displayed relatively small fold changes (<1.5) between the different DTF mutant groups. Only ten DMRs had fold changes ≥1.5. Table 2 lists the chromosomal position of the DMRs with a fold change ≥1.5 including the start and end positions, the observed fold change, the overlapping genes associated with the DMR, the location of the DMR with respect to the gene body and the methylation status in the S45F

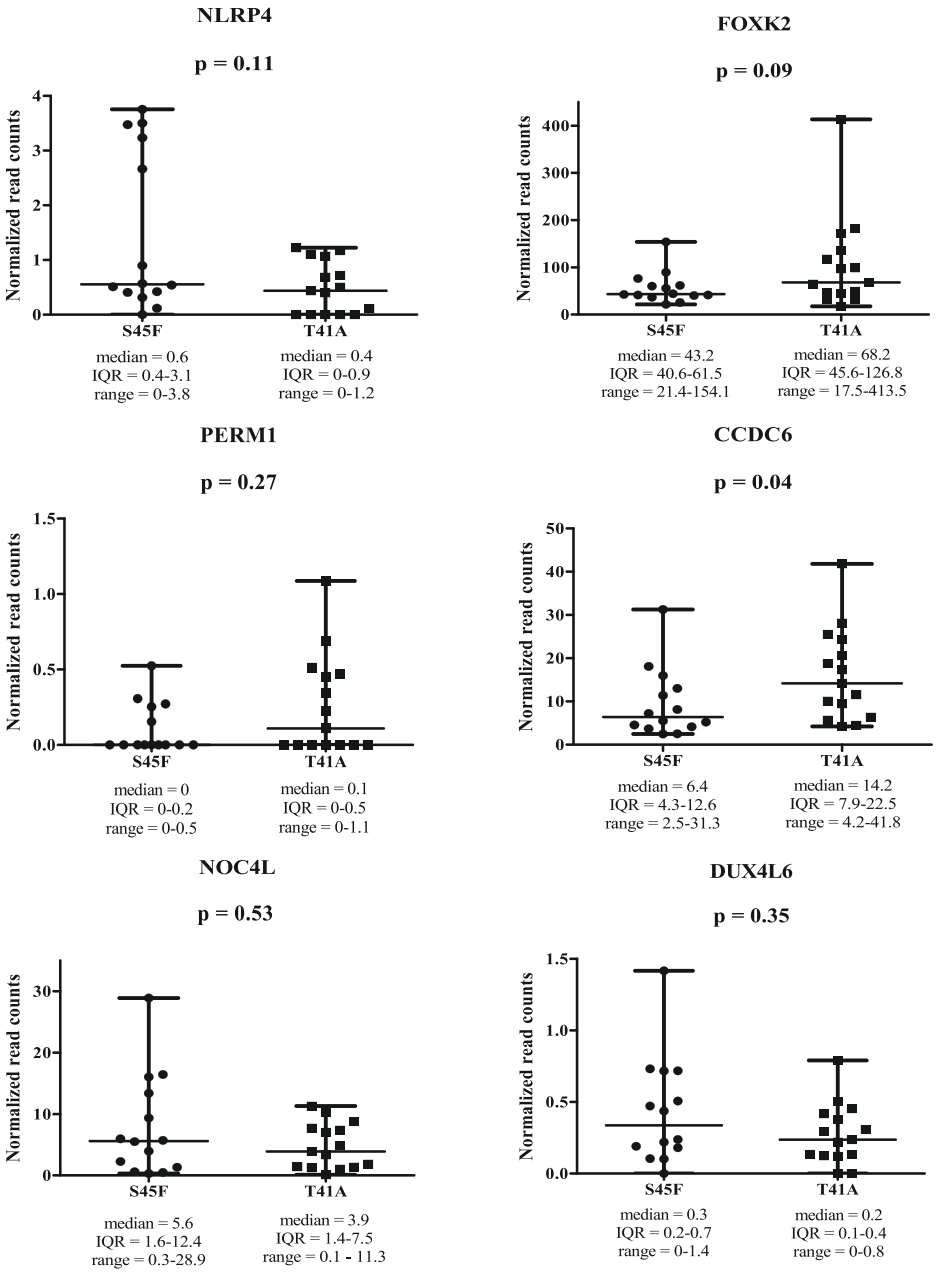

Figure 2. Read counts for DMRs in individual S45F and T41A DTF. Plots showing the normalized read counts for selected DMRs (fold-change ≥1.5) associated with NLRP4, FOXK2, PERM1, CCDC6, NOC4L and DUX4L6. The whiskers represent the minimum and maximum read counts. Dots (S45F) or squares (T41A) indicate individual data points, the horizontal line designates the median level. A Mann Whitney U test was used to assess statistical significance.

and T41A samples. Most genes, with the exception of DUX4L6, present with DMRs in the gene body. The DMRs and their location were visualized by loading the MeD-seq data into the Integrative Genomics Viewer (IGV) (Supplemental Figure 4A-E). The IGV graphs present the methylation patterns in a quantitative way in each DTF sample that was analysed. It was observed that the methylation profiles within a *CTNNB1* mutant class may differ considerable, with samples displaying almost a complete absence of methylation whereas other samples appear (partly) methylated. Figure 2 depicts the actual normalized read counts of the DMRs and associated genes detected between S45F and T41A DTF samples. A more stringent statistical analysis of the raw data revealed that only the DMR associated with CCDC6 remained statistically significant ($p = 0.04$).

Table 2. Overview of DMRs with a fold change ≥ 1.5 between S45F and T41A DTF.

Chromosome	Fold change	Position (start – end)	Methylation status		Over-lapping genes	Position
			S45F	T41A		
19	2.68	55850028-55850071	+	-	NLRP4	postTSS1KB-TES
17_GL383563v3_alt	2.27	130411-131133	-	+	-	
17	1.85	82585430-82587461	-	+	FOXK2	postTSS1KB-TES
16	1.79	85410202-85410284	+	-	-	
1	1.77	977947-977974	-	+	PERM1	postTSS1KB-TES
21		42956670-42958541	+	-	-	
10	1.70	59882022-59883084	-	+	CCDC6	postTSS1KB-TES
10	1.69	42089321-42090999	+	-	-	
12	1.65	132149685-132150607	+	-	NOC4L	postTSS1KB-TES
4	1.50	190075267-133745860	+	-	DUX4L6	TSS

DMRs located on sex (X- and Y) chromosomes were excluded; TSS, Transcription Start Site; TES, Transcription End Site; postTSS1KB-TES, indicates the region starting at 1 Kb after the TSS till the TES thus corresponding to the gene body without promoter region. - hypomethylation, + hypermethylation

Effects of DMRs on gene expression levels

Next, the effect of the initially detected DMRs with a fold change ≥ 1.5 on the expression of the associated genes was examined. DMR associated genes NLRP4, FOXK2, PERM1, CCDC6, NOC4L and DUX4L6 were identified in a publicly available mRNA expression dataset of 34 S45F and 45 T41A DTF samples. It was noted that the same genes are detected by multiple probes on the Affymetrix platform used (Table 3). A Mann-Whitney *U* test indicated that no significant expression differences were observed between S45F and T41A mutant DTF for most genes with the exception of *CCDC6* ($p = 0.034$, 1 out of 2 probes) and *FOXK2* ($p = 0.004$, 1 out of 4 probes) (Table 3). It was noted that the expression of *CCDC6*

and *FOXK2* in T41A samples was downregulated suggesting that the hypermethylation observed in T41A samples reduces mRNA expression.

Table 3. Expression levels of genes identified by DMRs between S45F and T41A DTF samples. Transcript levels are derived from publicly available Affymetrix based mRNA expression data (GSE58697) of 45 T41A and 34 S45F tumours.. Some genes are represented by multiple probe sets. Mann-Whitney *U* test was used to assess statistical significance in transcript levels of the respective genes between S45F and T41A tumours.

Gene name	Probe number	Median (IQR)	p-value
CCDC6	204716_at	203 (126-239)	**0.034**
	225010_at	617 (554.6-703.4)	0.579
FOXK2	242937_at	17 (15-21)	**0.004**
	242938_s_at	34 (32-39)	0.384
	226224_at	149 (141-158)	0.373
	203064_s_at	112 (101-127)	0.533
DUX4L6	216472_x_at	11 (8-14)	0.362
	208201_at	15 (11-20)	0.510
NLRP4	242334_at	2.7 (1.2-4.2)	0.149
NOC4L	218860	50 (44-55)	0.628
PERM1	224501_at	1 (1-1)	0.415

CTNNB1 (β-catenin) – DNMT1 protein interaction.

To verify whether the observed DMRs between S45F and T41A DTF samples could be the result of a differential regulatory interaction between β-catenin mutants and DNMT1, an immunoprecipitation experiment was performed. FLAG-tagged wild-type β-catenin and different β-catenin mutants (T41A, S45P, exon 3 deletion mutant, K335I) were transiently expressed in HEK293T and HCT116 cells. Western blot analysis of total lysates of the transfectants demonstrated expression of DNMT1 as well as the FLAG-tagged version of β-catenin and β-actin as loading control (Figure 3, Supplemental Figure 5). When FLAG-tagged β-catenin was immunoprecipitated no DNMT1 was co-precipitated. We tentatively conclude that we cannot verify a protein-protein interaction between β-catenin wild-type or mutants and DNMT1.

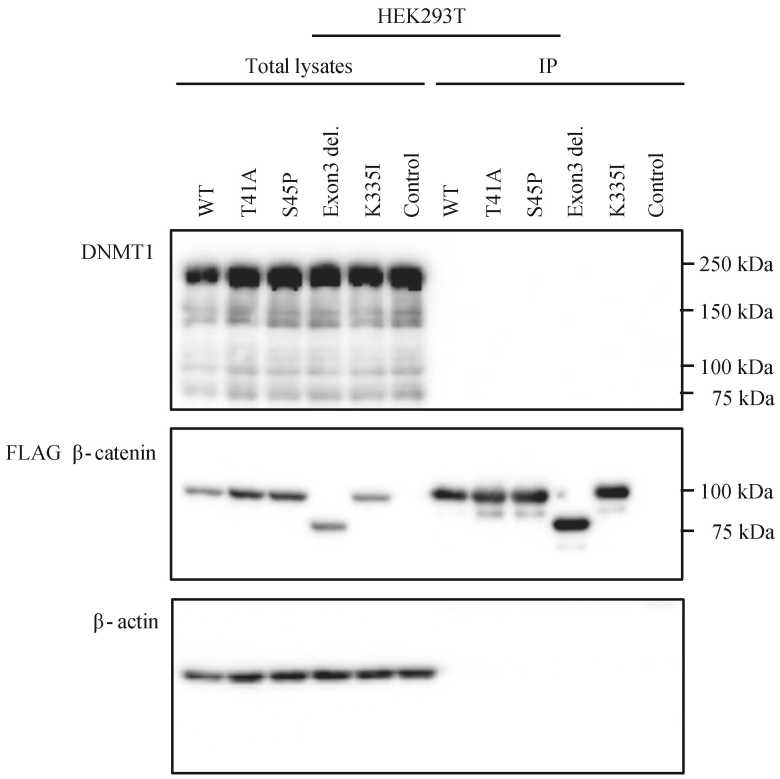

Figure 3. DNMT1 is not co-precipitated with wild-type or mutant CTNNB1 (β-catenin). HEK293T cells were transfected with plasmids driving the expression of FLAG-tagged wild-type β-catenin (WT) or FLAG-tagged mutant versions of β-catenin (T41A; S45P; Exon 3 deletion mutant; K335I). As a control cells were transfected with the empty vector. At 48 h post-transfection cell lysates were prepared from which the FLAG-tagged β-catenin variants were immunoprecipitated. Western Blot analysis was used to examine DNMT1, β-catenin and β-actin protein expression in the total lysates and immunoprecipitates.

Discussion

DNA methylation patterns are a good representation and reflection of molecular changes in the early stages of human cancer. The correlation between cancer and aberrant methylation patterns are described by various studies [22, 23, 26]. DNA hypomethylation of gene promoter regions is usually associated with tumour formation, activation of oncogenes and chromosomal instability [33, 34]. In addition, DNA hypermethylation of gene promoter regions may alter gene expression and can cause tumour suppressor silencing and promote cancer progression [35]. Also, gene body methylation is a widespread phenomenon, however, its functional consequences are less clear [36, 37]. There is experimental evidence that gene body methylation is also associated with transcriptional activity and can affect gene expression [3, 38]. Aberrant

methylation has been the subject of various sarcoma-related publications. A study by Röhrich et al. based the classification of peripheral nerve sheath tumours (benign versus malignant) on methylation patterns [39]. Tombolan et al. was able to distinguish metastatic and non-metastatic rhabdomyosarcomas based on their methylation patterns [18].

In this study, we hypothesized a role for aberrant methylation patterns based on the differences in clinical behaviour between the different *CTNNB1* mutations found in DTF. To our knowledge, this is the first study that explored DNA methylation patterns in DTF. Whole genome DNA methylation was examined using MeD-seq. This is a novel and powerful technique to perform genome-wide DNA methylation analysis [28]. Since this technique focusses the sequencing resources on methylated regions only, and because the restriction enzyme *LpnPI* is restricted by a short template size, the generated fragments are consistent in size which results in accurate identification of DMRs genome-wide [28]. The MeD-Seq method compared very favourable to other methods such as whole-genome bisulfite sequencing, MeDIP and the 450K Infinium bead-chip technology [28]. Furthermore, MeD-seq uses single base pair resolution which allows us to identify methylation on one specific CG site. In case of marker development, this would create the opportunity to use information from this single nucleotide for primer selection.

Here we focused on differences between *CTNNB1* T41A and S45F mutated tumours as they occur frequently, are mutually exclusive and are reported to exhibit a divergent clinical behaviour. Overall, the detected differences in DNA methylation were few, subtle and unable to discriminate between S45F and T41A tumours in a cluster analysis. Only some DMRs were found to be differentially methylated with a fold change ≥ 1.5, and only a single DMR, related to the *CCDC6* gene had a fold change of ≥ 2. Most of the DMRs (fold change ≥ 1.5) appeared to be situated within gene bodies. The relatively small fold changes in DMRs observed, the intertumoural heterogeneity and the low amount of statistically significant DMRs identified in this study, suggests that there is no distinct difference in DNA methylation patterns between S45F and T41A DTF tumours. In the current study the effects of differential methylation on gene expression were assessed using an independent Affymetrix mRNA expression dataset which only revealed significant expression differences between S45F and T41A tumours for *CCDC6* ($p = 0.034$) and *FOXK2* ($p = 0.004$) but not for all probes capable of detecting these genes. To explain these observations, one may speculate that gene body methylation affects differential splicing yielding splice products that hybridize only with some capture probe. To obtain biological insight why different β-catenin mutants would affect DNA methylation differently, the interaction between

β-catenin and DNMT1, as reported by Song et al., was investigated [15]. Despite the use of similar immunoprecipitation conditions and an identical cell line (HCT116) DNMT1 was not pulled-down with wild-type β-catenin or any of the β-catenin mutants tested.

No distinct cluster patterns were seen based on tumour size when a hierarchical clustering was performed using all 354 DMRs between S45F and T41A samples. Interestingly, when only the smallest and largest tumours were considered, DNA methylation patterns almost perfectly discriminated the two groups. Although tumour size depends on the measuring methods (radiological imaging or the dimensions of freshly resected surgical specimen) and can therefore be fairly subjective, our data suggest larger tumours display a different methylation pattern compared to smaller tumours. The phenomena that methylation patterns differ between tumour sizes has previously been described by Nishida et al., and may suggest that stepwise progression of methylation alterations may take place during the development of tumours [40].

This study has several limitations. The first limitation is the relatively small DTF sample size. DTF samples are challenging to obtain due to the rarity of these tumours and the current tendency to use an active surveillance approach instead of surgical resection [41]. Furthermore, obtaining paired control tissue such as fascia from which desmoids are believed to arise, is challenging as it would require an additional resection of adjacent fascia next to the tumour site. Due to the retrospective nature of the current study, we were not able to obtain paired control tissue samples. Furthermore, we opted not to include WT DTF samples as control as they comprise a heterogeneous group which often contains rare *CTNNB1* mutations or alterations in other genes [5].

Future research should focus on the integrated genomic and molecular characterization of DTF samples and include appropriate control tissues to further delineate and understand the biological mechanisms and epigenetic changes involved in the pathogenesis of DTF. The functional significance of the observed differential gene body methylation of CCDC6 in T41A and S45F DTF should be further validated and investigated. Exploration of the dynamic changes in DNA methylation patterns and their consequences for gene expression from tumour onset to tumour progression and/or regression is of interest too and may provide an explanation for the different clinical behaviours of DTF tumours.

Data Availability Statement

The datasets generated for this study can be found in the Sequence Read Archive (SRA) data repository under accession number PRJNA604749.

Conflict of Interest

The authors declare no conflict of interest or financial interests except for R.B., J.B., W.V.I. and J.G., who report being shareholder in Methylomics B.V., a commercial company that applies MeD-seq to develop methylation markers for cancer staging.

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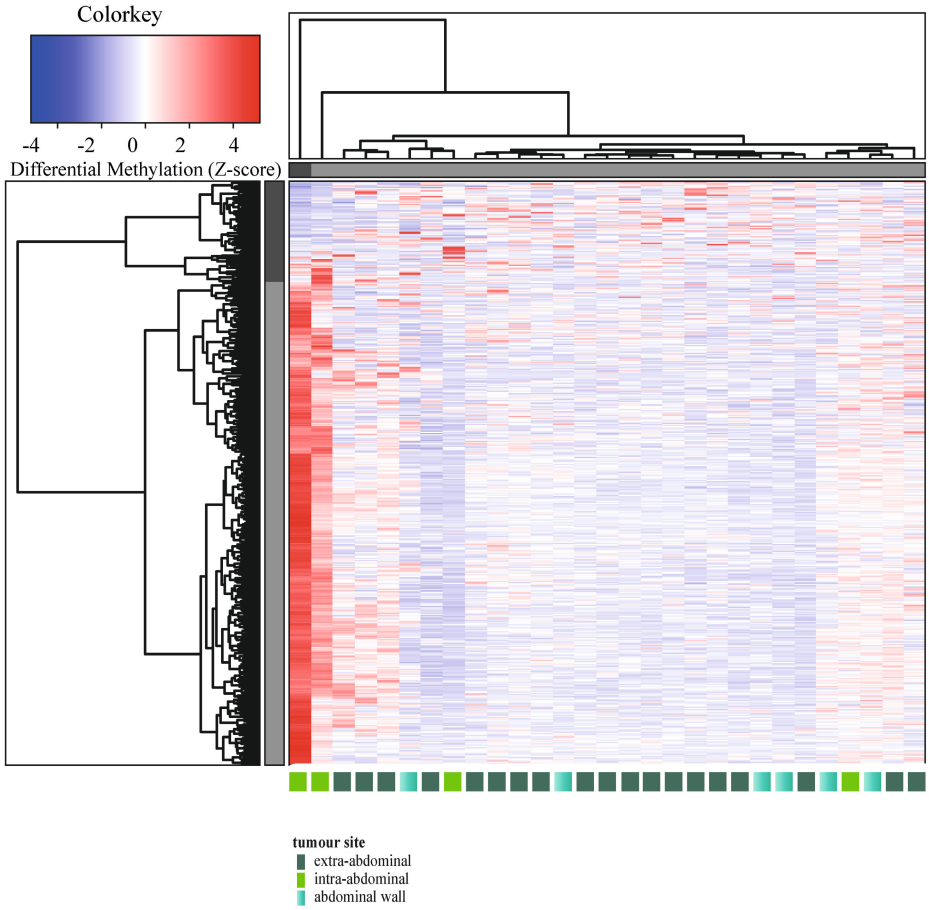
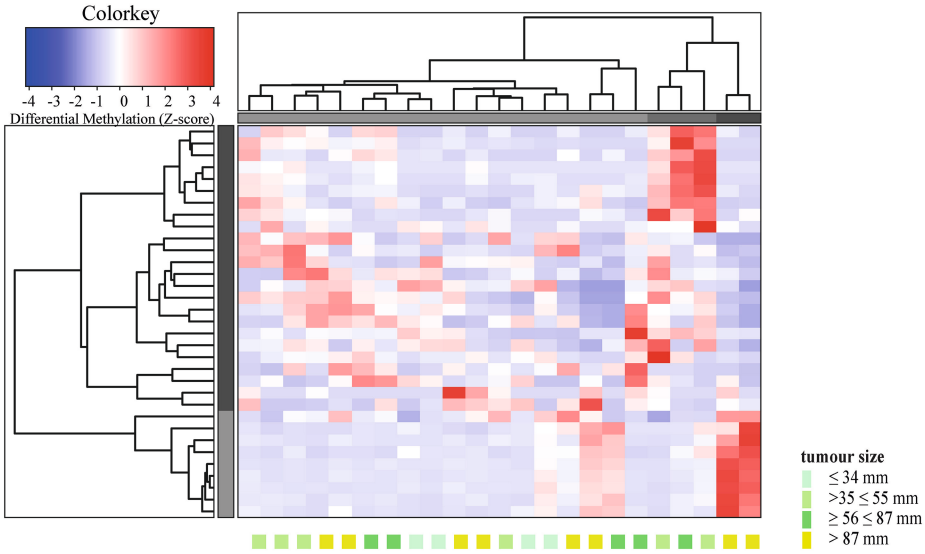
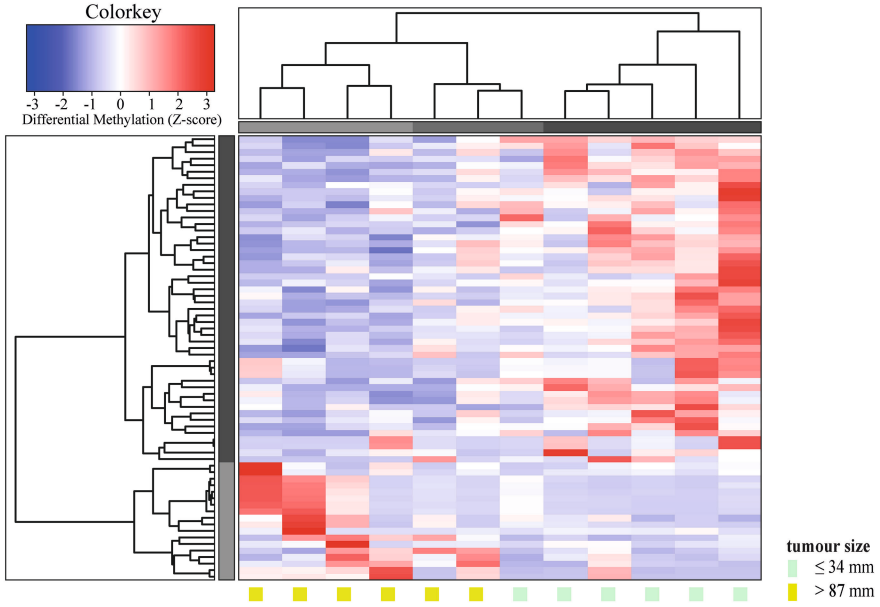


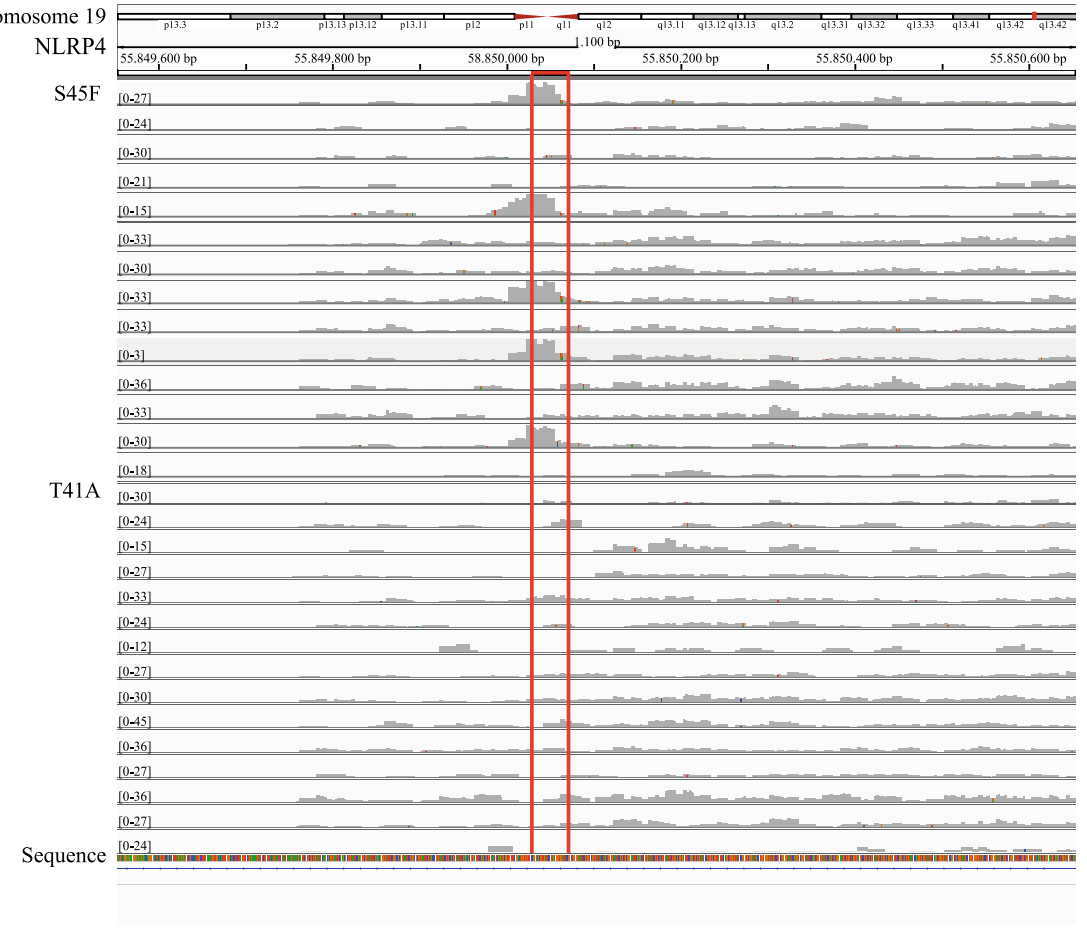
Supplemental Figure 1. Supervised hierarchical clustering based on differentially methylated regions (DMRs) between DTF tumour locations. DTF samples were obtained from tumours at extra-abdominal sites, intra-abdominal sites or the abdominal wall.



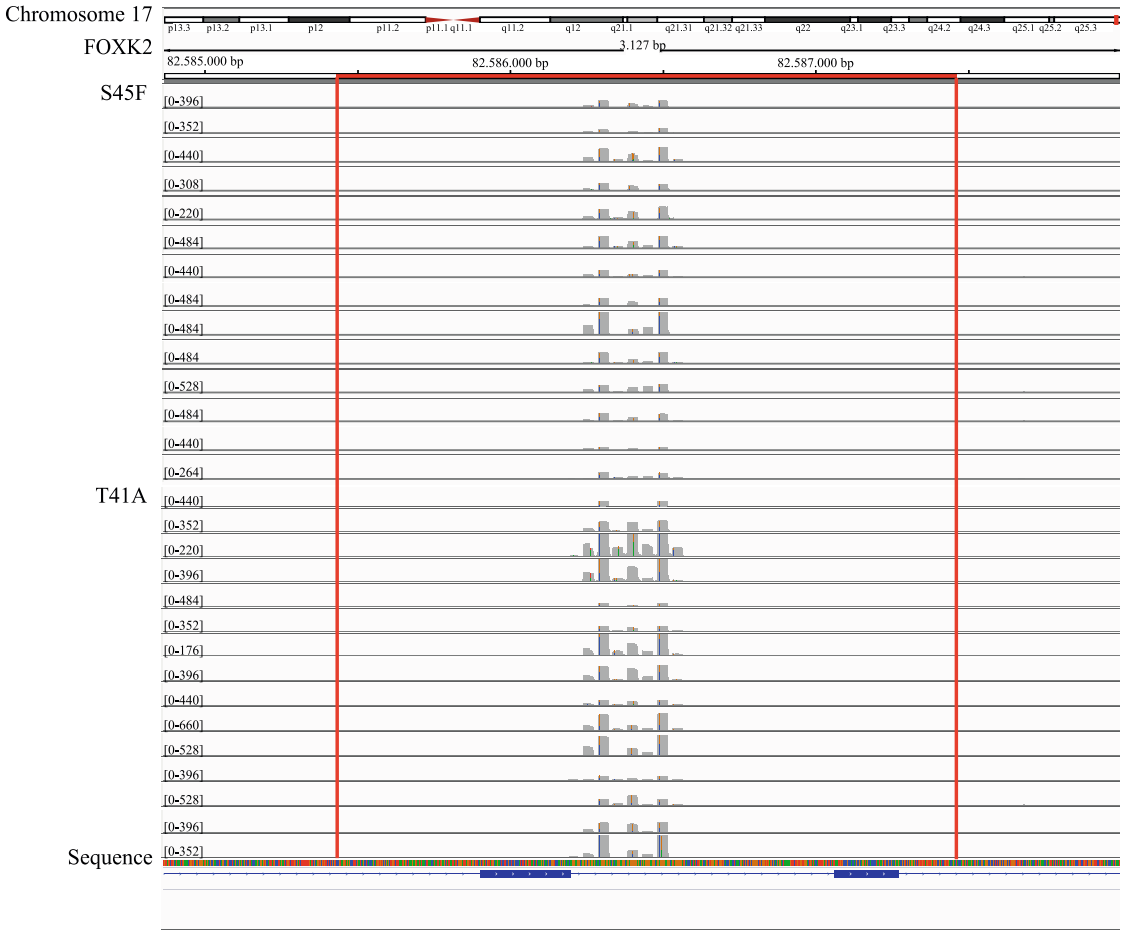
Supplemental Figure 2. Supervised hierarchical clustering based on differentially methylated regions (DMRs) between DTF tumour sizes. Tumour sizes were based on initial imaging data obtained at diagnosis. The following size classes were analysed ≤ 34 mm; >35mm ≤ 55 mm; ≥56 mm ≤ 87 mm; > 87 mm.



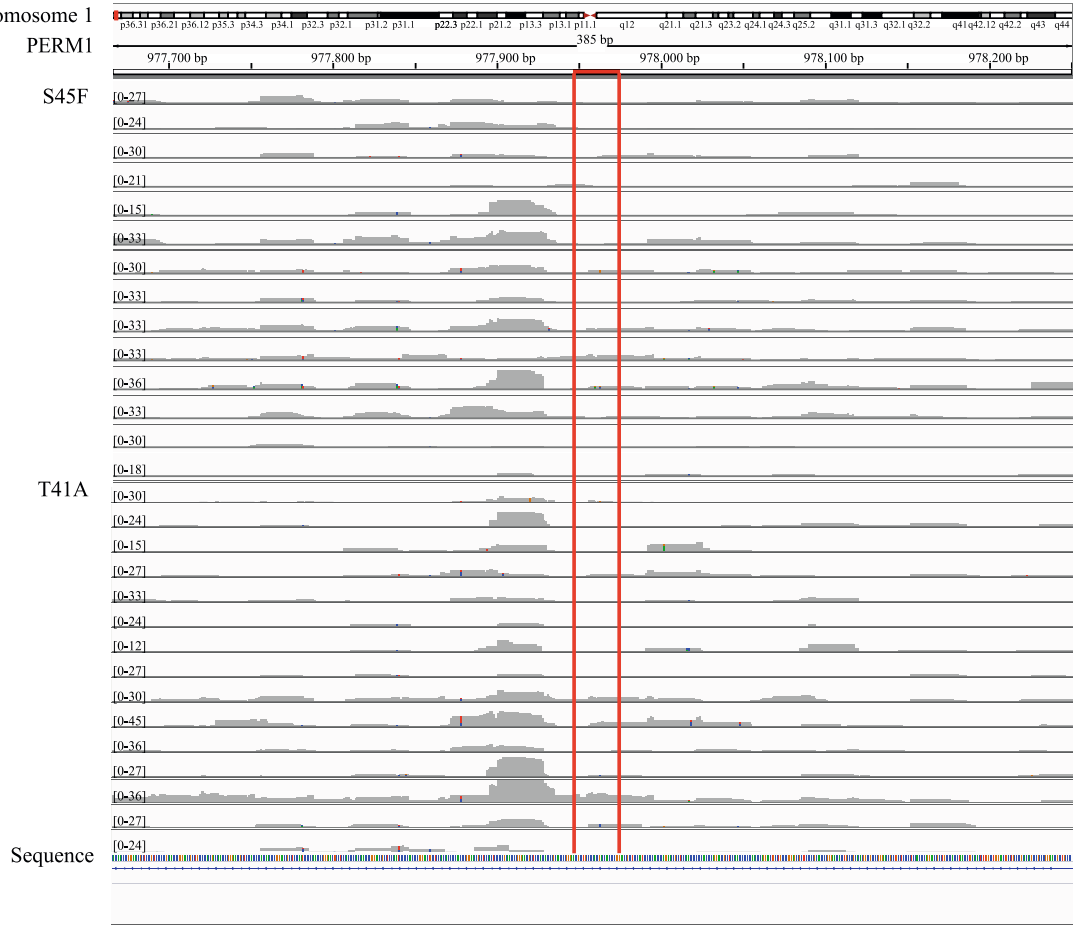
Supplemental Figure 3. Supervised hierarchical clustering based on differentially methylated regions (DMRs) between DTF tumour size extremes. Tumour sizes were based on initial imaging data obtained at diagnosis. The smallest tumours ≤ 34 mm were compared to the largest tumours > 87 mm.



Supplemental Figure 4A. Quantitative visualization of the differentially methylated regions with a fold-change ≥ 1.5 between S45F and T41A DTF. The DMRs are associated with NLRP4. In the top panel, the chromosome involved with the location of the gene indicated by a small red block is depicted. The panels below represent the read counts found in either the S45F or T41A samples with the red box designating the location of the DMR.

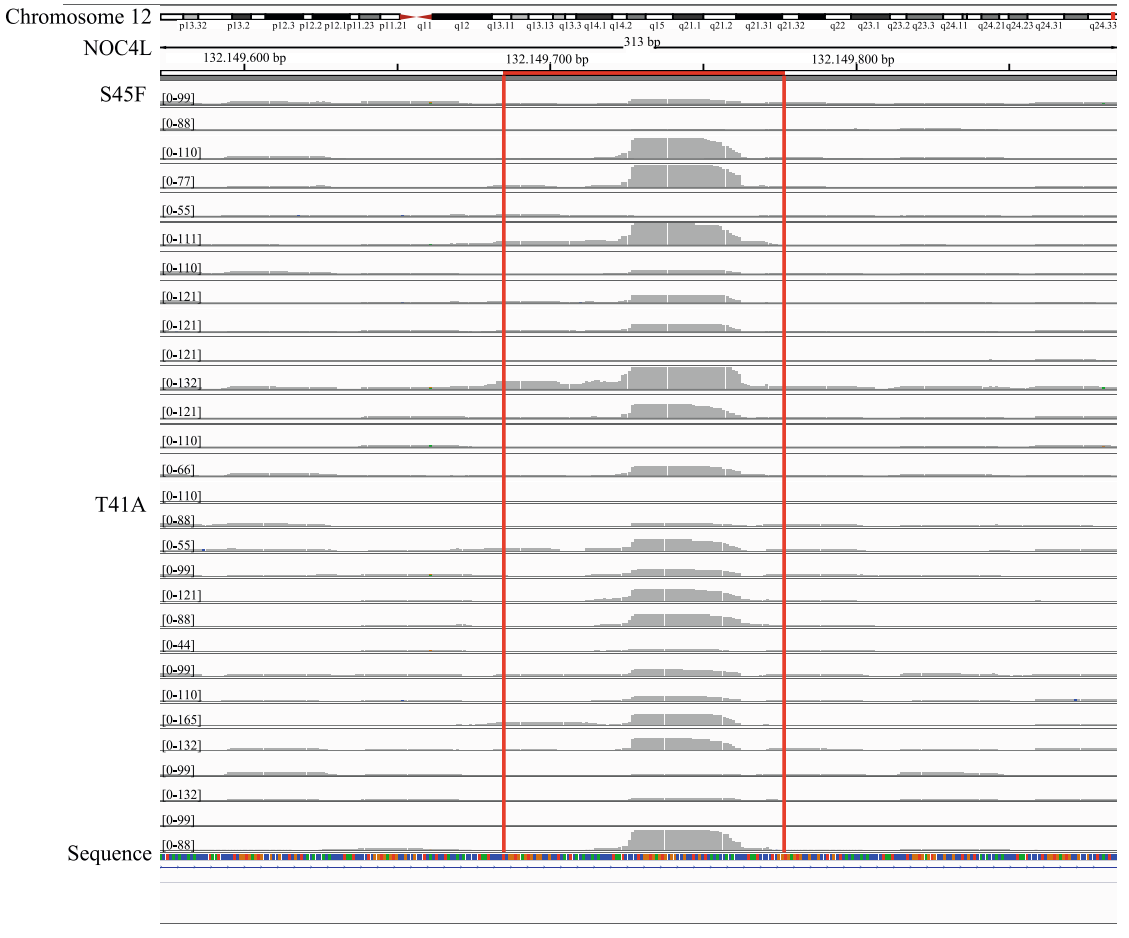


Supplemental Figure 4B. Quantitative visualization of the differentially methylated regions with a fold-change ≥ 1.5 between S45F and T41A DTF. The DMRs are associated with FOXK2. In the top panel, the chromosome involved with the location of the gene indicated by a small red block is depicted. The panels below represent the read counts found in either the S45F or T41A samples with the red box designating the location of the DMR.

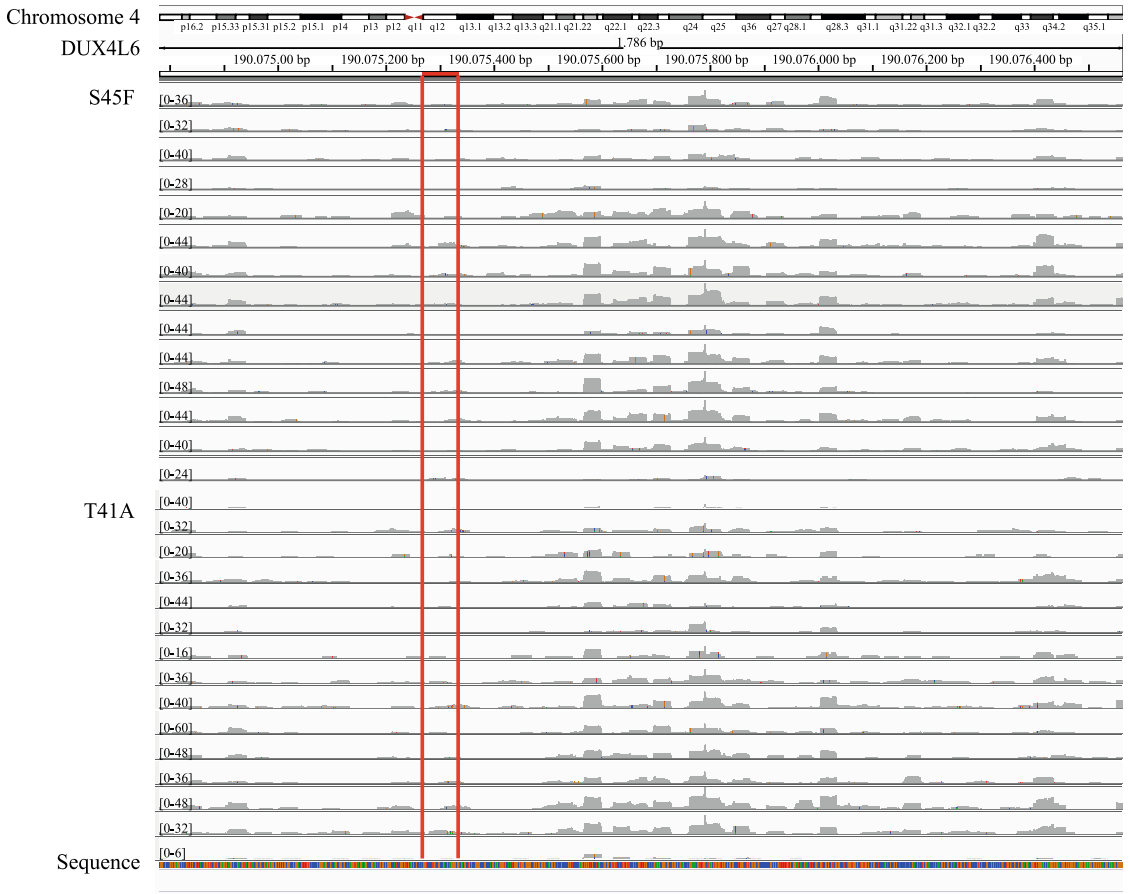


Supplemental Figure 4C. Quantitative visualization of the differentially methylated regions with a fold-change ≥ 1.5 between S45F and T41A DTF. The DMRs are associated with PERM1. In the top panel, the chromosome involved with the location of the gene indicated by a small red block is depicted. The panels below represent the read counts found in either the S45F or T41A samples with the red box designating the location of the DMR.

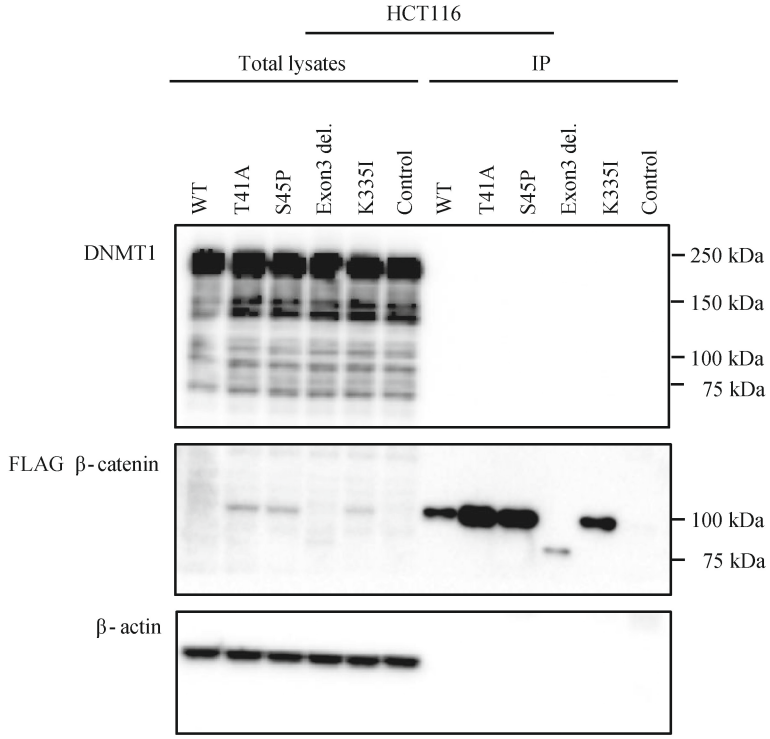
Supplemental Figure 4D. Quantitative visualization of the differentially methylated regions with a fold-change ≥ 1.5 between S45F and T41A DTF. The DMRs are associated with CCDC6. In the top panel, the chromosome involved with the location of the gene indicated by a small red block is depicted. The panels below represent the read counts found in either the S45F or T41A samples with the red box designating the location of the DMR.



Supplemental Figure 4E. Quantitative visualization of the differentially methylated regions with a fold-change ≥ 1.5 between S45F and T41A DTF. The DMRs are associated with NOC4L. In the top panel, the chromosome involved with the location of the gene indicated by a small red block is depicted. The panels below represent the read counts found in either the S45F or T41A samples with the red box designating the location of the DMR.



Supplemental Figure 4F. Quantitative visualization of the differentially methylated regions with a fold-change ≥ 1.5 between S45F and T41A DTF. The DMRs are associated with DUX4L6. In the top panel, the chromosome involved with the location of the gene indicated by a small red block is depicted. The panels below represent the read counts found in either the S45F or T41A samples with the red box designating the location of the DMR.



Supplemental Figure 5. DNMT1 is not co-precipitated with wild-type or mutant CTNNB1 (β-catenin). HCT116 cells were transfected with plasmids driving the expression of FLAG-tagged wild-type β-catenin (WT) or FLAG-tagged mutant versions of β-catenin (T41A; S45P; Exon 3 deletion mutant; K335I). As a control cells were transfected with the empty vector. At 48 h post-transfection cell lysates were prepared from which the FLAG-tagged β-catenin variants were immunoprecipitated. Western Blot analysis was used to examine DNMT1, β-catenin and β-actin protein expression in the total lysates and immunoprecipitates.

II

Diagnosis and Treatment

5

Differential diagnosis and mutation stratification of desmoid-type fibromatosis on MRI using radiomics

Milea J.M. Timbergen*, Martijn P.A. Starmans*, Guillaume A. Padmos, Dirk J. Grünhagen, Geert J.L.H. van Leenders, David F. Hanff, Cornelis Verhoef, Wiro J. Niessen, Stefan Sleijfer, Stefan Klein, Jacob J. Visser

** equal contribution*

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Abstract

Background

Diagnosing desmoid-type fibromatosis (DTF) requires an invasive tissue biopsy with β-catenin staining and *CTNNB1* mutational analysis, and is challenging due to its rarity. The aim of this study was to evaluate radiomics for distinguishing DTF from soft tissue sarcomas (STS), and in DTF, for predicting the *CTNNB1* mutation types.

Materials and methods

Patients with histologically confirmed extremity STS (non-DTF) or DTF and at least a pretreatment T1-weighted (T1w) MRI scan were retrospectively included. Tumours were semi-automatically annotated on the T1w scans, from which 411 features were extracted. Prediction models were created using a combination of various machine learning approaches. Evaluation was performed through a 100x random-split cross-validation. The model for DTF versus non-DTF was compared to classification by two radiologists on a location matched subset.

Results

The data included 203 patients (72 DTF, 131 STS). The T1w radiomics model showed a mean AUC of 0.79 on the full dataset. Addition of T2w or T1w post-contrast scans did not improve the performance. On the location matched cohort, the T1w model had a mean AUC of 0.88 while the radiologists had an AUC of 0.80 and 0.88, respectively. For the prediction of the *CTNNB1* mutation types (S45F, T41A and wild-type), the T1w model showed an AUC of 0.61, 0.56, and 0.74.

Conclusions

Our radiomics model was able to distinguish DTF from STS with high accuracy similar to two radiologists, but was not able to predict the *CTNNB1* mutation status.

Introduction

Sporadic desmoid-type fibromatosis (DTF) is a rare borderline, soft tissue tumour arising in musculoaponeurotic structures [1]. Worldwide epidemiological data is lacking, but population studies in Scandinavia and the Netherlands show a low incidence of 2.4–5.4 cases per million per year [2, 3]. Early recognition and diagnosis of DTF is therefore challenging.

On MRI, DTF can display a wide variety of enhancement patterns [4]. DTF has imaging characteristics that are often associated with soft tissue sarcomas (STS), such as crossing fascial boundaries, an invasive growth pattern, little central necrosis, mildly hyperintense on T1-weighted (T1w) MRI, and hyperintense and heterogeneous on T2-weighted (T2w) MRI with hypointense bands [5]. Hence, the distinction between DTF and STS, i.e., non-DTF, can be difficult. An invasive tissue biopsy, with additional immunohistochemical staining for β-catenin and mutation analysis of the *CTNNB1* (β-catenin) gene, is therefore currently required to differentiate DTF from non-DTF [6].

As DTF is a borderline tumour who is unable to metastasize, and requires a different treatment regimen than malignant STS, this distinction is highly relevant. Differentiation between DTF and STS based on imaging would be beneficial because of the rarity of DTF, making clinical and pathological recognition challenging. Furthermore, DTF exhibits an aggressive growth pattern and growth might be stimulated after (surgical) trauma, including biopsies [7]. Avoiding (multiple) harmful biopsies which potentially cause tumour growth is therefore of great importance.

Several studies have addressed the prognostic role of the *CTNNB1* mutation in DTF [8-10], as serine 45 (S45F) tumours appear to have a higher risk of recurrence after surgery compared to threonine 41 (T41A) and wild-type (WT) (i.e. no *CTNNB1* mutation [11]) tumours [12]. Obtaining the *CTNNB1* mutation status is for diagnostic purposes and to guide the clinical work-up, but, for now, the *CTNNB1* mutation status has no therapeutic consequences [13]. The majority of DTF harbours a *CTNNB1* mutation at either T41A or S45F [8]. Assessment of the mutation status is currently done by Sanger Sequencing or Next Generation Sequencing, which are time consuming and expensive.

In radiomics, large amounts of quantitative imaging features are related to clinical outcome [14]. Radiomics may serve as a non-invasive surrogate to contribute to diagnosis, prognosis and treatment planning [15, 16]. Based on the results of previous studies in cancer [17], we hypothesized that radiomics may also be useful in DTF.

This study investigated whether a radiomics model based on MRI is able to 1) distinguish DTF from non-DTF in the extremities, and 2) to predict the *CTNNB1* mutation status of DTF. Additionally, in the DTF versus non-DTF distinction, we evaluated which of the included MRI sequences has the highest predictive value.

Material and methods

Data collection

Approval by the Erasmus Medical Center (MC) institutional review board (MEC-2016-339) was obtained. Patients diagnosed or referred to the Erasmus MC between 1990-2018 with a histologically proven primary or recurrent DTF were included. This resulted in a multicentre imaging dataset as patients referred to our sarcoma expert institute often received imaging at their referring hospital. The most frequently used imaging modality prior to treatment was T1w-MRI, and its availability was used as an inclusion criterion [18]. When available, other sequences such as T2w, T1w post-contrast, dynamical contrast enhanced (DCE), proton density (PD) and diffusion weighted imaging (DWI) MRI were collected.

For the differential diagnosis (DTF versus non-DTF), histologically confirmed malignant extremity STS were included. Benign STS were excluded, because this distinction is clinically less relevant. Non-extremity STS were excluded because of the infrequent use of MRI. Although DTF tumours commonly occur in the abdominal wall, their differential diagnosis is broad and includes pseudo-tumours such as myositis, nodular fasciitis and hematomas, and tumours such as lipomas, STS, endometriosis, carcinomas, lymphomas and metastasis [19]. Hence, we decided to focus on the distinction between DTF and STS, and included patients with a histologically proven primary fibromyxosarcoma, myxoid liposarcoma or leiomyosarcoma of the extremities. Similar to the DTF, patients with at least a pre-treatment T1w-MRI were retrospectively included.

Sex, age at diagnosis, and tumour site were collected. For the DTF, in case of a missing *CTNNB1* mutation status, Sanger Sequencing was performed after review of formalin-fixed paraffin-embedded tumour sections by a pathologist. Cases with a known *CTNNB1* mutation did not undergo additional review by a pathologist. Poor scan quality (e.g., artefacts), poor DTF DNA quality with failure of sequencing, and *CTNNB1* mutation other than S45F, T41A or WT led to exclusion.

Radiomics feature extraction

The tumours were all manually segmented once on the T1w-MRI by one of two clinicians under supervision of a musculoskeletal radiologist (4 years of experience). A subset of 30 DTF tumours was segmented by both clinicians, in which intra-observer variability was evaluated through the pairwise Dice Similarity Coefficient (DSC), with DSC > 0.70 indicating good agreement [20]. To transfer the segmentations to the other sequences, all sequences were automatically aligned to the T1w-MRI using image registration with the Elastix software [21]. For each lesion, per MRI sequence, 411 features quantifying intensity, shape and texture were extracted. Details can be found in Supplemental Materials 1 and Supplemental Table 1.

Decision model creation

To create a decision model from the features, the WORC toolbox was used, see Figure 1 [22-24]. In WORC, the decision model creation consists of several steps, e.g., feature selection, resampling, and machine learning. WORC performs an automated search amongst a variety of algorithms for each step and determines which combination of algorithms maximizes the prediction performance on the training set. More details can be found in Supplemental Materials 2.

Evaluation

Evaluation of all models was done through a 100x random-split cross-validation. In each iteration, the data was randomly split in 80% for training and 20% for testing in a stratified manner, to make sure the distribution of the classes in all sets was similar to the original (Supplemental Figure 1). Within the training set, model optimization was performed using an internal cross-validation (5x). Hence, all optimization was done on the training set to eliminate any risk of overfitting on the test set.

For the differential diagnosis cohort, a binary classification model was created using a variety of machine learning models. For the DTF cohort (predicting the *CTNNB1* mutation), a multiclass classification model was created using random forests.

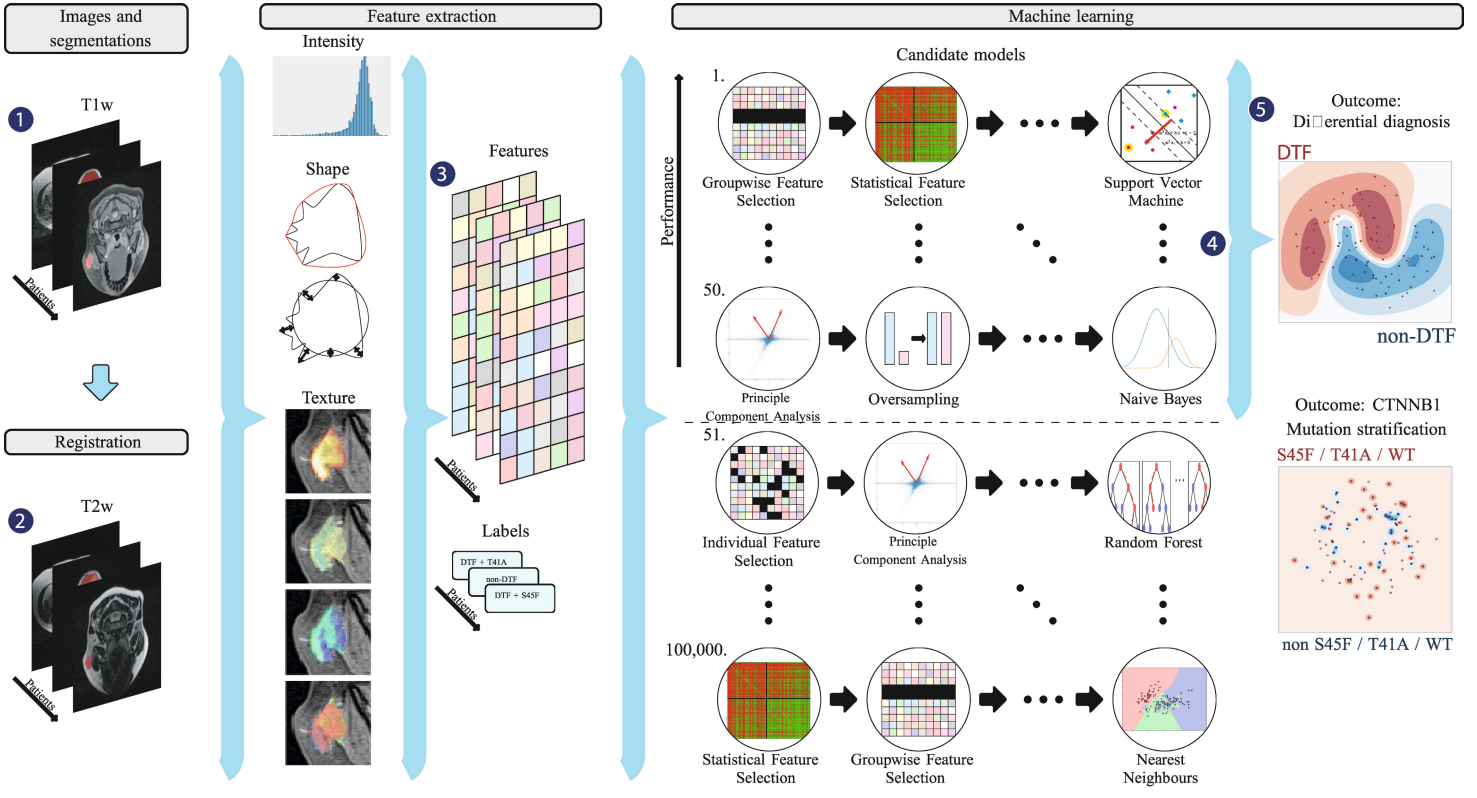

Figure 1. Schematic overview of the radiomics approach: adapted from 24. Processing steps include segmentation of the tumour on the T1-weighted (T1w) MRI (1), registration of the T1w to the T2-weighted (T2w) MRI to transform this segmentation to the T2w-MRI (2), feature extraction from both the T1w-MRI and the T2w-MRI (3) and the creation of machine learning decision models (5), using an ensemble of the best 50 workflows from 100,000 candidates (4), where the workflows are different combinations of the processing and analysis steps. *DTF, desmoid-type fibromatosis*

Performance was evaluated using the Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) curve, balanced classification accuracy (BCA), sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV). For the multiclass models, we reported the multiclass AUC [25] and overall BCA [26]. The positive classes included: DTF in the differential diagnosis, and the presence of the mutation in the mutation analysis. The 95% confidence intervals were constructed using the corrected resampled t-test, thereby taking into account that the samples in the cross-validation splits are not statistically independent [27]. Both the mean and the confidence intervals are reported. ROC confidence bands were constructed using fixed-width bands [28].

To assess the predictive value of the various features, models were trained based on: 1) volume; 2) age and sex; 3) T1w-MRI imaging; 4) T1w-MRI imaging, age and sex. Model 1 was created to verify that the imaging models were not solely based on volume. Model 2 was created to evaluate potential age and gender biases. In model 4, the imaging and clinical characteristics are combined by using both the imaging features and age and sex as features for a total of 413 features. This allows WORC to combine the imaging and clinical characteristics in the most optimal way. Additionally, a model was made for each combination of T1w-MRI and one of the other included MRI sequences (e.g., based on T1w-MRI and T2w-MRI) to evaluate the added value of these other sequences. When a sequence was missing for a patient, feature imputation was used to estimate the missing values.

The code for the feature extraction, model creation and evaluation has been published open-source [29].

Model insight

To explore the predictive value of individual features, the Mann-Whitney *U* univariate statistical test was used. P-values were corrected for multiple testing using the Bonferroni correction, and were considered statistically significant at a p-value < 0.05. Feature robustness to variations in the segmentations was assessed on the subset of 30 DTF segmented by two observers using the intra-class correlation coefficient (ICC), were an ICC > 0.75 indicated good reliability [30]. To evaluate model reliability, a separate model was trained using only these features with a good reliability. To gain insight into the models, the patients were ranked based on the consistency of the model predictions. Typical examples for each class consisted of the patients that were correctly classified in all cross-validation iterations; atypical vice versa.

Classification by radiologists

To compare the models with clinical practice, the tumours were classified by two musculoskeletal radiologists (5 and 4 years of experience), which had access to all available MRI sequences, age, and sex. They were specifically instructed to distinguish between STS and DTF. Classification was made on a ten-point scale to indicate the radiologists' certainty. As only extremity STS were selected for the non-DTF group, a location-matched database was used. This included all extremity DTF and the same number of non-DTF. Agreement between the radiologists was evaluated using Cohen's kappa. The radiomics models were evaluated as well in this cohort. In each cross-validation iteration, these models were trained on 80% of the full dataset, but tested only on patients from the location-matched cohort in the other 20% of the dataset. The DeLong test was used to compare the AUCs [31].

Results

Study selection and population

The dataset included 203 patients; see Table 1 for the clinical characteristics. The differential diagnosis cohort consisted of 64 fibromyxosarcomas, 31 leiomyosarcomas, 36 myxoid liposarcomas, and 72 DTFs (65 primary, 7 recurrent), of which 61 were suitable for the mutation analysis.

The dataset originated from 68 scanners, resulting in a large heterogeneity in the acquisition protocols, see Table 2. From the 72 patients in the DTF cohort, there were 30 T1w post-contrast (42%), 49 T1w post-contrast FatSat (68%), 34 T2w (47%), 33 T2w FatSat (46%), 3 proton density (PD) (4%), 18 DCE (25%) and 3 DWI (4%) MRI scans. Due to the limited availability of the PD, DCE, and DWI sequences, besides the T1w-MRI, only the T1w post-contrast and T2w (with/without FatSat) sequences were analysed.

On the subset of 30 DTF that was segmented by both observers, the mean DSC was 0.77 (standard deviation of 0.20), indicating good agreement. An example of the image registration results is depicted in Figure 2.

Table 1. Clinical characteristics of both cohorts.

	Differential diagnosis cohort				Mutation analysis cohort
	DTF n = 72	**Fibro-myxosarcoma n = 64**	**Leiomyo-sarcoma n = 31**	**Myxoid liposarcoma n = 36**	**DTF n = 61**
Sex					
Male	16 (22%)	41 (64%)	19 (61 %)	22 (61%)	15 (25%)
Female	56 (78%)	23 (36%)	12 (39%)	14 (39%)	46 (75%)
Age median (IQR)	36 (23-47)	67 (54-77)	66 (55-73)	42 (35-56)	36 (22-47)
Tumour site					
Head / neck	12 (17%)	-	-	-	11 (18%)
Chest aperture	4 (6%)	-	-	-	3 (5%)
Abdominal wall	24 (33%)	-	-	-	16 (26%)
Back	11 (15%)	-	-	-	10 (16 %)
Intra-abdominal	1 (1%)	-	-	-	1 (2%)
Upper extremity	5 (7%)	6 (9%)	7 (23%)	1 (3%)	5 (8%)
Lower extremity	15 (21%)	58 (91%)	24 (77%)	35 (97 %)	15 (25%)
Tumour size in cm[a] median (IQR)	6.3 (4.1-9.8)	7.0 (4.9-12.9)	8.3 (5.2-9.4)	12.8 (8.5-15.3)	6.3 (4.1-9.5)
Volume in cl median (IQR)	2.0 (0.5-9.8)	5.6 (1.1-34.1)	8.2 (1.7-11.4)	16.8 (5.2-37.4)	2.2 (0.7-9.6)
Mutation type					
T41A	NA	NA	NA	NA	24 (39%)
S45F	NA	NA	NA	NA	16 (26%)
Wild-type	NA	NA	NA	NA	21 (34%)
MRI sequences					
T2w FS	33 (46%)	37 (58%)	15 (48%)	16 (44%)	26 (43%)
T2w non-FS	32 (70%)	37 (64%)	19 (39%)	19 (43%)	26 (61%)
T1w PC FS	49 (70%)	32 (50%)	19 (48%)	22 (51%)	43 (70%)
T1w PC non-FS	30 (43%)	24 (48%)	11 (23%)	17 (33%)	25 (35%)

DTF, desmoid-type fibromatosis; IQR, interquartile range; cm, centimetre; cl, centilitre; MRI, magnetic resonance imaging; FS, FatSat; PC, post-contrast.

[a] *maximum diameter automatically measured in three planes.*

Percentages might not add up to 100% in total because of rounding.

Table 2. Properties of the acquisition protocols of the 203 T1-weighted MRI sequences in the dataset.

Property	**Number**	**%**		
Magnetic field strength				
1T	20	10		
1.5T	167	82		
3T	16	8		
Manufacturer				
Siemens	93	46		
Philips	79	39		
General Electrics	27	13		
Toshiba	4	2		
Setting (Unit)	**Mean**	**Std.**	**Min**	**Max**
Slice thickness (mm)	4.66	1.45	1.0	11.0
Repetition time (ms)	619	533	0.0	4620
Echo time (ms)	14	7	2.0	94.0

T, tesla; Std, standard deviation; mm, millimetre; ms, milliseconds

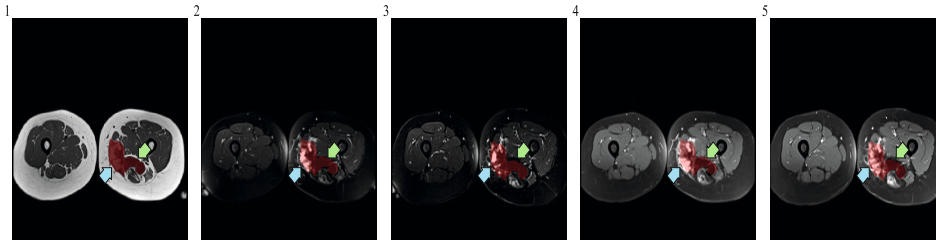

Figure 2. Segmentations on various MRI sequences before and after applying image registration in a desmoid-type fibromatosis case. The arrows are at the same position in each image and point at two details where the (mis)alignment is evident. (1) Original T1-weighted (T1w) MRI; (2) Original T2w-MRI; (3) Registered T2w-MRI; (4) Original T1w post-contrast MRI ; (5) Registered T1w post-contrast MRI

Differential diagnosis

The performance of models 1-6 for the differential diagnosis is shown in Table 3. Model 1, based on volume, showed little predictive value (mean AUC of 0.69). Model 2, based on age and sex, performed better (mean AUC of 0.86). Model 3, based on T1w-MRI, had a mean AUC of 0.79, thus performing worse than age and sex, but better than volume alone. Model 4, combining the T1w-MRI, age, and sex, showed little improvement in terms of mean AUC (0.88) over model 2. Addition of a T2w-MRI, i.e. model 5, or T1 post-contrast MRI, i.e. model 6, both with or without FatSat, both yielded a minor overall improvement over model 3 (mean AUC of 0.84 and 0.84, respectively). These observations were confirmed by the ROC curves in Figure 3. The models using either only non-FatSat or FatSat scans, both for the T2w and T1w post-contrast MRI, faired similar, see Supplemental Table 2.

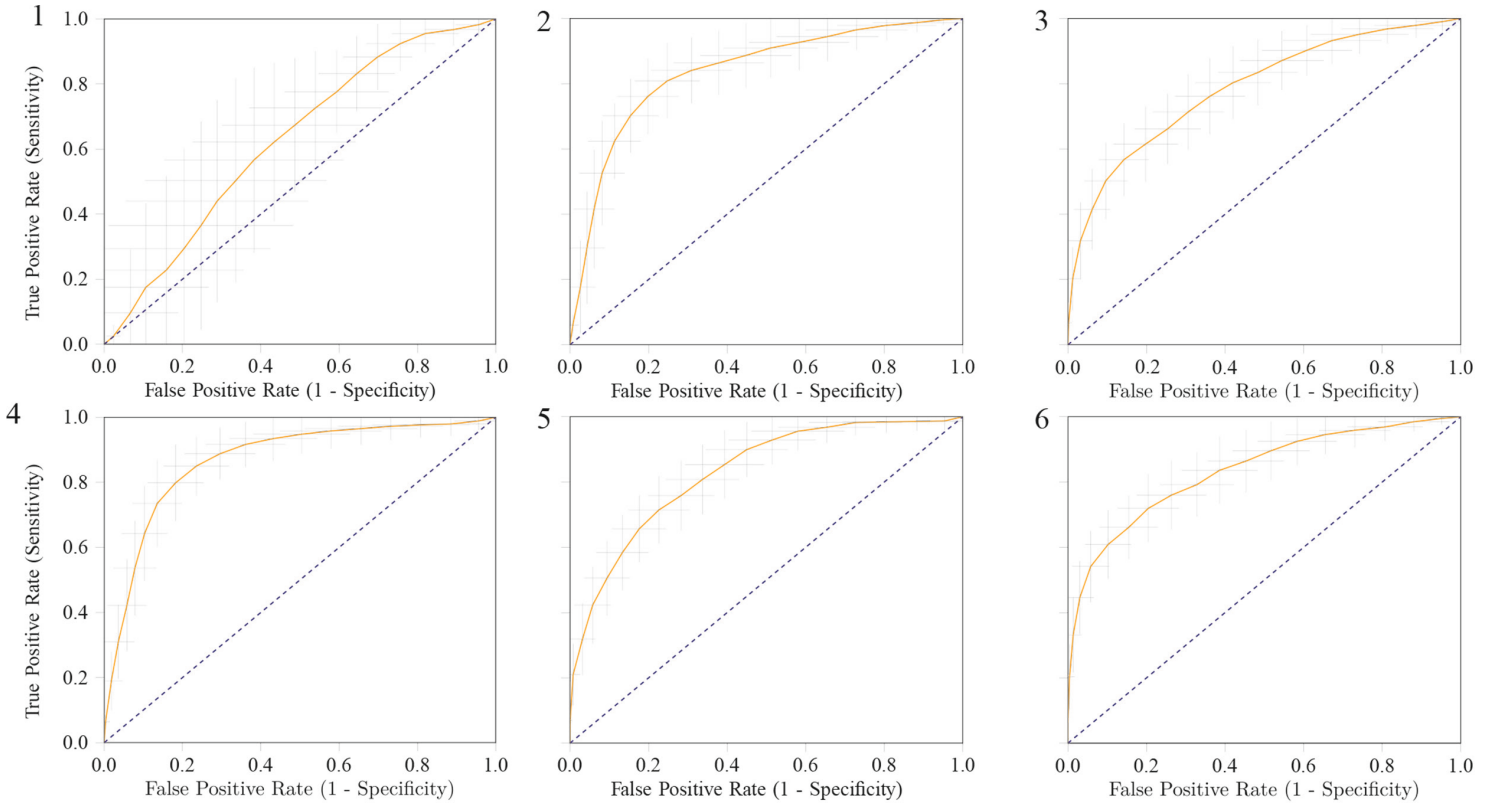

Figure 3. Receiver operating characteristic curves of the radiomics models based on volume (1); age and sex (2); T1-weighted (T1w) features (3); T1w features, age, and sex (4); T1w + T2weighted imaging features (5); and T1w + T1w post-contrast imaging features (6). The grey crosses identify the 95% confidence intervals of the 100x random-split cross-validation; the orange curve depicts the mean.

Table 3. Performance of the radiomics models for the DTF differential diagnosis based on: model 1: volume only; model 2: age and sex only; model 3: T1w imaging features, including volume; model 4: the combination of T1w imaging features and age and sex; model 5: the combination of T1w and T2w imaging features; and model 6: the combination of T1w and T1w post-contrast imaging features. Outcomes are presented with the 95% confidence interval.

	Model 1 Volume	**Model 2** Age + Sex	**Model 3** T1w	**Model 4** T1w + Age + Sex	**Model 5** T1w + T2w	**Model 6** T1w + T1w post-contrast
AUC	0.69 [0.61, 0.76]	0.86 [0.79, 0.92]	0.79 [0.73, 0.85]	0.88 [0.82, 0.93]	0.84 [0.78, 0.89]	0.84 [0.78, 0.90]
BCA	0.59 [0.53, 0.65]	0.78 [0.71, 0.86]	0.71 [0.65, 0.77]	0.79 [0.72, 0.86]	0.68 [0.62, 0.75]	0.75 [0.69, 0.81]
Sensitivity	0.80 [0.70, 0.91]	0.78 [0.66, 0.90]	0.61 [0.49, 0.72]	0.70 [0.57, 0.83]	0.43 [0.31, 0.55]	0.62 [0.52, 0.73]
Specificity	0.39 [0.28, 0.49]	0.79 [0.71, 0.87]	0.81 [0.73, 0.89]	0.88 [0.82, 0.94]	0.94 [0.88, 0.99]	0.88 [0.82, 0.95]
NPV	0.50 [0.71, 0.89]	0.88 [0.81, 0.94]	0.80 [0.76, 0.75]	0.85 [0.80, 0.91]	0.76 [0.72, 0.80]	0.81 [0.76, 0.85]
PPV	0.41 [0.36, 0.46]	0.72 [0.57, 0.76]	0.64 [0.53, 0.75]	0.76 [0.67, 0.86]	0.80 [0.66, 0.94]	0.76 [0.65, 0.88]

T1w, T1-weighted; T2w, T2-weighted; AUC, area under the receiver operator characteristic curve; BCA, balanced classification accuracy; NPV, negative predictive value; PPV, positive predictive value

Comparison with radiologists

As described in the methods, for the comparison with radiologists, a location-matched cohort consisting of all extremity DTFs and an equal amount of extremity non-DTF was used. To this end, all 20 extremity DTFs and 20 randomly selected extremity non-DTFs were included in the location-matched cohort. The performance of radiomics and the radiologists in this cohort is shown in Table 4: model 1 and 5-6 were omitted from the results for brevity. The AUCs of the r0adiomics models (model 2: 0.93; model 3: 0.88; model4: 0.98) were generally higher than both radiologists 1 (0.80) and 2 (0.88). This is confirmed by the ROC curves in Figure 4. Cohen's kappa between the two radiologists was 0.40, indicating intermediate observer agreement. A DeLong power analysis of the AUCs resulted in a power of only 0.1. Due to the limited power, the p-values of the DeLong test were omitted.

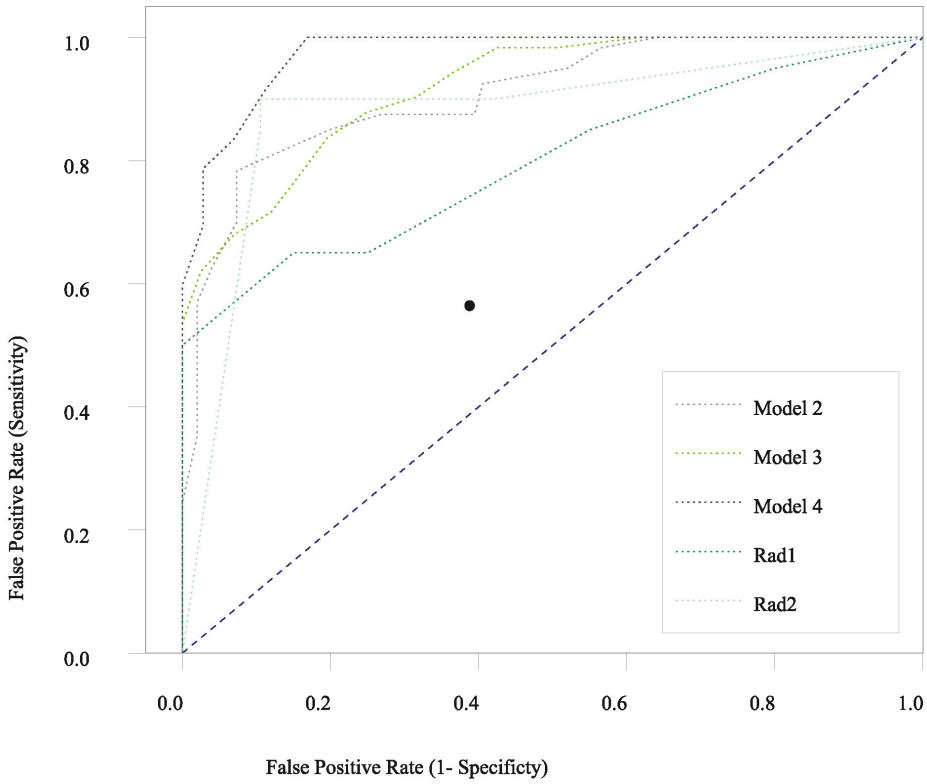

Figure 4. Receiver operating characteristic curves of the radiomics models based on age and sex (model 2); imaging (model 3); and imaging, age and sex (model 4); and those of the radiologists (Rad1 and Rad2), in the location-matched cohort.

Table 4. Performance of the two radiologists and the radiomics models in differentiating between DTF (n=20) and non-DTF (n = 20) in the location-matched cohort. Outcomes are presented with the 95% confidence interval.

	Model 2 Age +Sex	**Model 3** T1w	**Model 4** T1w + Age + Sex	**Rad 1**	**Rad 2**
AUC	0.93 [0.84, >1]	0.87 [0.73, >1]	0.98 [0.92, >1]	0.80	0.88
BCA	0.85 [0.71, 1.00]	0.71 [0.56, 0.87]	0.88 [0.77, 0.99]	0.75	0.90
Sensitivity	0.79 [0.57, >1]	0.49 [0.21, 0.77]	0.78 [0.57, 1.00]	0.65	0.90
Specificity	0.90 [0.71, >1]	0.93 [0.78, >1]	0.98 [0.91, >1]	0.85	0.89
NPV	0.82 [0.61, >1]	0.65 [0.43, 0.76]	0.82 [0.64, >1]	0.71	0.89
PPV	0.91 [0.72, >1]	0.81 [0.47, >1]	0.98 [0.91, >1]	0.81	0.90

T1w: T1-weighted; AUC: area under the receiver operator characteristic curve; BCA: balanced classification accuracy; PPV: positive predictive value; NPV: negative predictive value

CTNNB1 mutation status stratification

Table 5 depicts the performance of the radiomics models for the *CTNNB1* mutation stratification. Model 4, using T1w-MRI, age, and sex, had a high specificity (S45F: 0.83, T41A: 0.59 and WT: 0.72), but a sensitivity similar to guessing (S45F: 0.15, T41A: 0.49 and WT: 0.56). This indicates a strong bias in the models towards the negative classes, i.e. not-S45F, not-T41A and not-WT. As model 4 did not perform well, models 1, 2, and 3 were omitted from the results, as these contain a subset of these features. Adding the T2w or T1w post-contrast imaging, i.e. models 5 and 6, did not improve the performance. Hence, the models using either only non-FatSat or FatSat scans were omitted, as these contain subsets of the scans from models 5 and 6.

Model insight

As the *CTNNB1* mutation status stratification models did not perform well, the model insight analysis was only conducted for the differential diagnosis. The p-values from the Mann-Whitney *U* test between the DTF and non-DTF patients of all features are shown in Supplemental Table 3. In the feature importance analysis, 76 T1w-MRI features had significant p-values ($5.4x10^{-8}$ to $4.8x10^{-2}$). These included two intensity features (entropy and peak), two shape features (radial distance and volume), and 72 texture features. The p-value of age ($1x10^{-11}$) was lower than that of all imaging features. The ICC values of all T1w-MRI features are shown in Supplemental Table 4. Of the 411 features, 270 (66%) had an ICC > 0.75 and thus good reliability. Only using these features with a good reliability in model 3 did not alter the performance.

Table 5. Performance of the random forest multilabel radiomics models for the DTF CTNNB1 mutation stratification based on; model 4: T1w imaging features, age and sex; model 5: T1w + T2w imaging features; and model 6: T1w + T1w post-contrast imaging features. Model 4 was evaluated for a single class (S45F, T41A, and WT) or the overall performance (All). Outcomes are presented with the 95% confidence interval.

	Model 4 - S45F T1w+ age +sex	**Model 4 - T41A** T1w + age + sex	**Model 4 - WT** T1w + age + sex	**Model 4 - All** T1w + age + sex	**Model 5 - All** T1w + T2w	**Model 5 - All** T1w + T1w post-contrast
AUC	0.61 [0.44, 0.77]	0.56 [0.43, 0.68]	0.74 [0.60, 0.87]	0.63 [0.54, 0.72]	0.63 [0.53, 0.72]	0.60 [0.50, 0.69]
BCA	0.48 [0.35, 0.61]	0.53 [0.42, 0.64]	0.65 [0.54, 0.75]	0.56 [0.47, 0.64]	0.57 [0.48, 0.66]	0.53 [0.44, 0.61]
Sensitivity	0.15 [<0, 0.37]	0.49 [0.27, 0.71]	0.56 [0.35, 0.77]	NA	NA	NA
Specificity	0.83 [0.67, 0.98]	0.59 [0.41, 0.76]	0.72 [0.55, 0.89]	NA	NA	NA
NPV	0.76 [0.70, 0.82]	0.65 [0.53, 0.77]	0.73 [0.64, 0.82]	NA	NA	NA
PPV	0.17 [<0, 0.45]	0.42 [0.28, 0.56]	0.59 [0.40, 0.77]	NA	NA	NA

T1w, T1-weighted MRI; T2w,: T2-weighted MRI; AUC, area under the receiver operator characteristic curve; BCA, balanced classification accuracy; PPV, positive predictive value; NPV, negative predictive value; WT, wild-type, NA, not applicable

As we are mostly interested in which imaging features define typical DTF, and not age and sex, the patient ranking was conducted for model 3. Of the 203 patients, 104 tumours (24 DTFs, 80 non-DTFs) were always classified correctly by model 3, i.e. in all 100 cross-validation iterations. Nineteen tumours (17 DTFs, 2 non-DTFs) were always classified incorrectly. In Figure 5, MRI slices of such typical and atypical examples of DTFs are shown.

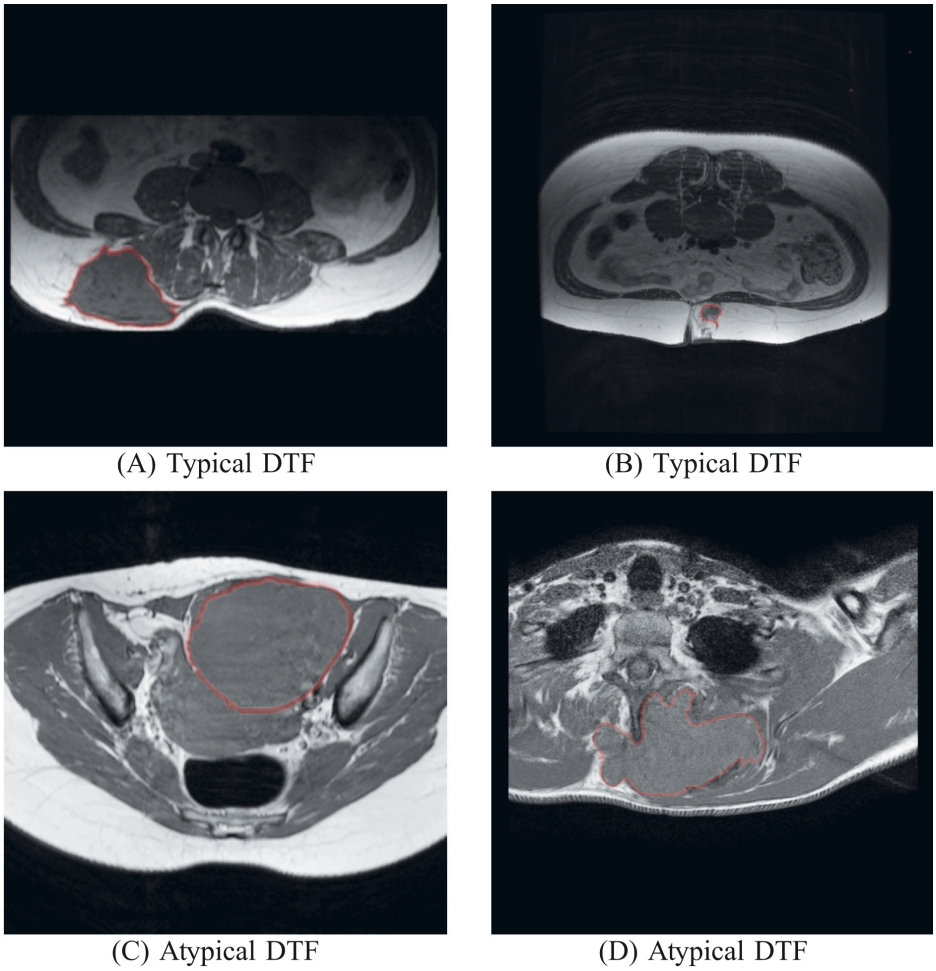

Figure 5. The typical examples (A and B) are two cases always classified correctly by the T1-weighted (T1w) imaging model; the atypical examples (C and D) are two cases always classified incorrectly by the T1w imaging model.

Discussion

This study showed that radiomics based on T1w-MRI can distinguish DTF from STS. Adding T2w or T1w post-contrast MRI did not substantially improve the model. The DTF *CTNNB1* mutation status could not be predicted through radiomics. To our knowledge, this is the first study to evaluate the DTF differential diagnosis and mutation status through an automated radiomics approach.

Age and sex appeared to be strong predictors for the diagnosis of DTF, performing better than T1w-MRI. The combination of imaging, age and sex did not improve the model. This implies that age and sex are sufficient for distinguishing DTF from STS. In line with previous nationwide DTF cohort studies, females represented the majority of our cohort, with a lower median age compared to the median age of the patients from the non-DTF group [2, 32]. The relation in our database may however be too strong, and thereby not representative of clinical practice. For example, above 63 years of age, our database included 60 non-DTF and only a single DTF. While the peak incidence of DTF is between 20 to 40 years, DTF can affect patients of all ages with reported ranges from 2 to 90 years [32]. Simply classifying all tumours in patients above 63-years as non-DTF, regardless of any tumour (imaging) information, is unfeasible. Such a model cannot be applied in the general population, while the model purely based on T1w-MRI imaging, as it does not use any population-based information. Our cohort might be biased due to the focus on MRI and the extremity as a site, while other modalities (e.g., CT or ultrasound) may be used for certain tumour sites or for certain types of patients. Further research should include the expansion of our dataset to make especially the age distribution more representative.

To estimate the clinical value of our model, we compared the performance with the assessment of two radiologists. The model based on imaging performed similar to the radiologists. The model combining age, sex and imaging features, using the same dataset as the radiologist, had a higher AUC than the musculoskeletal radiologists. However this model may suffer from the selection bias as mentioned in the previous section. The agreement between the radiologists was intermediate, indicating observer dependence in the prediction. The radiomics model is observer independent, assuming the segmentation is reproducible as indicated by the high DSC and ICCs, and will always give the same prediction on the same image.

The DTF differential diagnosis is highly important for treatment decisions, but difficult on imaging due to its rarity, while using invasive biopsies brings risks such as tumour

growth. The use of our T1w-MRI radiomics model may therefore aid early recognition and diagnosis of DTF, thus shortening the diagnostic delay by enabling direct referral to an STS expertise centre. Since all routine MRI protocols include a T1w-MRI, our radiomics method is generalizable, feasible and applicable for use in daily clinical practice. After further model optimization, it may serve as a quick, non-invasive, and low-cost alternative for a biopsy, currently limited to extremities due to the used dataset.

Additionally, we investigated the predictive value of sequences other than T1w-MRI. The number of available sequences was however limited due to the multicentre imaging dataset. Although T2w-MRI is often used to correlate DTF signal intensity with prognosis or response to therapy [33-36], in the current study T2w-MRI added little predictive value to the T1w-MRI, similar to the T1w post-contrast MRI. This may however be attributed to the fact that these sequences were only available for a subset of the patients. Our cohort contained too few patients with PD, DCE, or DWI sequences to be analysed. However, there is little to no indication of the added value of these sequences in DTF [37-39].

The second aim of this study was to predict the DTF *CTNNB1* mutation status. Our radiomics model was not able to stratify the *CTNNB1* mutation type, which is in line with the absence of literature linking DTF MRI appearance to the *CTNNB1* mutation.

The current study enclosed several limitations. First, due to the rarity of DTF, the DTF sample size was limited and possibly too small for the mutation stratification model to learn from. This also resulted in little statistical power for the mutation analysis, as shown by the large width of our confidence intervals, and for the comparison with the radiologists in the differential diagnosis. Besides primary tumours, the DTF cohort contained also recurrent tumours. As this number was low, and to our knowledge, there are no indications that recurrent DTF appear different on MRI than primary DTF, the expected influence is small. Within the DTF cohort, the WT group was relatively large and might have been subjected to incorrect allocation, as Sanger Sequencing is not always sensitive enough to detect all mutations [11]. The results of the *CTNBB1* mutation status stratification showed a strong bias towards the majority classes, which may be attributed to the class imbalance. Although we exploited commonly used imbalanced learning strategies such as resampling and ensembling. other strategies may improve the performance. Second, only extremity DTFs were included for comparison with STS. This was due to the limited availability of MRI in non-extremity soft tissue tumours. However, this is not representative for the entire DTF population, which also occurs frequently in the abdominal wall and trunk [3].

Third, the current radiomics approach requires manual annotations. While accurate, this process is also time consuming and subject to some observer variability as indicated by our DSC, and thus limits the transition to clinical practice. Automatic segmentation methods, for example deep learning, may help to overcome these limitations [40]. Lastly, the dataset originated from 68 different scanners, which resulted in substantial heterogeneity in the acquisition protocols. The lack of standard imaging parameters can be problematic as these can affect the appearance of the tumour and thus the radiomics performance. However, our method was successfully able to create diagnostic models despite these differences. As these models were trained on a variety of imaging protocols, there is an increased chance that the reported performance can be reproduced in a routine clinical setting when using other MRI scanners. Using a single-scanner with dedicated tumour protocols may improve the model performance, but will limit the generalizability.

Future work should firstly focus on the prospective validation of our findings. Although we did use a multicentre imaging dataset and performed a rigorous cross-validation experiment strictly separating training from testing data, we did not validate our model on an independent, external dataset. Afterwards, the radiomics model could be used to predict clinical outcomes of DTF receiving active surveillance or systemic treatment.

Conclusions

Our radiomics approach is capable of distinguishing DTF from non-DTF tumours on T1w-MRI, and can potentially aid diagnosis and shorten diagnostic delay. The performance of the model was similar to that of two experienced musculoskeletal radiologists. The model was not able to predict *CTNNB1* mutation status of DTF tumours. Further optimization and external validation of the model is needed to incorporate radiomics in clinical practice.

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Competing Interests

Wiro J. Niessen is founder, scientific lead and stock holder of Quantib BV. The other authors do not declare any conflicts of interest.

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Supplemental Material 1. Radiomics feature extraction

This Supplemental material is similar to [1], but details relevant for the current study are highlighted.

A total of 411 radiomics features were used in this study. All features were extracted using the defaults for MRI scans from the Workflow for Optimal Radiomics Classification (WORC) toolbox [2], which internally uses the PREDICT [3] and PyRadiomics [4] feature extraction toolboxes. The code to extract the features for this specific study has been published open-source [5]. An overview of all features is depicted in Supplemental Table 2. For details on the mathematical formulation of the features, we refer the reader to Zwanenburg et al. (2020) [6]. More details on the extracted features can be found in the documentation of the respective toolboxes, mainly the WORC documentation [7].

The features can be divided in several groups. Twelve histogram features were extracted using the histogram of all intensity values within the Regions of Interest (ROIs), i.e. the tumours, and included several first-order statistics such as the mean, standard deviation and kurtosis. To create the histogram, the images were binned using a fixed number of 50 bins. Seventeen shape features were extracted based only on the ROI, i.e. not using the image, and included shape descriptions such as the volume, compactness, roundness and circular variance. The orientation of the ROI was described by three features, which represent the three major axis angles of a 3-D ellipse fitted to the ROI. Lastly, 379 texture features were extracted using the Gray Level Co-occurrence Matrix (144 features), Gray Level Size Zone Matrix (16 features), Gray Level Run Length Matrix (16 features), Gabor filters (72 features), Laplacian of Gaussian filters (36 features), vessel (i.e. tubular structure) filters (36 features) [8], local phase filters (36 features) [9], Local Binary Patterns (18 features), and the Neighborhood Grey Tone Difference Matrix (5 features).

Most of the texture features include parameters to be set for the extraction. Beforehand the values of the parameters which will result in features with the highest discriminative power for the classification at hand (e.g. DTF vs non-DTF) is not known. Including these parameters in the workflow optimization, see Supplemental Material 2, would lead to repeated computation of the features, resulting in a redundant decrease in computation time. Therefore, alternatively, these features are extracted at a range of parameters as is default in WORC. The hypothesis is that the features with high discriminative power will be selected by the feature selection methods and/or the machine learning methods as described in Supplemental Material 2. The parameters used are described in Supplemental Table 1.

The dataset used in this study is highly heterogeneous in terms of acquisition protocols. Especially the variations in slice thickness may cause feature values to be highly dependent on the acquisition protocol as this varied between 1.0 mm and 11 mm,. Hence, extracting robust 3D features may be hampered by these variations, especially for low resolutions. To overcome this issue, all features were extracted per 2D axial slice and aggregated over all slices. Afterwards, several first-order statistics over the feature distributions were evaluated and used in the machine learning approach. The images were not resampled, as this would result in interpolation errors. Due to variations in especially the magnetic field strength, echo time, and repetition time, the image contrast highly varies, which would affect the feature values. To partially overcome this, each 3D MRI was normalized using z-scoring before feature extraction. These settings are also the default in WORC.

Supplemental Material 2. Adaptive workflow optimization for automatic decision model creation

This Supplemental Material is similar to [1], but details relevant for the current study are highlighted.

The Workflow for Optimal Radiomics Classification (WORC) toolbox [2] makes use of adaptive algorithm optimization to create the optimal performing workflow from a variety of methods. WORC defines a workflow as a sequential combination of algorithms and their respective parameters. To create a workflow, WORC includes algorithms to perform feature scaling, feature imputation, feature selection, oversampling, and machine learning. If used, as some of these steps are optional as described below, these methods are performed in the same order as described in this Supplemental materials. More details can be found in the WORC documentation [7].

Feature scaling was performed to make all features have the same scale, as otherwise the machine learning methods may focus only on those features with large values. This was done through z-scoring, i.e., subtracting the mean value followed by division by the standard deviation, for each individual feature. In this way, all features had a mean of zero and a variance of one.

In the analysis including the T2w or T1w post contrast sequences, in case of a missing sequence, feature imputation was used to estimate replacement values for the missing sequence. Strategies for imputation included 1) the mean; 2) the median; 3) the most frequent value; and 4) a nearest neighbour approach.

Feature selection was performed to eliminate features which were not useful to distinguish between the classes, e.g., DTF vs. non-DTF. These included; 1) a variance threshold, in which features with a low variance (<0.01) are removed. This method was always used, as this serves as a feature sanity check with almost zero risk of removing relevant features; 2) optionally, a group-wise search, in which specific groups of features (i.e. intensity, shape, and the subgroups of texture features as defined in Supplemental material 1) are selected or deleted. To this end, each feature group had an on/off variable which is randomly activated or deactivated, which were all included as hyperparameters in the optimization; 3) optionally, individual feature selection through univariate testing. To this end, for each feature, a Mann-Whitney *U* test is performed to test for significant differences in distribution between the labels (e.g., DTF vs non-DTF). Afterwards, only features with a p-value above a certain threshold are selected. A Mann-Whitney *U* test was chosen as features may not be normally distributed and the samples (i.e., patients) were independent; and 4) optionally, principal component analysis (PCA), in which either only those linear combinations of features were kept which explained 95% of the variance in the features or a limited amount of components (between 10 – 50). These feature selection methods may be combined by WORC, but only in the mentioned order.

Oversampling was used to make sure the classes were balanced in the training dataset. These included; 1) random oversampling, which randomly repeats patients of the minority class; and 2) the synthetic minority oversampling technique (SMOTE) [10], which creates new synthetic "patients" using a combination of the features in the minority class. Randomly, either one of these methods or no oversampling method was used.

Lastly, machine learning methods were used to determine a decision rule to distinguish the classes. These included; 1) logistic regression; 2) support vector machines; 3) random forests; 4) naive Bayes; and 5) linear and quadratic discriminant analysis.

Most of the included methods require specific settings or parameters to be set, which may have a large impact on the performance. As these parameters have to be determined before executing the workflow, these are so-called "hyperparameters". In WORC, all parameters of all mentioned methods are treated as hyperparameters, since they may all influence the decision model creation. WORC simultaneously estimates which combination of algorithms and hyperparameters performs best. A comprehensive overview of all parameters is provided in the WORC documentation [7].

By default in WORC, the performance is evaluated in a 100x random-split train-test cross-validation. In the training phase, a total of 100,000 pseudo-randomly generated workflows is created. These workflows are evaluated in a 5x random-split cross-validation on the training dataset, using 85% of the data for actual training and 15% for validation of the performance. All described methods were fit on the training datasets, and only tested on the validation datasets. The workflows are ranked from best to worst based on their mean performance on the validation sets using the F1-score, which is the harmonic average of precision and recall. Due to the large number of workflows executed, there is a chance that the best performing workflow is overfitting, i.e. looking at too much detail or even noise in the training dataset. Hence, to create a more robust model and boost performance, WORC combines the 50 best performing workflows into a single decision model, which is known as ensembling. These 50 best performing workflows are re-trained using the entire training dataset, and only tested on the test datasets. The ensemble is created through averaging of the probabilities, i.e., the chance of a patient being DTF or non-DTF, of these 50 workflows.

A full experiment consists of executing 50 million workflows (100,000 pseudo-randomly generated workflows times a 5x train-validation cross-validation times 100x train-test cross-validation), which can be parallelized. The computation time of training or testing a single workflow is on average less than a second, depending on the size of the dataset both in terms of samples (i.e., patients) and features. The largest experiment in this study, i.e. the differential diagnoses including 203 patients with both a T1w and T2w MRI had a computation time of approximately 32 hours on a 32 CPU core machine. The contribution of the feature extraction to the computation time is negligible. The code for the model creation, including more details, has been published open-source as well [5].

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Supplemental Table 1. Overview of the 411 features used in this study. GLCM and GLCMMS features were calculated in four different directions (0, 45, 90, 135 degrees) using 16 gray levels and pixel distances of 1 and 3. LBP features were calculated using the following three parameter combinations: 1 pixel radius and 8 neighbours, 2 pixel radius and 12 neighbours, and 3 pixel radius and 16 neighbours. Gabor features were calculated using three different frequencies (0.05, 0.2, 0.5) and four different angles (0, 45, 90, 135 degrees). LoG features were calculated using three different widths of the Gaussian (1, 5 and 10 pixels). Vessel features were calculated using the full mask, the edge, and the inner region. Local phase features were calculated on the monogenic phase, phase congruency and phase symmetry.

Histogram (12 features):	min max mean median std skewness kurtosis peak range energy quartile range entropy	**Shape (17 features):**	compactness (mean + std) radius (mean + std) roughness (mean + std) convexity (mean + std) circular variance (mean + std) principal axes ratio (mean + std) elliptic variance (mean + std) solidity (mean + std) volume
GLCM (6*4*2=48 features):	contrast dissimilarity homogeneity angular second moment (ASM) energy correlation	**GLCMMS (12*4*2=96 features):**	contrast (mean + std) dissimilarity (mean + std) homogeneity (mean + std) ASM (mean + std) energy (mean + std) correlation (mean + std)
NGTDM (5 features):	busyness coarseness complexity contrast strength	**Gabor (6*4*3=72 features):**	mean std min max skewness kurtosis
GLSZM (16 features):	GrayLevelNonUniformity GrayLevelNonUniformityNormalized GrayLevelVariance HighGrayLevelZoneEmphasis LargeAreaEmphasis LargeAreaHighGrayLevelEmphasis LargeAreaLowGrayLevelEmphasis LowGrayLevelZoneEmphasis SizeZoneNonUniformity SizeZoneNonUniformityNormalized SmallAreaEmphasis SmallAreaHighGrayLevelEmphasis SmallAreaLowGrayLevelEmphasis ZoneEntropy ZonePercentage ZoneVariance	**GLRM (16 features):**	GrayLevelNonUniformity GrayLevelNonUniformityNormalized GrayLevelVariance HighGrayLevelRunEmphasis LongRunEmphasis LongRunHighGrayLevelEmphasis LongRunLowGrayLevelEmphasis LowGrayLevelRunEmphasis RunEntropy RunLengthNonUniformity RunLengthNonUniformityNormalized RunPercentage RunVariance ShortRunEmphasis ShortRunHighGrayLevelEmphasis ShortRunLowGrayLevelEmphasis

Supplemental Table 1. (continued)

LoG (12*3=36 features)	min max mean median std skewness kurtosis peak range energy quartile entropy	**Vessel (12*3=36 features)**	min max mean median std skewness kurtosis peak range energy quartile entropy
LBP (6*3=18 features):	mean std median kurtosis skewness peak	**Local phase (12*3=36 features)**	min max mean median std skewness kurtosis peak range energy quartile entropy
Orientation (3 features):	theta_x theta_y theta_z		

GLCM, gray level co-occurrence matrix; GLCMMS, GLCM multislice; NGTDM, neighbourhood gray tone difference matrix; GLSZM, gray level size zone matrix; GLRLM, gray level run length matrix; LBP, local binary patterns; LoG, Laplacian of Gaussian; std, standard deviation.

Supplemental Table 2. Performance of the radiomics models for the DTF differential diagnosis based on T1w and T2w non-FatSat imaging features; T1w and T2w FatSat imaging features; T1w and T1w post-contrast non-FatSat imaging features; and T1w and T1w post-contrast FatSat imaging features. Outcomes are presented with the 95% confidence interval.

	T1w + T2 non-FatSat	**T1w + T2w FatSat**	**T1w + T1w post-contrast non-FatSat**	**T1w + T1w post-contrast FatSat**
AUC	0.83 [0.76, 0.89]	0.83 [0.77, 0.89]	0.80 [0.74, 0.85]	0.82 [0.75, 0.88]
BCA	0.64 [0.58, 0.71]	0.66 [0.59, 0.72]	0.73 [0.67, 0.79]	0.72 [0.66, 0.79]
Sensitivity	0.32 [0.19, 0.44]	0.34 [0.20, 0.47]	0.60 [0.49, 0.72]	0.59 [0.48, 0.70]
Specificity	0.97 [0.92, >1]	0.97 [0.94, 1.00]	0.85 [0.79, 0.92]	0.86 [0.79, 0.94]
NPV	0.74 [0.70, 0.77]	0.74 [0.70, 0.78]	0.79 [0.74, 0.84]	0.79 [0.74, 0.83]
PPV	0.87 [0.68, >1]	0.88 [0.71, >1]	0.71 [0.60, 0.82]	0.72 [0.61, 0.84]

T1w, T1-weighted images, T2w, T2-weighted images; AUC, area under the receiver operator characteristic curve; BCA, balanced classification accuracy; PPV, positive predictive value; NPV, negative predictive value

Supplemental Table 3. P-values after Bonferroni correction of features in a Mann-Whitney *U* test between desmoid type fibromatosis (DTF) and non-DTF patients. Only the features with significant p-values ($p < 0.05$) are depicted. Besides the feature names, several of the labels also include the parameters used. More details on the features can be found in Supplementary Material 1.

Feature label	P-value	Feature label	P-value
tf_Gabor_0.5A1.57mean_Features_0	5,39E-08	tf_GLRLM_RunPercentage_Features_0	2,36E-05
logf_energy_sigma10_Features_0	7,98E-07	tf_Gabor_0.5A1.57skew_Features_0	3,69E-05
tf_GLSZM_ LargeAreaHighGrayLevelEmphasis_ Features_0	1,18E-06	sf_rad_dist_avg_2D_Features_0	4,06E-05
logf_energy_sigma1_Features_0	1,64E-06	vf_Frangi_full_peak_SR(1.0, 10.0)_SS2.0_ Features_0	4,89E-05
logf_peak_sigma5_Features_0	1,69E-06	vf_Frangi_edge_peak_SR(1.0, 10.0)_SS2.0_ Features_0	4,89E-05
tf_Gabor_0.5A1.57max_Features_0	1,87E-06	tf_GLRLM_RunVariance_Features_0	5,09E-05
logf_peak_sigma10_Features_0	1,93E-06	phasef_monogenic_peak_WL3_N5_ Features_0	5,23E-05
logf_energy_sigma5_Features_0	2,30E-06	phasef_monogenic_energy_WL3_N5_ Features_0	5,74E-05
tf_GLRLM_ LongRunHighGrayLevelEmphasis_ Features_0	3,08E-06	tf_GLSZM_ZoneVariance_Features_0	5,82E-05
tf_GLRLM_LongRunEmphasis_Features_0	4,97E-06	tf_GLSZM_ LargeAreaLowGrayLevelEmphasis_ Features_0	6,73E-05
tf_GLSZM_LargeAreaEmphasis_Features_0	5,66E-06	tf_Gabor_0.5A1.57kurt_Features_0	6,82E-05
hf_peak_Features_0	7,41E-06	hf_entropy_Features_0	2,29E-04
logf_peak_sigma1_Features_0	7,47E-06	tf_GLRLM_RunEntropy_Features_0	2,41E-04
phasef_phasesym_peak_WL3_N5_ Features_0	7,74E-06	tf_GLRLM_GrayLevelNonUniformity_ Features_0	2,99E-04
tf_Gabor_0.5A1.57std_Features_0	1,15E-05	tf_GLCMMS_correlationd1.0A1.0std_ Features_0	3,10E-04
vf_Frangi_inner_peak_SR(1.0, 10.0)_SS2.0_ Features_0	1,54E-05	tf_GLCMMS_correlationd1.0A1.0mean_ Features_0	3,97E-04
semf_Gender_Features_0	1,58E-05	tf_Gabor_0.5A2.36std_Features_0	4,02E-04
phasef_phasecong_peak_WL3_N5_ Features_0	1,60E-05	tf_GLCMMS_dissimilarityd1.0A1.0mean_ Features_0	5,21E-04
tf_Gabor_0.5A1.57min_Features_0	1,89E-05	logf_entropy_sigma1_Features_0	6,88E-04
tf_Gabor_0.5A2.36mean_Features_0	1,92E-05	tf_GLCMMS_dissimilarityd1.0A1.0std_ Features_0	7,95E-04

Supplemental Table 3. *(continued)*

Feature label	P-value	Feature label	P-value
tf_Gabor_0.2A0.0skew_Features_0	1,12E-03	tf_Gabor_0.5A2.36min_Features_0	9,93E-03
tf_GLRLM_ LongRunLowGrayLevelEmphasis_ Features_0	1,19E-03	tf_Gabor_0.05A0.0mean_Features_0	1,02E-02
tf_GLRLM_ RunLengthNonUniformityNormalized_ Features_0	1,73E-03	tf_Gabor_0.5A0.79min_Features_0	1,21E-02
tf_GLCMMS_homogeneityd1.0A1.0mean_ Features_0	2,18E-03	tf_Gabor_0.2A0.79min_Features_0	1,39E-02
vf_Frangi_edge_min_SR(1.0, 10.0)_SS2.0_ Features_0	2,59E-03	tf_GLCM_correlationd1.0A1.0_Features_0	1,41E-02
vf_Frangi_full_min_SR(1.0, 10.0)_SS2.0_ Features_0	2,59E-03	tf_GLRLM_ShortRunEmphasis_Features_0	1,44E-02
sf_volume_2D_Features_0	2,71E-03	tf_GLCM_homogeneityd3.0A3.0_Features_0	1,59E-02
vf_Frangi_edge_mean_SR(1.0, 10.0)_SS2.0_ Features_0	3,55E-03	tf_Gabor_0.2A0.0mean_Features_0	1,61E-02
vf_Frangi_full_mean_SR(1.0, 10.0)_SS2.0_ Features_0	3,55E-03	tf_GLSZM_ZonePercentage_Features_0	1,96E-02
tf_GLCMMS_contrastd1.0A1.0std_ Features_0	3,63E-03	vf_Frangi_inner_min_SR(1.0, 10.0)_SS2.0_ Features_0	2,15E-02
tf_GLCMMS_contrastd1.0A1.0mean_ Features_0	4,25E-03	tf_Gabor_0.05A0.0kurt_Features_0	2,40E-02
vf_Frangi_full_median_SR(1.0, 10.0)_ SS2.0_Features_0	4,83E-03	tf_Gabor_0.05A0.0max_Features_0	3,38E-02
vf_Frangi_edge_median_SR(1.0, 10.0)_ SS2.0_Features_0	4,83E-03	logf_entropy_sigma10_Features_0	3,41E-02
phasef_phasecong_energy_WL3_N5_ Features_0	5,85E-03	tf_Gabor_0.05A0.0skew_Features_0	3,76E-02
logf_entropy_sigma5_Features_0	7,59E-03	tf_GLRLM_ ShortRunLowGrayLevelEmphasis_Features_0	3,88E-02
vf_Frangi_edge_quartile_range_SR(1.0, 10.0)_SS2.0_Features_0	7,84E-03	tf_Gabor_0.05A0.0std_Features_0	4,53E-02
vf_Frangi_full_quartile_range_SR(1.0, 10.0)_SS2.0_Features_0	7,84E-03	tf_GLCMMS_correlationd3.0A3.0std_ Features_0	4,60E-02
phasef_phasesym_median_WL3_N5_ Features_0	7,99E-03	tf_GLCM_homogeneityd1.0A1.0_Features_0	4,81E-02
tf_GLCMMS_homogeneityd1.0A1.0std_ Features_0	9,21E-03		

GLCM, gray level co-occurrence matrix; GLCMMS, GLCM multislice; NGTDM, neighbourhood gray tone difference matrix; GLSZM, gray level size zone matrix; GLRLM, gray level run length matrix; LBP, local binary patterns; LoG, Laplacian of Gaussian; std, standard deviation.

Supplemental Table 4. Intra-class correlation coefficient (ICC) values of all features among segmentations of two clinicians in a set of 30 desmoid type fibromatosis patients. Only the features with an ICC > 0.75, which are considered as reliable, are included. Besides the feature names, several of the labels also include the parameters used. More details on the features can be found in Supplemental Material 1.

Feature label	ICC	Feature label	ICC
tf_Gabor_0.05A0.79std_M0	0,75	tf_GLCMMS_ASMd1.0A0.79mean_M0	0,81
tf_Gabor_0.5A2.36kurt_M0	0,75	tf_NGTDM_Contrast_M0	0,81
tf_GLSZM_ SizeZoneNonUniformityNormalized_M0	0,75	tf_GLCMMS_dissimilarityd1.0A1.57mean_M0	0,81
tf_GLCM_ASMd1.0A2.36_M0	0,76	tf_GLCMMS_dissimilarityd1.0A0.79mean_M0	0,81
logf_mean_sigma1_M0	0,76	tf_GLCMMS_energyd1.0A1.57mean_M0	0,81
tf_Gabor_0.5A2.36skew_M0	0,76	tf_GLCMMS_ASMd1.0A2.36mean_M0	0,81
tf_GLRLM_GrayLevelVariance_M0	0,76	vf_Frangi_inner_quartile_range_SR(1.0, 10.0)_ SS2.0_M0	0,81
tf_GLRLM_ GrayLevelNonUniformityNormalized_M0	0,76	tf_LBP_std_R8_P24_M0	0,81
tf_GLCMMS_contrastd1.0A0.0std_M0	0,76	tf_GLCMMS_homogeneityd1.0A0.0std_M0	0,82
tf_Gabor_0.5A0.79mean_M0	0,76	tf_GLCMMS_dissimilarityd1.0A0.0std_M0	0,82
tf_GLCM_ASMd1.0A0.0_M0	0,76	tf_GLCMMS_homogeneityd1.0A0.79mean_M0	0,82
phasef_phasesym_median_WL3_N5_M0	0,77	tf_Gabor_0.2A2.36std_M0	0,82
tf_GLCM_ASMd1.0A1.57_M0	0,77	tf_GLCMMS_homogeneityd1.0A2.36mean_M0	0,83
tf_LBP_skew_R8_P24_M0	0,77	tf_GLCMMS_energyd1.0A0.0mean_M0	0,83
tf_Gabor_0.5A0.79max_M0	0,77	tf_GLCMMS_dissimilarityd1.0A0.79std_M0	0,83
tf_GLSZM_ SmallAreaHighGrayLevelEmphasis_M0	0,77	tf_GLCMMS_energyd1.0A0.0std_M0	0,83
tf_Gabor_0.5A0.79skew_M0	0,77	tf_GLCMMS_correlationd1.0A0.0std_M0	0,83
tf_GLCMMS_ASMd1.0A1.57mean_M0	0,78	tf_GLCMMS_energyd1.0A1.57std_M0	0,83
tf_GLCMMS_contrastd1.0A0.79std_M0	0,78	tf_Gabor_0.5A2.36std_M0	0,84
tf_GLCMMS_contrastd1.0A0.79mean_M0	0,78	tf_GLCMMS_energyd1.0A0.79mean_M0	0,84
tf_Gabor_0.5A0.79std_M0	0,78	hf_median_M0	0,84
tf_GLSZM_SmallAreaLowGrayLevelEmphasis_M0	0,79	vf_Frangi_inner_max_SR(1.0, 10.0)_SS2.0_M0	0,84
tf_GLCMMS_dissimilarityd1.0A2.36mean_M0	0,79	tf_GLCMMS_energyd1.0A0.79std_M0	0,84
tf_GLCMMS_ASMd1.0A0.0mean_M0	0,79	tf_Gabor_0.5A2.36mean_M0	0,84
tf_Gabor_0.05A0.79min_M0	0,79	tf_GLCMMS_energyd1.0A2.36std_M0	0,84
hf_entropy_M0	0,79	tf_GLCMMS_homogeneityd1.0A0.79std_M0	0,84
tf_Gabor_0.5A0.0kurt_M0	0,79	tf_GLCMMS_homogeneityd1.0A2.36std_M0	0,84
tf_GLCMMS_ASMd1.0A2.36std_M0	0,80	tf_GLCMMS_homogeneityd1.0A1.57mean_M0	0,84
tf_GLCMMS_homogeneityd1.0A1.57std_M0	0,80	tf_GLSZM_ZoneEntropy_M0	0,84
tf_GLCMMS_dissimilarityd1.0A2.36std_M0	0,80	vf_Frangi_inner_range_SR(1.0, 10.0)_SS2.0_M0	0,85
tf_GLSZM_SmallAreaEmphasis_M0	0,80	tf_GLCMMS_energyd1.0A2.36mean_M0	0,85
tf_GLCMMS_ASMd1.0A0.79std_M0	0,80	phasef_phasecong_kurtosis_WL3_N5_M0	0,85
tf_GLCMMS_dissimilarityd1.0A1.57std_M0	0,80	tf_GLCMMS_correlationd1.0A0.79mean_M0	0,85
tf_GLCMMS_ASMd1.0A0.0std_M0	0,80	logf_median_sigma1_M0	0,85
tf_Gabor_0.5A0.0std_M0	0,80	tf_Gabor_0.2A1.57min_M0	0,85
tf_GLCMMS_contrastd1.0A0.0mean_M0	0,80	tf_Gabor_0.2A1.57kurt_M0	0,86
vf_Frangi_edge_std_SR(1.0, 10.0)_SS2.0_M0	0,80	tf_GLCMMS_correlationd1.0A0.0mean_M0	0,86
vf_Frangi_full_std_SR(1.0, 10.0)_SS2.0_M0	0,80	tf_GLCMMS_dissimilarityd1.0A0.0mean_M0	0,86
tf_GLCMMS_ASMd1.0A1.57std_M0	0,81	tf_GLCMMS_homogeneityd1.0A0.0mean_M0	0,86
tf_LBP_mean_R8_P24_M0	0,81	tf_GLCMMS_correlationd1.0A0.79std_M0	0,86

Supplemental Table 4. *(continued)*

Feature label	ICC	Feature label	ICC
tf_Gabor_0.5A0.0max_M0	0,86	tf_Gabor_0.2A1.57std_M0	0,91
tf_GLCMMS_correlationd1.0A1.57std_M0	0,87	phasef_phasecong_skewness_WL3_N5_M0	0,91
tf_LBP_mean_R15_P36_M0	0,87	hf_min_M0	0,91
tf_Gabor_0.05A1.57kurt_M0	0,87	tf_Gabor_0.2A0.79kurt_M0	0,91
sf_cvar_avg_2D_M0	0,87	phasef_phasesym_max_WL3_N5_M0	0,91
vf_Frangi_inner_std_SR(1.0, 10.0)_SS2.0_M0	0,87	phasef_phasesym_range_WL3_N5_M0	0,91
phasef_phasecong_mean_WL3_N5_M0	0,87	tf_Gabor_0.2A0.79std_M0	0,91
tf_LBP_std_R3_P12_M0	0,87	phasef_phasesym_mean_WL3_N5_M0	0,91
tf_LBP_std_R15_P36_M0	0,88	hf_quartile_range_M0	0,91
tf_GLCM_energyd3.0A0.79_M0	0,88	tf_Gabor_0.2A2.36min_M0	0,91
tf_GLCM_energyd3.0A1.57_M0	0,88	vf_Frangi_edge_max_SR(1.0, 10.0)_SS2.0_M0	0,92
tf_Gabor_0.2A2.36skew_M0	0,88	vf_Frangi_full_max_SR(1.0, 10.0)_SS2.0_M0	0,92
tf_Gabor_0.5A1.57std_M0	0,88	vf_Frangi_edge_range_SR(1.0, 10.0)_SS2.0_M0	0,92
tf_GLCMMS_correlationd1.0A2.36std_M0	0,88	vf_Frangi_full_range_SR(1.0, 10.0)_SS2.0_M0	0,92
tf_GLCM_energyd3.0A0.0_M0	0,88	logf_skewness_sigma1_M0	0,92
phasef_monogenic_mean_WL3_N5_M0	0,88	sf_prax_avg_2D_M0	0,92
tf_GLCM_energyd1.0A0.79_M0	0,88	tf_Gabor_0.05A2.36max_M0	0,92
tf_GLRLM_LongRunEmphasis_M0	0,88	tf_GLCMMS_correlationd1.0A1.57mean_M0	0,92
tf_GLCMMS_correlationd1.0A2.36mean_M0	0,88	tf_Gabor_0.2A0.0skew_M0	0,92
tf_Gabor_0.05A0.79max_M0	0,89	hf_max_M0	0,92
tf_GLCM_energyd3.0A2.36_M0	0,89	tf_Gabor_0.2A0.79max_M0	0,93
tf_GLSZM_HighGrayLevelZoneEmphasis_M0	0,89	phasef_phasecong_std_WL3_N5_M0	0,93
tf_GLSZM_LowGrayLevelZoneEmphasis_M0	0,89	phasef_phasesym_std_WL3_N5_M0	0,93
tf_GLCM_energyd1.0A2.36_M0	0,89	tf_GLRLM_RunEntropy_M0	0,93
tf_Gabor_0.2A2.36max_M0	0,89	hf_range_M0	0,93
tf_GLCM_energyd1.0A0.0_M0	0,89	tf_Gabor_0.05A2.36skew_M0	0,93
tf_GLRLM_ LongRunHighGrayLevelEmphasis_M0	0,89	tf_Gabor_0.05A0.79kurt_M0	0,93
vf_Frangi_inner_entropy_SR(1.0, 10.0)_ SS2.0_M0	0,89	tf_Gabor_0.05A0.0std_M0	0,93
tf_Gabor_0.5A1.57max_M0	0,89	hf_std_M0	0,93
tf_GLCM_energyd1.0A1.57_M0	0,89	tf_Gabor_0.2A1.57skew_M0	0,94
tf_Gabor_0.2A0.79skew_M0	0,89	sf_rad_dist_std_2D_M0	0,94
tf_Gabor_0.05A0.79skew_M0	0,90	tf_Gabor_0.05A1.57min_M0	0,94
phasef_monogenic_median_WL3_N5_M0	0,90	tf_Gabor_0.05A2.36std_M0	0,94
logf_mean_sigma5_M0	0,90	tf_Gabor_0.2A1.57mean_M0	0,94
tf_Gabor_0.05A2.36min_M0	0,90	tf_Gabor_0.05A2.36kurt_M0	0,94
hf_mean_M0	0,90	tf_Gabor_0.2A0.0std_M0	0,94
tf_Gabor_0.5A0.0min_M0	0,90	tf_Gabor_0.05A0.0max_M0	0,94
tf_Gabor_0.2A0.0kurt_M0	0,90	tf_Gabor_0.2A2.36mean_M0	0,94
tf_GLRLM_ LongRunLowGrayLevelEmphasis_M0	0,90	phasef_phasesym_quartile_range_WL3_N5_M0	0,95
tf_Gabor_0.2A0.79min_M0	0,90	logf_min_sigma1_M0	0,95

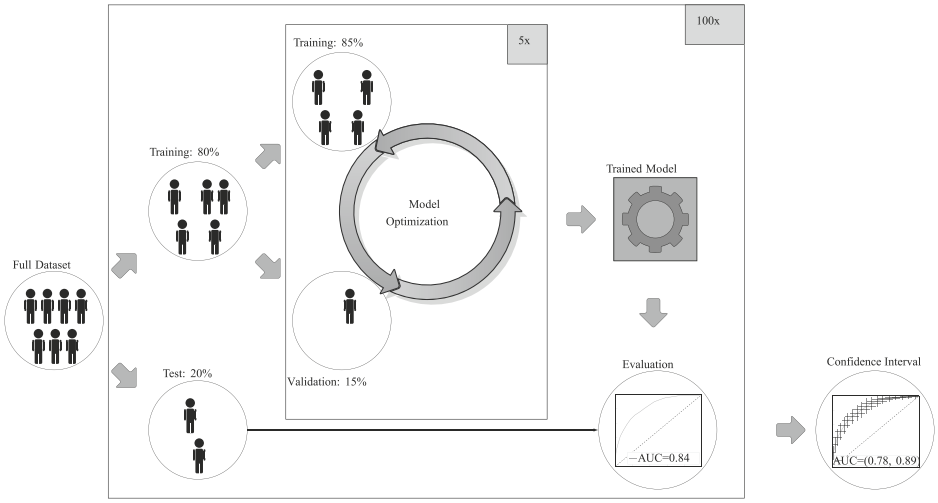
Supplemental Table 4. *(continued)*

Feature label	ICC	Feature label	ICC
tf_Gabor_0.2A2.36kurt_M0	0,95	tf_GLCM_contrastd1.0A1.57_M0	0,97
tf_GLCM_contrastd3.0A2.36_M0	0,95	tf_Gabor_0.2A0.0min_M0	0,97
phasef_monogenic_min_WL3_N5_M0	0,95	tf_Gabor_0.05A0.0mean_M0	0,97
logf_range_sigma1_M0	0,95	tf_GLCM_dissimilarityd1.0A0.0_M0	0,97
tf_GLCM_homogeneityd3.0A0.79_M0	0,95	tf_Gabor_0.5A0.0mean_M0	0,97
hf_peak_M0	0,95	tf_GLCM_dissimilarityd3.0A2.36_M0	0,97
tf_GLCM_homogeneityd3.0A0.0_M0	0,95	phasef_phasesym_entropy_WL3_N5_M0	0,97
tf_Gabor_0.2A0.79mean_M0	0,96	phasef_monogenic_entropy_WL3_N5_M0	0,97
tf_GLCM_homogeneityd3.0A1.57_M0	0,96	logf_kurtosis_sigma1_M0	0,98
vf_Frangi_edge_entropy_SR(1.0, 10.0)_SS2.0_M0	0,96	tf_GLCM_dissimilarityd1.0A2.36_M0	0,98
vf_Frangi_full_entropy_SR(1.0, 10.0)_SS2.0_M0	0,96	tf_GLCM_dissimilarityd1.0A1.57_M0	0,98
tf_GLCM_contrastd3.0A0.0_M0	0,96	tf_GLCM_dissimilarityd3.0A1.57_M0	0,98
logf_max_sigma1_M0	0,96	tf_Gabor_0.2A1.57max_M0	0,98
phasef_monogenic_range_WL3_N5_M0	0,96	logf_range_sigma5_M0	0,98
tf_Gabor_0.05A1.57std_M0	0,96	vf_Frangi_inner_skewness_SR(1.0, 10.0)_SS2.0_M0	0,98
tf_GLCM_homogeneityd3.0A2.36_M0	0,96	tf_GLCM_dissimilarityd1.0A0.79_M0	0,98
tf_GLCM_contrastd3.0A1.57_M0	0,96	tf_GLCM_dissimilarityd3.0A0.79_M0	0,98
tf_Gabor_0.05A2.36mean_M0	0,96	logf_std_sigma5_M0	0,98
tf_GLRLM_RunVariance_M0	0,96	tf_GLCM_dissimilarityd3.0A0.0_M0	0,98
tf_GLCM_contrastd1.0A0.0_M0	0,96	tf_Gabor_0.05A1.57mean_M0	0,98
phasef_phasecong_entropy_WL3_N5_M0	0,96	logf_std_sigma1_M0	0,98
logf_min_sigma5_M0	0,96	logf_mean_sigma10_M0	0,98
logf_max_sigma10_M0	0,96	tf_Gabor_0.5A1.57mean_M0	0,98
tf_GLCM_contrastd3.0A0.79_M0	0,96	hf_energy_M0	0,98
tf_GLCM_homogeneityd1.0A0.79_M0	0,96	logf_entropy_sigma1_M0	0,98
phasef_monogenic_max_WL3_N5_M0	0,96	tf_Gabor_0.05A0.0min_M0	0,98
tf_GLCM_contrastd1.0A2.36_M0	0,96	logf_std_sigma10_M0	0,98
tf_Gabor_0.05A1.57skew_M0	0,96	logf_entropy_sigma10_M0	0,98
tf_GLCM_contrastd1.0A0.79_M0	0,96	logf_median_sigma10_M0	0,98
phasef_phasecong_max_WL3_N5_M0	0,96	tf_Gabor_0.2A0.0mean_M0	0,99
phasef_phasecong_range_WL3_N5_M0	0,96	tf_Gabor_0.2A0.0max_M0	0,99
logf_range_sigma10_M0	0,96	logf_entropy_sigma5_M0	0,99
tf_GLCM_homogeneityd1.0A2.36_M0	0,97	phasef_phasecong_energy_WL3_N5_M0	0,99
logf_min_sigma10_M0	0,97	sf_rad_dist_avg_2D_M0	0,99
phasef_monogenic_std_WL3_N5_M0	0,97	phasef_phasesym_energy_WL3_N5_M0	0,99
phasef_monogenic_quartile_range_WL3_N5_M0	0,97	phasef_monogenic_kurtosis_WL3_N5_M0	0,99
tf_GLCM_homogeneityd1.0A0.0_M0	0,97	logf_max_sigma5_M0	0,99
vf_Frangi_inner_kurtosis_SR(1.0, 10.0)_SS2.0_M0	0,97	tf_Gabor_0.05A1.57max_M0	0,99
tf_GLCM_homogeneityd1.0A1.57_M0	0,97	vf_Frangi_inner_energy_SR(1.0, 10.0)_SS2.0_M0	0,99

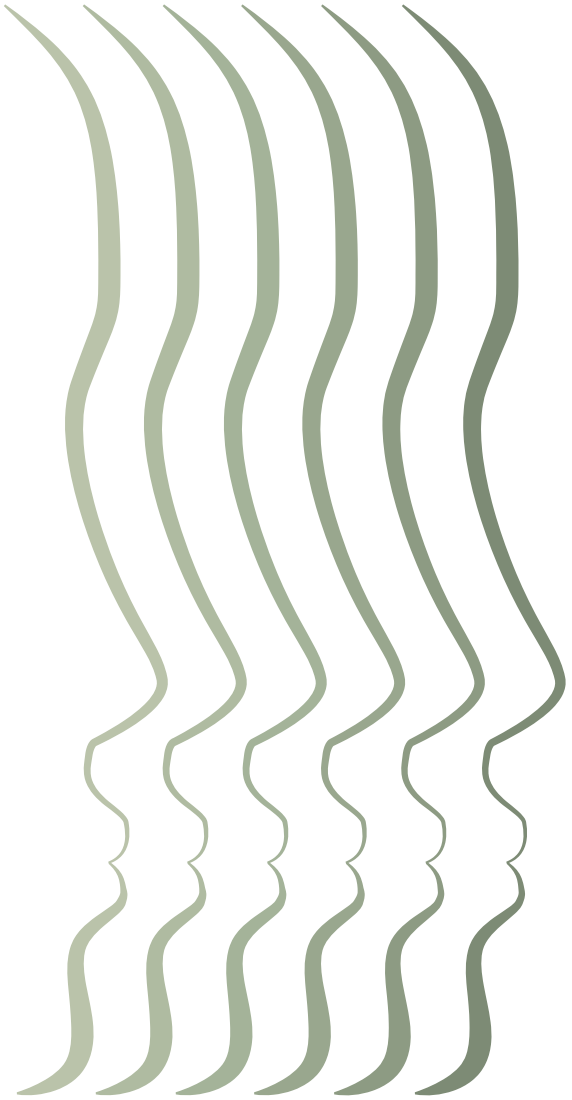
Supplemental Table 4. *(continued)*

Feature label	ICC	Feature label	ICC
logf_skewness_sigma5_M0	0,99	logf_energy_sigma5_M0	1,00
logf_quartile_range_sigma10_M0	0,99	phasef_monogenic_peak_WL3_N5_M0	1,00
tf_GLSZM_GrayLevelNonUniformity_M0	0,99	vf_Frangi_inner_peak_SR(1.0, 10.0)_SS2.0_M0	1,00
logf_energy_sigma1_M0	0,99	tf_Gabor_0.5A1.57min_M0	1,00
logf_quartile_range_sigma5_M0	1,00	phasef_phasesym_peak_WL3_N5_M0	1,00
logf_kurtosis_sigma5_M0	1,00	phasef_monogenic_energy_WL3_N5_M0	1,00
logf_kurtosis_sigma10_M0	1,00	phasef_phasecong_peak_WL3_N5_M0	1,00
logf_skewness_sigma10_M0	1,00	vf_Frangi_edge_peak_SR(1.0, 10.0)_SS2.0_M0	1,00
logf_peak_sigma10_M0	1,00	vf_Frangi_full_peak_SR(1.0, 10.0)_SS2.0_M0	1,00
tf_GLSZM_SizeZoneNonUniformity_M0	1,00	tf_GLRLM_GrayLevelNonUniformity_M0	1,00
logf_energy_sigma10_M0	1,00	phasef_phasecong_min_WL3_N5_M0	1,00
logf_quartile_range_sigma1_M0	1,00	phasef_phasesym_min_WL3_N5_M0	1,00
sf_volume_2D_M0	1,00	tf_LBP_median_R8_P24_M0	1,00
tf_GLRLM_RunLengthNonUniformity_M0	1,00	tf_LBP_peak_R15_P36_M0	1,00
logf_peak_sigma1_M0	1,00	tf_LBP_peak_R3_P12_M0	1,00
logf_peak_sigma5_M0	1,00	tf_LBP_peak_R8_P24_M0	1,00

GLCM, gray level co-occurrence matrix; GLCMMS, GLCM multislice; NGTDM, neighbourhood gray tone difference matrix; GLSZM, gray level size zone matrix; GLRLM, gray level run length matrix; LBP, local binary patterns; LoG, Laplacian of Gaussian; std, standard deviation.



Supplemental Figure 1. Visualization of the 100x random split-cross validation, including a second cross validation within the training set.



6

Active surveillance in desmoid-type fibromatosis: A systematic literature review

Milea J.M. Timbergen, Anne-Rose W. Schut, Dirk J. Grunhagen,
Stefan Sleijfer, Cornelis Verhoef

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Abstract

Background

This study evaluates the results of the active surveillance (AS) approach in adult patients with desmoid-type fibromatosis (DTF), because AS is advocated as a front-line approach for DTF in the European consensus guidelines.

Materials and methods

A systematic literature search was conducted (December 19th, 2019, updated on April 14th, 2020). Studies describing the outcomes of the AS approach were included. The PRISMA guidelines were used.

Results

Twenty-five articles were included for data-retrieval. Forty-two percent of reported patients (1480 of 3527 patients) received AS, the majority were females and the majority had a primary tumour. The median age at diagnosis ranged from 28 to59 years. Common tumour sites were; the extremities/girdles (n = 273), the abdominal wall (n = 253), and the trunk (n = 153). The median reported percentage of progressive disease, stable disease and partial response was 20% (interquartile range [IQR] 13-35%), 59% (IQR: 37-69%) and 19% (IQR: 3-23%), respectively. In 640 patients, the outcome was not specified. The median reported percentage of shifting to an active form of treatment was 29%, most commonly to systemic treatment (n=195) and surgery (n=107). The reported median follow-up time ranged between 8 and 73 months. The reported median time to progression and/or initiation of the subgroup shifting from AS to 'active' therapy ranged from 6.3 months to 19.7 months.

Conclusions

The majority of patients undergoing AS have either stable disease or a partial response, and about one-third of patients shift to an active form of treatment. Selecting patients who will benefit from active surveillance upfront should be the priority of future studies.

Introduction

Desmoid-type fibromatosis (DTF) is an uncommon, soft-tissue tumour arising in musculoaponeurotic structures and mainly affecting young adults aged between 20 and 40 years [1]. DTF is characterised by unpredictable, invasive growth. Rapid growth is often seen in the early phase of the disease, but also in response to pregnancy or hormonal manipulation [2, 3]. After an initial period of growth, many patients experience prolonged stabilisation of the desmoid tumour.

Up to ten years ago, surgical treatment was the mainstay of treating DTF leading to significant morbidity and high recurrence rates [4-6]. Other forms of active treatments, such as radiotherapy and systemic therapy, mainly have a role in case of progressive and symptomatic tumours located at sites which are difficult to treat surgically [7]. However, these therapies can lead to treatment-related toxicities [7]. The term 'active surveillance' (AS) for the management of DTF was introduced in the 1990s. Initially, AS was only offered to patients with recurrent tumours, but after 2005 also patients with primary tumours were exposed to this approach [8, 9]. As a results, a decrease in the use of these 'active treatments' over the past years has been reported in several nation-wide cohort studies [4, 5]. AS for DTF is justified as it has no metastatic potential and spontaneous tumour regression is reported in up to 30% of patients who undergo initial AS [10]. A large retrospective study showed no difference in event-free survival (EFS) comparing surgery with the AS approach (53% versus 58%, $p = 0.415$) [6]. The first European consensus guideline dates from 2015, and advocates using AS as an upfront approach, to minimise overtreatment and to prevent unnecessary morbidity [11]. This recommendation was based on the results of several retrospective series [8, 10, 12-14]. A systematic review to summarise and to evaluate the published results of the AS approach can be helpful to select patients who benefit from this approach, while awaiting the results of three ongoing, prospective clinical trials from Europe (NCT01801176, NCT02547831, and NTR 4714) [15-17].

The aim of the current study was to systematically review published studies reporting the results of the AS approach in adult DTF patients. Furthermore, Response Evaluation Criteria in Solid Tumors, (RECIST) classification of DTF tumours during the AS approach was evaluated, prognostic factors for a successful AS approach were identified, the median time to shift to an active form of treatment and the median duration of the AS approach were analysed and lastly, the forms of active treatment after the initial AS approach were assessed.

Material and methods

This study uses the PRISMA guidelines for reporting a systematic literature review.

Information sources

On December 19th 2019, a systematic literature search was performed by an expert librarian. The search was updated on April 14th which yielded one additional inclusion. Used databases include Embase.com, Ovid MEDLINE, Web of Science, Cochrane CENTRAL, PsychInfo Ovid and Google Scholar. Duplicated records were removed. Case reports were excluded and an English language filter was applied. There were no constrains on publication dates. Supplemental Materials 1 depicts the search strategy.

Eligibility criteria

Studies with sporadic DTF as a main subject and full-text availability were included by two researchers (MJMT, AWS). Papers reporting outcomes (either using RECIST [18] or number of patients shifting to 'active treatment') were included in this systematic literature review. Cross-referencing was carried out ensuring inclusion of all relevant articles. The flowchart depicting the study selection procedure is available in Supplemental Materials 2.

Study selection

Table 1. Inclusion and exclusion criteria of study selection procedure

Inclusion criteria	Exclusion criteria
• Primary and recurrent DTF • Active surveillance (or other similar terms such as wait and see, expectative management etc.) as a primary treatment • Adult (aged ≥ 18 years) patients • English language • Reporting the outcomes of active surveillance in terms of reporting the success rate of active surveillance, numbers of patients needed to shift to active treatment, RECIST outcomes during active surveillance	• Studies with patients receiving solely active forms of treatment such as surgery, systemic treatment, local therapy (e.g., cryotherapy), and radiotherapy • Case reports, case series ≤ 5 patients • Pre-clinical studies describing molecular features of DTF • Diagnostic studies describing imaging features of DTF • Non-original reports (e.g., editorials, study protocols, reviews etc.) • Non-full text available (e.g., conference abstracts etc.) • Studies describing solely paediatric cohorts • Studies describing solely FAP or Garner syndrome • Other subjects than DTF (e.g., soft tissue sarcoma) • Language other than English

DTF, desmoid-type fibromatosis; RECIST, Response Evaluation Criteria in Solid Tumors; FAP, familial adenomatous polyposis

The retrieved articles were assessed for potential inclusion by the first and second author based on the review of title and abstract. Next, full-text articles were evaluated in accordance with the predetermined inclusion criteria and exclusion criteria for this systematic literature review (listed in Table 1).

Data Extraction

Data was collected by two researchers (MJMT, AWS) using a pre-defined Excel sheet statin the year of publication, the first author, the journal, the publication title, whether the publication fulfilled the inclusion criteria, the inclusion period, the type of study, the total number of participants, the number of participants receiving AS, the number of patients with familial adenomatous polyposis (FAP)/Gardner syndrome, the number of primary tumours, and the number of recurrent tumours. Of the AS group, the following variables were extracted: the reported mean/median follow-up (range, interquartile range [IQR], 95% confidence interval [CI]), the reported median/mean age (with range or IQR), the sex distribution, the tumour sites, the number of patients with progressive disease (PD), stable disease (SD), partial response (PR), complete response (CR), the number of patients who shifted to active treatment, reasons for shifting to an active form of treatment, and whether RECIST were used for determination of these outcomes. For responses not evaluated by RECIST but by using similar terms, tumour response was categorised based on the RECIST categories; PD, SD, PR and CR. PD included the terms 'increase', 'evolution', 'enlarged', SD included the terms 'stable', 'arrested', and 'non-progressive', PR included the terms 'decreased', 'regressed', 'disease free survival', 'responding disease', and 'spontaneous remission', and CR included the terms 'disappeared', and 'complete regression'. Not specified (NS) was used in case a variable was missing.

Tumour sites were classified as: the extremity/girdle region (including upper extremity, lower extremity, shoulder, buttock, thigh and hip), intra-abdominal (including mesenteric), trunk (including paraspinal and thoracic wall), abdominal wall, head/neck region and other (including inguinal region and not further specified sites). When age and follow-up (in months of years) were reported for each individual patient, the median age and median follow-up with range were extracted and calculated from these data.

A shift to 'active treatment' was defined as 'ceasing active surveillance'. The following therapies were categorised as 'active treatments': systemic treatment (including hormonal treatment, chemotherapy, and tyrosine kinase inhibitors), surgery, radiotherapy, combination

therapies, and local therapies such as radiofrequency or cryotherapy. The category of "NS" was used when information was lacking about the type of active treatment. Shift to 'active treatment' is reported as the percentage of patients shifting to active treatment from each separate study, and compiled as an overall median percentage of patients shifting to active treatment with IQR, compiling all study results. The same was done for the types of active treatments. Variables such as median follow-up of the AS group, the time to intervention, the time to progression, the time to stabilisation, the time to regression, progression-free survival (PFS), and EFS were extracted in case they were stated by the included studies.

Results

Systematic literature search

The search was performed on December 19, 2019 and updated on April 14, 2020. The search strategy yielded a total of 940 papers, after deduplication, 589 papers remained. Title and abstract were screened leading to the exclusion of 551 papers. A total of 38 papers were reviewed based on full-text and 25 studies were finally included for further analysis. The study selection procedure is depicted in Supplemental Materials 2. No randomised controlled trials reporting about AS in DTF were identified. Several reviews, discussing the current status and treatments of DTF addressed the AS approach, but none of these reviews included a systematic literature review solely focussing on the outcomes of the AS approach.

Study design and quality assessment.

All included studies were published after 2005. All studies were retrospective case series which are generally considered to have a high risk of bias and a low certainty [19, 20]. Of note, nine studies potentially used overlapping patient cohorts based on author names, affiliations and inclusion time period [2, 9, 14&6, 10, 12, 21-23].

Table 2. Overview of studies reporting the results of the active surveillance approach in desmoid-type fibromatosis

First author, year of publication, inclusion. period	Total N	FAP/ Gardner N	P/R total	ASG N	P/R ASG	Median age ASG	Sex M/F ASG	Site ASG	Median FU (r/ IQR/95% CI) ASG	PD	SD	PR	CR	NS	Shift to AT	AT
Dalén, 2006 [24] NS	8	NS	6/2 [a]	**8**	6/2[a]	32.5	3/5	5 AW 1 EG 2 TR	4.6 year (r: 0.8-7.5)	2	1	2	3	0	NS	NS
Bertagnolli, 2008 [25] 2001-2006	52	21	NS	**4**	NS	NS	4 NS	4 IA	NS	0	4	0	0	0	0/4	NA
Bonvalot, 2008 [12] 1988-2003	112	NS	112/0	**11**	11/0	NS	11 NS	11 NS	NS	3	NS	NS	NS	8	3/11	1 ST 2 ST+SG
Nakayama, 2008 [26] 1992-2003	11	NS	9/2	**11**	9/2	28	2/9	2 AW 7 EG 2 HN	56 months (r: 16-132)	1	7	3	0	0	3/11	2 SG 1 ST
Fiore, 2009 [14] 1995-2008	142	6	74/68	**83**	54/29	NS	22/61	33 AW 27 EG 3 HN 6 IA 14 TR	NS	29	35	3	NS	16	26/83	10 NS 6 SG 10 ST
Barbier, 2010 [27] 1989-2009	26	0	11/15	**26**	11/15	34.5	5/21	26 EG	8 months (r: 0-80)	1	24	0	1	0	0/26	NA
Salas, 2011 [22] 1965-2008	426	0	426/0	**27**	27/0	NS	27 NS	27 NS	52 months (95% CI 43.6-61.6%)	6	16	5	0	0	NS	NS
Bonvalot, 2013 [10] 1993-2012	147	0	147/0	**102**	102/0	NS	102 NS	102 AW	NS	NS	NS	NS	NS	102	37/102	15 SG 22 ST
Fiore, 2014 [2] 1985-2011	44 [b]	0	44/0	**27**	27/0	NS	0/27	27 NS	NS	17	NS	NS	NS	10	12/44	6 SG 6 ST

Table 2. *(continued)*

First author, year of publication, inclusion. period	Total N	FAP/ Gardner N	P/R total	ASG N	P/R ASG	Median age ASG	Sex M/F ASG	Site ASG	Median FU (r/ IQR/95% CI) ASG	PD	SD	PR	CR	NS	Shift to AT	AT
Huang, 2014 [28] 1987-2009	214	NS	153/61	**20**	9/11	NS	20 NS	20 NS	45 months (r: 24-90)	4	14	2	0	0	NS	NS
Roussin, 2015 [23] 1992-2014	31	0	NS	**11**	NS	50	1/10	11 TR	23 months (r: 3-144)	2	NS	NS	NS	9	3/11	1 SG 2 ST
Colombo, 2015 [9] 1992- 2012	216	0	216/0	**70**	70/0	41	22/48	26 EG 10 IA 2 HN 32 TR	39 months (r 15-62)	28	24	15	NS	3	28/70	3 RT 3 SG 22 ST
Burtenshaw, 2016 [29] 1980-2012	194 [c]	80	176/18[a]	**120**	109/11	NS	120 NS	120 NS	NS	NS	NS	NS	NS	120 [d]	53/120	16 SG 33 ST 2 ST + SG 2 RT + SG
Park, 2016 [30] 2008-2015	47	NS	39/8	**20**	20/0	40.2	6/14	9 EG 1 HN 1 IA 9 TR	NS	1	13	5	1	0	1/20	1 SG
Cassidy, 2018[31] 2008-2015	160	NS	118/ 42[a]	**72**	50/22 [a]	NS	22/50	19 AW 21 EG 21 IA 6 NS 5 TR	25.1 months (r: 1.8-177)	10	NS	NS	NS	62	42/72 [e]	42 NS

Table 2. *(continued)*

First author, year of publication, inclusion. period	Total N	FAP/ Gardner N	P/R total	ASG N	P/R ASG	Median age ASG	Sex M/F ASG	Site ASG	Median FU (r/ IQR/95% CI) ASG	PD	SD	PR	CR	NS	Shift to AT	AT
Van Broekhoven, 2018 [32] 1993-2013	91	6	91/0	**37**	37/0	36	9/28	17 AW 4 EG 3 HN 13 TR	16 months (IQR: 7-31)	5	21	4	2	5	15/37	15 NS
De Bruyns, 2019 [33] 1990-2013	227	14	NS	**59**	NS	NS	59 NS	59 NS	NS	NS	20	13	9	17	NS	NS
Duazo-Cassin, 2019 [21] 1998-2016	63	0	63/0	**17**	17/0	59	1/16	17 TR	42.2 months (r: 0-214)	2	9	6	0	0	2/17	2 SG
Krieg, 2019 [34] NR	96	NS	NS	**15**	NS	NS	15 NS	15 NS	3.4 year (r: 2.4-11.6)	3	9	3	0	0	3/15	1 SG+RT 2 NS
Shen, 2019 [35] 2010-2018	29	2	27/2	**3**	NS	NS	3 NS	3 NS	NS	3	0	0	0	0	1/3	1 SG
Van Houdt, 2019 [36] 1998-2016	168	0	168/0	**168**	168/0	42.2	50/118	61 AW 51 EG 15 IA 11 NS 30 TR	40.5 months	60	60	33	12	3	78/168	40 SG 36 ST 2 RT
Kim, 2020 [37] 1995-2015	76	0	46/30	**76**	30/46	30.2[m]	29/47	39 EG 37 NS	50.4 months (r 12-226)[m]	NS	54	8	1	13	NS	NS

Table 2. *(continued)*

First author, year of publication, inclusion. period	Total N	FAP/ Gardner N	P/R total	ASG N	P/R ASG	Median age ASG	Sex M/F ASG	Site ASG	Median FU (r/ IQR/95% CI) ASG	PD	SD	PR	CR	NS	Shift to AT	AT
Reported concurrent use of NSAID's and/or hormonal therapy during the AS approach																
Non-narcotic analgesics and non-steroidal anti-inflammatory drugs (NSAID's) were offered to symptomatic patients																
Briand, 2014 [8] NS	73	0	52/21	**55**	31/24	35	20/35	42 EG 1 HN 12 TR	73 months	7	42	NS	5	1	5/55	3 SG 1 ST 1 SG + RT
With or without administration of NSAID's																
Penel, 2017 [6] 2010-2016	771	NS	771/0	**388**	388/0	NS	388 NS	388 NS	NS	117	NS	NS	NS	271	71/338	3 CrT 2 SG 61 ST 1 RF 4 RT
Conversion to hormonal therapy was not considered failure of AS treatment																
Turner, 2019 [38] 2004-2015	103	0	103/0	**50**	50/0	41[m]	13/37	14 AW 20 EG 3 HN 3 IA 8 TR 2 NS	NS	21	29	0	0	0	19/50	9 SG 9 RT 1 SG+ RT + ST

ASG, active surveillance group; AT, active treatment; AW, abdominal wall; CrT, cryotherapy; EG, extremity/girdles; IA, intra-abdominal; IQR, interquartile range; NA, not applicable; NS, not specified; NSAID's, non-steroidal anti-inflammatory drugs; P, primary disease; R, recurrent disease; RF, radiofrequency; RT, radiotherapy; SG, surgery; ST, systemic treatment; TTI, time to intervention; TR, trunk

r, range

[a] including residual tumours; [b] only group A, B and C included in this table; [c] only group A (primary tumours) and C (recurrent tumours) included in this table; [d] n=51 shift due to tumour growth, symptom escalation or patient preference for intervention; [e] n = 72 received AS, n = 37 patients had available Response Evaluation Criteria In Solid Tumors (RECIST); [m] mean value instead of median

Clinical characteristics and outcomes of active surveillance

The clinical characteristics and outcomes of patients treated with AS of the included studies are shown in Table 2. Most studies only included sporadic DTF, whilst seven studies also included FAP-related DTF. It was mostly unclear whether these FAP-patients were included in the AS groups, and no study published separate results for the AS approach in FAP-related DTF patients. Treatment strategy comparisons included surgery with or without adjuvant radiotherapy, isolated limb perfusion, cryotherapy, radiotherapy, and systemic treatments including chemotherapy, tyrosine kinase inhibitors, and hormonal treatment. One study compared three groups categorised by surgical margins [28], another study categorised groups based on their pregnancy status [2]. From the later, only groups A, B and C (representing patients with diagnosed during pregnancy [A], diagnosed within 6 months after delivery [B], and previously diagnosed and still in situ at the time of pregnancy [C]) were included in the analysis. Group D (resected before pregnancy without clinical evidence of residual or recurrent disease), was excluded from the results owing to lack of reporting of clinical outcome and shift to active treatment. One study only reported the outcome of 37 patients with RECIST whilst they had 72 patients undergoing AS (Table 2) [31]. Furthermore, one study also described a group of patients with resected tumours (group B). This group was excluded from analysis and only groups A and C from this study were included [29].

Few studies solely included patients receiving AS [26, 27, 36, 37]. Ten studies provided the type and interval of imaging during the AS approach. Most studies used intervals of two to six months after the first evaluation with either computed tomography (CT) [25] or magnetic resonance imaging [8, 10, 23, 27, 37], or a combination. Few studies used additional ultrasound [9, 36, 37]. Two studies stated to 'change to annual visits' after tumour stabilisation or after two years of follow-up [30, 36].

Active surveillance as a single treatment

The total number of patients was 3527, of which 1480 (42%) received AS. Three studies allowed the use of non-steroidal anti-inflammatory drugs (NSAIDs) in symptomatic patients during the AS approach or did not consider shift to hormonal therapy as a "failure of AS" (Table 2) [6, 8, 38]. As the use of NSAIDs could be under reported by both patients and researchers, the results of these studies were included in the analysis of this paper.

The number of patients receiving AS approach ranged from 3 to 388 per included study. The total group receiving AS consisted of 205 males and 526 females (reported in fifteen studies), for the remaining patients (n = 749), the sex was not further specified. The median percentage of females in each reported study was 72% (IQR: 67-78%). The reported median

age at diagnosis of the AS group (available in twelve studies) ranged from 28 to 59 years. Twenty studies reported the number of primary and recurrent tumours included in their AS group (Table 2). In these studies, the majority of patients had a primary tumour with a median percentage of primary tumours of 100% (IQR: 68-100%). The remaining had a recurrent tumour. Based on the reported information, no distinction in numbers of patients needing shift to active treatment could be made between primary and recurrent tumours.

Tumour response to active surveillance

Fourteen out of twenty-five studies stated to use RECIST (either 1.0 or 1.1) [18] to objectively measure tumour response [2, 6, 8, 14, 23, 25, 29-33, 35, 36, 38], however only a part of those studies actually reported the radiological response per treatment type in accordance with RECIST. Other studies used similar approaches describing the disease outcome as PD, SD, PR or CR.

A total of 21 studies reported PD in 322 patients. The median percentage of PD reported in these studies was 20% (IQR: 13-35%). A total of eighteen studies reported SD in 382 patients. The median percentage of SD reported in these studies was 59% (IQR: 37-69%). Seventeen studies reported PR in 102 patients. The median percentage of PR reported in these studies was 19% (IQR: 3-23%). CR was reported sixteen studies in 34 patients. The median percentage of CR reported in these studies was 0% (IQR: 0-6%) (Table 3).

Indications for start of treatment

Table 3. Overview of RECIST outcomes and shift to active treatment

	Number of studies reporting this variable	Number of patients	Median % of patients (IQR) reported in all studies
RECIST outcomes			
Progressive disease	21	322	20% (13-35%)
Stable disease	18	382	59% (37-69%)
Partial response	17	102	19% (3-23%)
Complete response	16	34	0% (0-6%)
Active treatment			
Shifting to an active form treatment	20	402	29% (17-40%)
Surgery	17	107	41% (11-62%)
Systemic treatment	17	195	33% (0-52%)
Local therapies [a]	16	4	0% (0%)
Radiotherapy	16	18	0% (0-1%)
Combination of therapies [b]	20	8	0% (0-3%)

[a] *radiofrequency, cryotherapy;* [b] *surgery + radiotherapy, systemic therapy + surgery*

Pain, with or without radiological evidence of progression, functional symptoms, or patient request, were frequently mentioned reasons for shifting to an 'active' treatment [10]. A total of 402 patients (reported in twenty studies) shifted to 'active' treatment. The median percentage of patients shifting in these studies was 29% (IQR: 17-40%). The type of 'active' treatment was systemic treatment in 195 cases, surgery in 107 cases, radiotherapy in 18 cases, a combination of therapies (e.g., systemic treatment with surgery, and systemic treatment with radiotherapy) in 8 cases and local therapy (e.g., radiofrequency and cryotherapy) in 4 cases. In 69 cases it was reported that patients shift to an active form of treatment but the type was unspecified (Table 3).

Progression and change in treatment strategy

The median follow-up time of patients with the AS approach was reported by twelve studies and ranged between 8 months and 73 months (Table 4). Most studies reported the median time to progression (n = 5) [9, 22, 28, 29, 32], and solely two studies reported median time to shifting from AS to 'active' therapy [31, 36]. Other studies used PFS [14, 30, 33, 38] or EFS [6, 28] to express the success rates of the AS approach. Two studies described time to SD [27, 37].

Van Broekhoven et al. described that the median duration of the AS approach was 22 months (IQR: 13-46) for patients with CR or PR [32]. Kim et al. reported that age younger than 40 and a recurrent tumour were significant predictive factors of longer time to disease stabilisation (p = 0.014 and p = 0.036, respectively) [37]. Penel et al. reported that 30.1% of patients in the AS group experienced an event (progression during AS, change in treatment strategy and/or disease-related death) [6]. Briand et al. reported a cumulative probability of dropping out from the AS approach of 5.7% (95% CI 1.5%-14.2%) at one year, and 9.6% (95% CI 3.5%- 19.6%) at 2, 5, and 10 years [8]. Bonvalot et al. stated that the percentage of patients shifting to another treatment was 33% (95% CI 24-43) at 1-year, and 41% at 3 years (95% CI 31%-52%) [10]. Fiore et al. reported that 89% of patients progressed within the first two years after referral, and reported a 5-year PFS rate of 47% (standard error [SE] 10.3%) for primary tumours and 54% (SE 11.6%) for recurrent tumours (p = 0.48) [14] (Table 4).

A description of the risk factors for progression or a change in treatment strategy is reported in Table 5. A larger tumour size, >5 cm vs. ≤5 cm, was associated with a shorter time to intervention (6.9 months versus 32.6 months, p = 0.02) [31], and shift to 'active' treatment was more likely in patients with "larger" tumours (≥ 7cm) with a hazard ratio (HR) of 2.0 (95% CI 1.3%- 3.2%, p = 0.002) [36], and >3.5 cm, p = 0.004 [10]. Furthermore, the initiation of 'active' treatment was more likely for patients with PD or SD than for patients with PR

($p < 0.001$) with a HR of 12.4 (95% CI 4.9%- 31.4%), and 4.8 (95% CI 1.8% to 12.6%), respectively [36]. Patients who experienced pain were also more likely ($p < 0.001$) to shift to an active form of treatment, with a HR of 2.55 (95% CI 1.63%-3.99%) [36]. Cassidy et al. found no association between intervention (i.e., shift to active treatment) and age ($p = 0.22$), as well as intervention and sex ($p = 0.07$) [31].

Table 4. Reported time intervals and survival data to express the success rate of the active surveillance approach

Ref.		Outcome
		Median time to intervention
Cassidy, 2018	[31]	11.7 months (±6.5)
Van Houdt, 2019	[36]	6.5 months
		Median time to progression
Salas, 2011	[22]	19.7 months (range: 7.8-46.2 months)
Huang, 2014	[28]	15.3 months (range 7:.8-41 months)
Colombo, 2015	[9]	16 months
Van Broekhoven, 2018	[32]	7.3 months (IQR: 4.1-11.9 months)
Krieg, 2019	[34]	1.2 years (range: 0.9-1.5 years)
		Median time to stable disease
Barbier, 2010	[27]	13.2 months (range: 6-30 months)
Kim, 2020	[37]	30.4 months (range: 7-112 months)[a]
		Median time to regression
Briand, 2014	[8]	54.8 months (range: 21-130 months)
		Median progression-free survival
Turner, 2019	[38]	10 months (range: 2-94 months)
		2-year progression-free survival
De Bruyns, 2019	[33]	71% (95% CI 0.6% to 0.84%)
		3-year progression-free survival
Turner, 2019	[38]	38%
Park, 2016	[30]	92%
		5-year progression-free survival
Fiore, 2009	[14]	47% (SE: 10.3%) primary tumours 54% (SE: 11.5%) recurrent tumours
		2-year event-free survival
Penel, 2017	[6]	85.7 (±9.6) core needle biopsy 52.8 (±4.6) open biopsy
		5-year event-free survival
Huang, 2014	[28]	71.2%

[a] *mean value instead of median*

CI, confidence interval; IQR, interquartile range; SE, standard error

Table 5. Published results regarding variables that are potentially associated with time to disease stabilisation, risk of progression or change in treatment strategy. Significant outcomes (p-value <0.05) are in bold.

	Ref	Outcome	p-value	Statistically significant identified risk factor
Barbier, 2010	[27]	*Time difference in evolution to stabilisation*		Longer evolution time before stabilization in recurrent tumours
		Primary vs. recurrent disease	**p = 0.0417**	
Kim, 2020	[37]	Age	**p = 0.022**	Age, < 40 years and recurrent tumours are predictive factors of longer time to disease stabilization
		Tumour status	**p = 0.041**	
		Tumour site (axial vs. extremity)	p = 0.148	
Bonvalot, 2013	[10]	*Change in treatment strategy*		Larger tumour size (>3.5)
		Pregnancy before the development of DTF	p = 0.27	
		Age	p = 0.27	
		Tumour size	**p = 0.004**	
		3.5-5.0 cm (HR 3.7, 95% CI 1.0% to 14%)		
		5-7 cm (HR 4.0, 95% CI 2.4% to 2.8%)		
		7-15.6 cm (HR 8.2, 95% CI 2.4% to 28%)		
Cassidy, 2018	[31]	*Change in treatment strategy*		
		Age	p = 0.22	
		Sex	p = 0.07	
		Documentation of symptoms at presentation	p = 0.35	
		PFS [a]		
		Age (HR 0.99)	p = 0.31	
		Tumour size (HR 1,027)	p= 0.13	
		Tumour site extremities/all other sites vs. abdominal wall	p = 0.54/ p = 0.38	
		Tumour site paraspinal/flank vs abdominal wall	**p = 0.01**	
Colombo, 2015	[9]	*Change in treatment strategy*		
		Sex	p = 0.565	
		Tumour site	p = 0.926	
		Size	p = 0.397	
Turner, 2019	[38]	*Progression*		
		Tumour site abdominal wall vs. other sites	p = 0.53	
Van Houdt, 2019	[36]	*Change in treatment strategy*		Larger tumour size (>7 cm), reporting pain, and stable disease or progressive disease are associated with a higher risk of initiation of an active form of treatment
		Tumour size >7 cm (HR 2.04, 95% CI 1.29% to 3.21%)	**p < 0.01**	
		Reporting pain	**p < 0.001**	
		PR vs. SD, PD	**p < 0.001**	
		Age	p = 0.13	
		Tumour site	p = 0.36	
		Sex	p = 0.84	

CI, confidence interval; HR, hazard ratio; PD, progressive disease; PFS. Progression-free survival; PR, partial response; SD, stable disease

[a] *only available for n = 37 patients with evaluable magnetic resonance imaging*

The influence of tumour site on initiation of active surveillance

Frequent reported tumour sites (available in sixteen studies) were the extremities/girdles (n = 273 patients, median percentage of incidence in studies 31% [IQR: 3-68%]), the abdominal wall (n = 253 patients, median percentage of incidence in studies 9% [IQR: 0-37%]), and the trunk (n = 153 patients, median percentage of incidence in studies 17% [IQR: 0-37%]). Intra-abdominal (n = 60) and head/neck (n = 15) tumours were less common, with a median percentage of incidence in studies of 0% (0-8%) and 0% (IQR: 0-4%) respectively. From a total of 1480 patients receiving AS, the tumour sites were not specified in 726 (49%) of patients (Table 2).

Cassidy et al. described that patients with abdominal wall tumours were often managed with AS (61%), whereas those with chest wall and intra-abdominal tumours more often received active treatment (80% and 60%, respectively) [31]. Fiore et al. also described that patients who received AS commonly had abdominal wall tumours ($p < 0.0001$) compared with patients who received other treatments [14], whilst Park et al. found no difference in tumour sites between groups managed with AS or surgery [30].

The influence of the tumour site on disease stabilisation, progression or a change of the treatment strategy

No differences in risk of progression during AS were found between abdominal wall tumours and other sites ($p = 0.53$) by Turner et al. [38] nor on a chance of spontaneous stabilisation among axial sites or extremity tumours ($p = 0.148$) by Kim et al. [37] (Table 5). The 5-year PFS of primary cases managed with AS of trunk/thoracic wall tumours and abdominal wall tumours was similar (53.9% [SE: 16.2%] versus 52.5%, [SE: 14.3%]) in the study from Fiore et al. [14]. Van Houdt et al. concluded that upper-extremity and chest wall DTF tumours have the highest percentage of progression (39% and 47%, respectively), although this difference was not significant compared with other locations [36].

Cassidy et al. described that tumours located paraspinal or flank were more commonly associated with a change in treatment than abdominal wall tumours ($p = 0.01$), but no differences were found comparing extremity, intra-abdominal or abdominal wall tumours [31]. Van Houdt et al concluded that there was no difference in initiation of active treatment between upper extremity and chest wall DTF ($p = 0.36$) [36]. This is in line with the findings of Colombo et al. who did not identify the tumour site as a predicting factor for progression and/or change in the treatment strategy among tumour sites ($p = 0.926$) [9]. No single conclusion could be reached regarding tumour site and the success or failure of the AS approach because of the heterogeneity of the cohorts of included studies.

Discussion

This systematic literature review evaluated the outcomes of the AS approach in sporadic DTF. Twenty-five articles, describing the outcomes of the AS in DTF, were identified. The majority of the reported patients experienced SD, and about one-third of the patients needed to shift to "active" treatment. The median time of follow-up was reported by twelve studies and ranged between 8 months and 73 months, and the median time to shift from AS to active treatment or to progression ranged from 6.5 months to 19.7 months.

AS has increasingly been advocated in for sporadic DTF [39]. This is underlined by the number of publications about this subject since the year of 2006. In the most recent European consensus paper, published by the Desmoid Tumor Work Group in 2020, AS is advocated as a first line treatment in symptomatic patients, independently of the tumour site or size. In case of progression, other treatments such as surgery or systemic therapies, and treatments (including AS), should preferably take place in an expert clinic with an experienced multidisciplinary sarcoma team [7]. A study by Eastly et al. showed that almost half of the clinicians prefer AS an initial management strategy for primary DTF for which function-sparing surgery is possible. In case of recurrent DTF after a previous complete resection without adjuvant treatment, this rate dropped to 20% [40]. This is illustrated by the current study as the majority of included patients have primary tumours.

The definition of AS varies widely between studies. Some studies also allowed the usage of non-narcotic analgesics, NSAIDs or hormonal treatment in the AS group [6, 8, 38]. Especially for NSAIDs, which are non-prescription drugs in many countries and mainly used for relieving pain symptoms, the usage of these drugs can be severely under-reported by patients, clinicians and researchers. Inclusion of these patients in studies evaluating the AS approach can distort the true outcomes because NSAIDs and hormonal treatment (e.g., tamoxifen) can be beneficial for DTF with a reported response rate of 85% [41].

The current study did not include the results of the phase 3 trial comparing sorafenib to placebo [42]. Whilst placebo treatment can be considered a form of AS, as patients do not receive an active form of treatment, we decided not to include this trial in the current study. This was because only patients with progressive, recurrent or primary disease which were deemed inoperable or required extensive surgical resection or were symptomatic were included in this clinical trial. In daily clinical practice, AS will not be offered as a front-line approach to these patients, and therefor3 this study was not included in the current review.

The selection of patients suitable for the AS approach remains challenging. The results of this systematic review suggest that AS is mainly described as a treatment for tumours localised in the extremity/girdles and in the trunk. This might be due to the predilection sites of DTF tumours to these locations [43], or due to a selection upfront because of the higher risk of recurrence after surgery for these groups [12]. Based on the current systematic review, drawing a single conclusion with regard to tumour sites and the success of AS remains challenging. This is mainly due to the inclusion of studies with homogeneous cohorts in terms of tumour site (e.g., mesenteric, or breast), or a preselection of patients upfront (e.g., inoperable tumours due to localisation adjacent to vital structures [e.g., nerves, blood vessels]). Furthermore, the exact tumour site was not specified in a large number of patients.

About one-third of the patients needed a shift to an 'active' form of treatment. Although no uniform results could be drawn from the current studies, several studies reported that larger tumours were more likely to shift [10, 36], whilst age, sex and pregnancy before the development of DTF were not associated with this shift [10, 31, 36]. Colombo et al. reported that the sex, tumour site and tumour size did not predict progression and/or shift to change in treatment; the non-surgical group (n = 106) also contained patients receiving medical treatments (n = 4) [9]. Few studies described β-catenin mutation of the included cohort, and none of these studies analysed the influence of these mutations on the success or failure of the AS approach [6, 21]. The same applies for FAP-related DTF tumours. The variable results from these retrospective studies highlight the need for the identification of predictive factors for progression and changes in treatment strategies.

In the current study, progression was often reported within two years after diagnosis [14], however the length of follow-up of the included studies varied highly. Few studies reported the median follow-up duration of the AS subgroup, and time to intervention was often lacking. The minimal available information about the type and frequency of follow-up during AS underlines the need for standardisation of the AS approach. This includes defining a follow-up schedule with the use of MRI or CT, depending on the tumour site. As few studies reported progression after stabilisation, a maximum AS term should be discussed with the patient.

The major limitation of the current study is the inclusion of retrospective, small sample-sized studies, which often evaluate several treatment regimens, with various follow-up schedules and limited information about disease outcomes, or reasons for shifting to 'active' treatment. Only part of the studies used and reported disease response based on RECIST [18]. Some included studies selected patients for the AS approach based on the fact that

the patients were unable to tolerate chemotherapy or radiotherapy [28], had unresectable asymptomatic mesenteric masses [25] or had masses that were not life-threatening or at risk for mutilation [22]. Moreover, some studies selected patients based on tumour sites (e.g., breast desmoids [21, 23]) or were interested in other study endpoints than the results of the AS approach (e.g., pregnancy status [2], or imaging characteristics [24, 31]). Another limitation is the relatively large number of studies included in this systematic review where there is potential cohort overlap (based on author names, affiliations and inclusion time period) [2, 9, 14&6, 10, 12, 21-23]. Despite these limitations, this systematic literature review was able to compile the available evidence for the use of the AS approach in adult DTF.

Currently, the results of three prospective European studies evaluating the efficacy of AS in DTF are awaited. The French study (NCT01801176) and the Italian study (NCT02547831) which started in May 2012 and July 2013, respectively, both evaluate 3-year PFS [15, 16]. The Dutch study (NTR 4714) which started in May 2014, evaluates tumour progression at 5-years follow-up [17]. These three studies will provide further insights into the natural growth of DTF, the differences in growth behaviour between various tumour sites, tumour sizes, and β-catenin mutation types as well as the indications and considerations for the start of 'active' treatment.

Conclusions

Active surveillance is the mainstay of treatment for sporadic DTF. This systematic literature review underlined the ongoing trend of the AS approach and indicates that a minority of patients need shift to an active form of treatment avoiding overtreatment and minimising potential morbidity.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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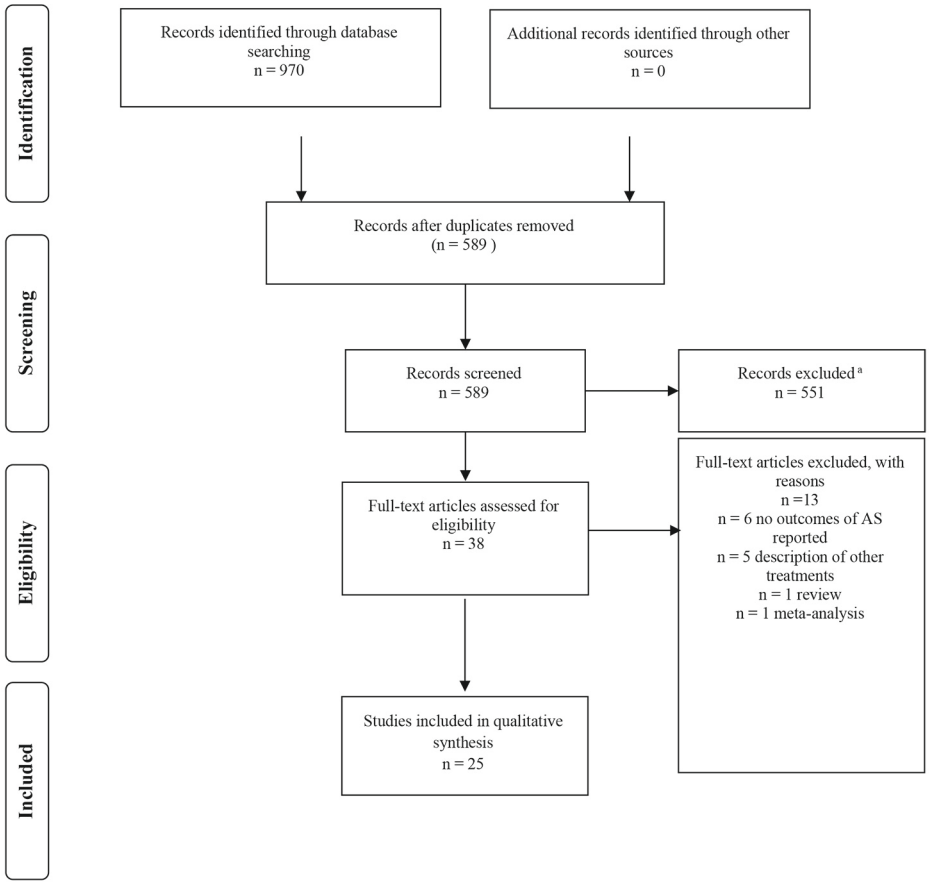
Supplemental Materials 1. Search strategy used in databases: Embase.com on December 20, 2019 and updated on April 14, 2020.

('desmoid tumor'/exp OR Fibromatosis/exp OR (desmoid* OR Fibromatos*):ab,ti,kw) AND ('conservative treatment'/de OR 'active surveillance'/de OR 'watchful waiting'/de OR 'expectant management'/de OR 'observation'/de OR 'clinical observation'/de OR (((activ*) NEAR/3 (surveill*)) OR ((conservativ* OR non-surgical* OR nonsurgical* OR non-operativ* OR nonoperativ* OR expectant* OR expectativ* OR expectiv*) NEAR/3 (treatm* OR therap* OR approach* OR managem* OR intervent*)) OR ((watchful*) NEAR/3 (waiting*)) OR ((wait) NEAR/3 (see OR watch)) OR wait-and-see OR ((without) NEXT/2 (intervent* OR treatm* OR therap*)) OR ((natural*) NEXT/3 (cours* OR behaviour* OR behavior*)) OR observation):ab,ti,kw) AND [ENGLISH]/lim NOT ((animal/exp OR animal*:de OR nonhuman/de) NOT ('human'/exp)) NOT ('case report'/exp OR (case-report*:ti)

Supplemental Material 2. Flow diagram of study selection: search performed on December 19, 2019 and updated on April 14, 2020



[a] reasons for exclusion:
n = 93, studies with patients receiving solely active forms of treatment such as surgery, systemic therapy, local therapy (e.g. cryoablation), and radiotherapy
n = 57, case reports, case series ≤ 5 patients
n = 12, pre-clinical studies
n = 14, diagnostic studies
n = 73, non-original reports (editorials, study protocols, reviews etc.)
n = 29, non-full text available (conference abstracts etc.)
n = 23, paediatric cohorts
n = 24, studies studying solely familial adenomatous polyposis (FAP) or Gardner syndrome
n = 223, other subject than solely DTF
n = 3, language other than English

7

The prognostic role of β-catenin mutations in desmoid-type fibromatosis undergoing resection only: A meta-analysis of individual patient data

Milea J.M. Timbergen, Chiara Colombo, Michel Renckens, Hee Sung Kim, Joost van Rosmalen, Sébastien Salas, John T. Mullen, Piergiuseppe Colombo, Yoshihiro Nishida, Erik A.C. Wiemer, Cornelis Verhoef, Stefan Sleijfer, Alessandro Gronchi, Dirk J. Grünhagen

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Abstract

Background

The majority of sporadic desmoid-type fibromatosis (DTF) tumours harbor a *CTNNB1* (ß-catenin) mutation: T41A, S45F and S45P or are wild-type (WT). Results are conflicting regarding the recurrence risk after surgery for these mutation types. This meta-analysis (PROSPERO CRD42018100653) uses individual patient data (IPD) to assess the association between recurrence and *CTNNB1* mutation status in surgically treated adult DTF patients.

Materials and methods

A systematic literature search was performed on June 6th, 2018. IPD from eligible studies was used to analyse differences in recurrence according to *CTNNB1* mutation status using Cox proportional hazards analysis. Predictive factors included: sex, age, mutation type, tumour site, tumour size, resection margin status, and cohort. The PRISMA-IPD guideline was used.

Results

Seven studies, describing retrospective cohorts were included and the IPD of 329 patients were used of whom 154 (46.8%) had a T41A mutation, 66 (20.1%) a S45F mutation, and 24 (7.3%) a S45P mutation, whereas 85 (25.8%) patients had a WT *CTNNB1*. Eighty-three patients (25.2%) experienced recurrence. Multivariable analysis, adjusting for sex, age and tumour site yielded a p-value of 0.011 for *CTNNB1* mutation. Additional adjustment for tumour size yielded a p-value of 0.082 with hazard ratios (HR) of 0.83 (95% confidence interval [CI]: 0.48-1.42), 0.37 (95% CI: 0.12-1.14) and 0.44 (95% CI: 0.21-0.92) for T41A, S45P and WT DTF tumours compared S45F DTF tumours. The effect modification between tumour size and mutation type suggest that tumour size is an important mediator for recurrence.

Conclusions

Primary sporadic DTFs harbouring a *CTNNB1* S45F mutation have a higher risk of recurrence after surgery compared to T41A, S45P and WT DTF, but this association appears to be mediated by tumour size.

Introduction

Desmoid-type fibromatosis (DTF) was first described 185 years ago by MacFarlane, and it was named desmoid in 1838 by Müller who referred to the Greek word "desmos", meaning "a tendon like structure" [1, 2]. Ever since, the understanding of this non-metastasizing and histologically benign tumour has grown remarkably [3]. Its potential to arise in musculoaponeurotic structures at virtually any body site and to invade surrounding structures poses therapeutic challenges. A histological biopsy, with nuclear ß-catenin staining, can confirm the diagnosis [4]. In recent years, there has been a tendency for an active surveillance approach in asymptomatic patients and several prospective clinical trials (NCT02547831, Italy, NTR 4714, the Netherlands, and NCT01801176, France) are conducted to evaluate the safety of this approach [5-9]. For progressive patients, surgical resection, isolated limb perfusion, radiotherapy and systemic therapy are available treatment options [10]. The variable growth behaviour with the possibility of tumour progression, growth arrest, or regression without treatment, makes this tumour unpredictable [5, 11, 12]. Local recurrence after surgery, especially in case of tumours located in the extremities, the head/neck region and intra-abdominal, occur frequently [13, 14].

The genetic roots of DTF have been extensively studied [15]. Desmoid tumours can occur as part of the inherited condition Familial Adenomatous Polyposis (FAP) and the FAP subtype; Gardner's syndrome [16]. Both conditions are associated with mutations found in the *adenomatous polyposis coli* gene on chromosome 5, and are known for the development of hundreds of pre-malignant colonic polyps. The development of mainly intra-abdominal DTF tumours is one of the associated manifestations, with a cumulative lifetime risk reaching 21% [17, 18]. Both syndromes will not be further discussed in this meta-analysis since the origin and clinical course of these diseases and the DTF tumours for which they predispose, differ from the sporadic variant of DTF. The sporadic variant is associated with extra-abdominal or abdominal wall desmoid tumours and finds its origin in the *CTNNB1* (ß-catenin) gene [15, 19-22]. β-catenin is involved in several downstream signaling pathways, functions as a transcriptional activator and is involved in cell-cell adhesion [19]. The mutations are located on exon 3, causing mostly the following amino acid changes: T41A, S45F and S45P [19, 23]. The remainder of tumours, less than 5%, that lack a mutation in the *CTNNB1* gene, and of which the underlying genetic aberrations are not entirely clear yet, are called wild-types (WT) [15].

The use of the *CTNNB1* mutation as a prognostic factor for recurrence after resection has been the subject of several studies. Some studies report that S45F-mutated DTF tumours exhibit a higher recurrence rate after primary resection than WT or other *CTNNB1*-mutated

tumours [19, 22-24]. However, others report conflicting results and could not reveal an impact of specific *CTNNB1* mutations on outcome [20, 25]. These contradictory results are almost certainly due to the fact that these are relatively small retrospective studies including heterogeneous patient cohorts, which hinders the assessment of the true prognostic value of specific *CTNNB1* mutations on outcome. Because the insight into prognostic factors can be crucial to come to a more personalized treatment approach for DTF patients who undergo a resection, we performed a meta-analysis using individual patient data (IPD) to study the impact of the *CTNNB1* mutation on the risk of local recurrence in a large series of sporadic DTF patients. The hypothesis is that S45F mutated DTF tumours have a higher risk of local recurrence than WT or other *CTNNB1*-mutated DTF tumours. The objective of this meta-analysis with IPD is to evaluate the impact of the *CTNNB1* mutation type on recurrence and recurrence-free survival (RFS) in adult patients with primary DTF tumours undergoing surgical resection alone.

Materials and methods

Protocol and registration

This study was approved by the local Medical Ethics Committee of the Erasmus Medical Center (MC) (MEC-2018-1386) Rotterdam, the Netherlands. The protocol of this meta-analysis was registered on PROSPERO (CRD42018100653) and can be accessed at www.crd.york.ac.uk/PROSPERO_[26].

Information sources

A systematic literature search was performed by an Erasmus MC librarian expert on June 6th, 2018. The following databases were used for the search: Embase.com, Medline Ovid, Web of science, Cochrane Central, Psych INFO Ovid and Google Scholar. Duplicated records were removed. No data or language filters were applied. The search strategy is provided in Supplemental Table 1.

Eligibility criteria

Studies with surgically treated sporadic DTF as a main subject were included. Papers describing follow-up, risk of recurrence, or recurrence-free survival (RFS) in primary DTF tumours with known *CTNNB1* mutational type were included for this meta-analysis. The flowchart depicting the study selection procedure is available in Supplemental Table 2.

Study selection

Two independent review authors (M.J.M.T. and M.R.) assessed the retrieved articles of the search for potential inclusion by reviewing title and abstract. Next, full articles were evaluated according to the predetermined inclusion and exclusion criteria for this meta-analysis (listed in Supplemental Table 3).

Assessment of study quality and risk of bias

The Oxford levels of Evidence (Oxford Centre for Evidence Based Medicine) were used to assess the quality of included articles [27]. The quality of included studies was assessed using the Quality In Prognostic Studies (QUIPS) tool [28]. This tool consists of six domains of potential biases: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, statistical analysis and reporting, rated as "low", "moderate", or "high" risk of bias. When all domains were considered to be "low" or "moderate" risk of bias, the risk of bias was considered to be low. If one or more domains were rated as "high" risk of bias, it was considered to be a high risk of bias. Two authors scored the risk of bias (M.J.M.T. and M.R.) and resolved discrepancies by discussion.

Data collection process

Two authors (M.J.M.T. and M.R.) independently extracted clinical and genetic information from the full text using a predefined extraction sheet. Subsequent cross-checks were performed. Inconsistencies were discussed and resolved. Patient overlap was defined as "the description of a cohort from one institute with overlapping time periods". In the latter case, the largest cohort was included and IPD was requested from the corresponding author.

Data items

For each eligible article, the corresponding authors were contacted via email to retrieve the IPD. They were asked to provide the data either in a template database or by providing their database. Requested variables included: sex, date of birth, date of diagnosis, age at diagnosis, tumour site (extra-abdominal, intra-abdominal, abdominal wall), tumour site specified, tumour size, treatment type (active surveillance, surgery, radiotherapy, systemic therapy, or combination therapy), date of surgery, resection margin status, presentation (primary or recurrent tumour), FAP status, *CTNNB1* mutation (WT, T41A, S45F, S45P or other), recurrence (yes or no), manner of identifying the recurrent tumour (radiological and/or pathological confirmation), date of recurrence on radiology and/or pathology, date of last follow-up, vital status (death from disease, death from other cause, alive without evidence of

disease, alive with evidence of disease, unknown), date of death, date of the last update of the database. Studies for which IPD was not provided were excluded from this analysis.

Inclusion of patients using individual patient data

Inclusion criteria for individual patients to be selected included: primary DTF tumour, age above eighteen years at diagnosis, treated with surgery alone, and known *CTNNB1* mutation. Patients receiving neoadjuvant or adjuvant treatment, patients with rare *CTNNB1* mutations, patients with intra-abdominal tumours or FAP, patients younger than 18 years, patients with missing data about recurrence, and patients with unknown *CTNNB1* mutation type were excluded from the analysis (Supplemental Table 2). With respect to tumour sites, all papers applied different definitions for tumour sites. For data synthesis the following categories of tumour sites were applied: trunk/back (including the following terms: trunk, trunk superficial, abdominal wall, rectus abdominis, chest aperture, chest wall, flank, back, dorsal, prevertebral, scapular area, vertebra), head and neck (including the following terms: head/neck, neck, cervical) and extremity (including the following terms: extremity, upper extremity distal, upper extremity proximal, ulna, arm, elbow, shoulder, axilla, groin, inguinal, buttock, gluteal, calf, knee, lower leg, leg, popliteal, thigh, lower extremity proximal, lower extremity distal). One study failed to specify the extra-abdominal tumour site and these patients were categorized as having extra-abdominal DTF not specified (NS).

In case the date of surgery was provided, age was calculated using date of birth and date of surgery. In case only the date of birth was provided, age was calculated using the date of birth and the date of diagnosis. In case age was provided in the cohort, it was defined in almost all cases as “age at surgery”. The median and mean age in years, the standard deviation (SD), and the interquartile range (IQR) were calculated for the total group and per cohort.

Tumour size was obtained from the provided databases; however, specifics on how this variable was measured were lacking for every cohort. A single measurement of tumour size reported was assumed to be the largest diameter of the tumour. In case tumour size was given in multiple dimensions, the largest tumour size was used. All tumour sizes were converted into millimetres (mm). The mean tumour size (in mm), SD, median and IQR were calculated for the total group and per cohort. In case of non-normal distribution, log transformed values were used. Additionally, the percentiles (25th, 50th, and 75th) were used to create age and tumour size categories.

R0 resection was assumed in case the surgical margin was provided as "0", "R0" or "clear". R1 resection was assumed in case the surgical margin was provided as a "1", or "involvement". In cases with "2", R2 resection was assumed and these patients were excluded from the analysis. Cases with unknown resection margin, stated as "not applicable" (NA), "unknown", "Rx" or "999" were marked as unknown.

RFS was calculated using the date of surgery and the date of recurrence. In case the date of surgery was not provided, the date of diagnosis and the date of recurrence were used to calculate this variable as we assumed that most patients underwent surgery within two months after diagnosis. End of follow-up was considered as "last date of follow-up", or "date life or death".

IPD integrity

Data of individual patients were not subjected to data checking since cohorts were from various countries and often included patients from various hospitals due to the rarity of DTF. Data integrity was checked by comparing published articles with shared databases containing data of individual patients.

Statistical analysis

An one-stage approach was used for this meta-analysis with IPD. All variables were collected in a single database. Categorical variables are summarized as frequencies and corresponding percentages. Continuous variables are summarized as mean values with SD or as median with IQR. Analysis of variance and the chi-square test were used to evaluate the associations between the variables tumour size, tumour site, sex, mutation type and cohort.

The Kaplan-Meier method was used to calculate RFS, which was defined as the time between the date of surgery (or the date of diagnosis in case the date of surgery was not available) and the date of recurrence (or the end of follow-up).

Univariable and multivariable Cox proportional hazards analyses were performed to assess the association between the outcome (recurrence) and the independent variables (age, sex, mutation type, tumour site, tumour size [log-transformed], resection margin status and cohort). The proportional hazards assumption was tested for each independent variable by including an interaction effect of the independent variable with time since surgery in a Cox regression with time-dependent covariates. In case of significance of the interaction effect, the proportional hazards assumption was considered not to be met and the variable was included as a stratification

variable. An interaction effect between tumour size and *CTNNB1* mutation type was tested in the multivariable Cox model adjusting for age, sex, mutation type, tumour site and tumour size; subgroup analyses based on tumour size were considered in case of considerable interaction. Two-sided $p < 0.05$ was considered statistically significant. SPSS Statistics (version 24) was used for all statistical analyses (IBM, Armonk, New York, USA).

Results

Study selection and study characteristics

Results of the search strategy are presented in the flowchart provided in Supplemental Table 2. A total of 47 articles were screened based on full text for eligibility. Studies were excluded for the following reasons: describing a pediatric cohort (n = 1), no *CTNNB1* mutation type data available (n = 14), no full text available (n = 3), conference abstract (n = 1), no recurrence data described (n = 6), no DTF as main subject (n = 1) and a review article (n = 3). Based on the origin of the patient cohort, eight studies had to be excluded for describing patient sets having a large overlap with series already published [19, 24, 25, 29-33]. The papers describing the largest cohort were included to request the IPD. From others we were not able to receive the IPD [15, 34, 35]. All included articles concerned retrospective cohort studies and received a score of 2b at the Oxford Levels of Evidence 2011[27]. The QUIPS tool was used for assessing the risk of bias (Supplemental Table 4) [28]. A detailed description of the published reports of the included studies can be found in Supplemental Table 5.

Individual patient data and clinical characteristics

A total of ten corresponding authors were contacted for exchanging IPD. Seven out of ten authors were willing to share the data. Data from individual patients were provided in a cohort template (n = 1), a database containing a selection of patients (n = 2) or by sharing the entire database (n = 4), and the cohorts were analysed by one author (M.J.M.T.). Patients were screened using the aforementioned inclusion and exclusion criteria for IPD, leaving a total number of surgically treated adult patients with primary DTF tumours of 329. The majority of the patients were female (n = 247, 75.1%) with a median age of 38 years (IQR: 31-50 years), and the most common tumour site was the trunk/back (n = 194, 59%). The majority (n = 154, 46.8%) of patients had a *CTNNB1* T41A mutated DTF tumour. Other clinical characteristics are summarized in Table 1. Missing values included unknown resection margin status for 18 (5.5%) patients, missing tumour size (in mm) for 7 patients (2.1%), and an extra-abdominal tumour site which was not further specified for 49 patients (14.9%). The latter was included as separate category for the variable tumour site.

Table 1. Clinical characteristics of the total cohort and of individual patient data of included studies

	Total n=329	Kim et al. (n = 49)[43]	Salas et al. (n = 39)[49]	Nishida et al.[42] (n = 3)	Mullen et al.[20] (n = 63)	Mussi et al.[41] (n = 17)	Van Broekhoven et al.[22] (n = 47)	Colombo et al.[23] (n = 111)
Sex								
Male	**82** (24.9%)	18 (36.7%)	8 (20.5%)	1 (33.3%)	13 (20.6%)	2 (11.8%)	13 (27.7%)	27 (24.3%)
Female	**247** (75.1%)	31 (63.3%)	31 (79.5%)	2 (66.7%)	50 (79.4%)	15 (88.2%)	34 (72.3%)	84 (75.7%)
Tumour Site								
Trunk / back	**194** (59%)	28 (57.1%)	23 (59%)	3 (100%)	29 (46%)	17 (100 %)	32 (68.1%)	62 (55.9%)
Head / neck	**9** (2.7%)	1 (2%)	4 (10.3%)	0	1 (1.6%)	0	3 (6.4%)	0
Extremity	**77** (23.4%)	20 (40.8%)	12 (30.8%)	0	33 (52.4%)	0	12 (25.5%)	0
EA NS	**49** (14.9%)	0	0	0	0	0	0	49 (44.1%)
Mean age in years (SD)								
	41 (14.3)	40 (13.1)	47 (16.5)	35 (10)	38 (13.6)	37 (7.3)	39 (12.7)	43 (15.2)
Median age in years (IQR)								
	38 (31-50)	37 (29-49)	46 (36-60)	36 (25-45)[a]	36 (27-46)	36 (33.5-39)	38 (30-46)	38 (32-54)
Age in years								
≤30 years	**81** (24.6%)	13 (26.5%)	9 (23.1%)	1 (33.3%)	22 (34.9%)	2 (11.8%)	12 (25.5%)	22 (19.8%)
31>38 years	**79** (24%)	13 (26.5%)	2 (5.1%)	1 (33.3%)	13 (20.6%)	9 (52.9%)	11 (23.4%)	30 (27%)
≥38<50 years	**78** (23.7%)	10 (20.4%)	9 (23.1%)	1 (33.3%)	17 (27%)	5 (29.4%)	16 (34%)	20 (18%)
≥ 50 years	**91** (27.7%)	13 (26.5%)	19 (48.7%)	0	11 (17.5%)	1 (5.9%)	8 (17%)	39 (65.1%)
Mean tumour size in mm (SD)								
	67 (44)	77.9 (45.5)	74.5 (52)	48.3 (2.9)	71.8 (38.5)	44.2 (33.3)	40.7 (31.4)	70.9 (46.2)
Median tumour size in mm (IQR)								
	55 (36.8-90)	70 (42.5-110)	65 (40-95)	50 (45-50)[a]	70 (40-95)	26 (19-66.5)	35 (23-45)	60 (40-90)

Table 1. *(continued)*

	Total n=329	Kim et al. (n = 49)[43]	Salas et al. (n = 39)[49]	Nishida et al.[42] (n = 3)	Mullen et al.[20] (n = 63)	Mussi et al.[41] (n = 17)	Van Broekhoven et al.[22] (n = 47)	Colombo et al.[23] (n = 111)
Tumour size in mm [b]								
≤37 mm	**82** (24.9%)	7 (14.3%)	6 (15.4%)	0	11 (17.5%)	9 (52.9%)	29 (61.7%)	20 (18%)
>37≤55 mm	**82** (24.9%)	11 (22.4%)	10 (25.6)	3 (100%)	15 (23.8%)	4 (23.5%)	10 (21.3%)	29 (26.1%)
>55≤90 mm	**89** (27.1%)	14 (28.6%)	12 (30.8%)	0	21 (33.3%)	1 (5.9%)	7 (14.9%)	34 (30.6%)
> 90 mm	**69** (21%)	17 (34.7%)	9 (23.1%)	0	16 (25.4%)	3 (17.6%)	1 (2.1%)	23 (20.7%)
Unknown	**7** (2.1 %)	0	2 (5.1%)	0	0	0	0	5 (4.5%)
Resection margin status								
R0	**185** (56.2%)	26 (53.1%)	20 (50%)	0	26 (41.3%)	16 (94.1%)	27 (57.4%)	70 (63.1%)
R1	**126** (38.3%)	16 (32.7%)	17 (45%)	3 (100%)	37 (58.7%)	0	15 (31.9%)	38 (34.2%)
Unknown	**18** (5.5%)	7 (14.3%)	2 (5%)	0	0	1 (5.9%)	5 (10.6%)	3 (2.7%)
Mutation type								
S45F	**66** (20.1%)	6 (12.2%)	9 (23.1%)	0	14 (22.2%)	1 (5.9%)	10 (21.3%)	26 (23.4%)
T41A	**154** (46.8%)	29 (59.2%)	17 (43.6%)	3 (100%)	31 (49.2%)	6 (35.3%)	23 (48.9%)	45 (40.5%)
S45P	**24** (7.3%)	3 (6.1%)	7 (17.9%)	0	1 (1.6%)	1 (5.9%)	3 (6.4%)	9 (8.1%)
WT	**85** (25.8%)	11 (22.4%)	6 (15.4%)	0	17 (27%)	9 (52.9%	11 (23.4%)	31 (27.9%)
Recurrence								
Yes	**83** (25.2%)	16 (32.7%)	8 (20.5%)	0	15 (23.8%)	1 (5.9%)	9 (19.1%)	34 (30.6%)
No	**246** (74.8%)	33 (67.3%)	31 (79.5%)	3 (100%)	48 (76.2%)	16 (94.1%)	38 (80.9%)	77 (69.4%)

EA NS, extra-abdominal not specified; SD, standard deviation; WT, wild-type;
Missing values in tumour size (n = 7) are not included in mean and median tumour size calculation
[a] *due to a limited number of patients this value represents the minimum and maximum values*
[b] *categories based on quartiles*

Follow-up and recurrence

The median follow-up time was 49 months (IQR: 21-94 months). Of 329 patients, 83 patients (25.2%) experienced a recurrence. Of these 83 patients, the median time to recurrence was 16 months (IQR: 10-31 months) and the mean time to recurrence was 26 months (SD: 30 months). Table 2 summarizes the number and corresponding frequencies of local tumour recurrence according to the *CTNNB1* mutation type.

Table 2. Cross-tabulation of recurrence and *CTNNB1* mutation type

***CTNNB1* mutation**	**n**	**S45F**	**T41A**	**S45P**	**WT**
No recurrence	246 (74.8%)	38 (58%)	115 (75%)	20 (83%)	73 (86%)
Recurrence	83 (25.2%)	28 (42%)	39 (25%)	4 (17%)	12 (14%)
Total	**329**	**66**	**154**	**24**	**85**

WT, wild-type

Survival

There was a statistically significant ($p = 0.019$) violation of the proportional hazards assumption for the variable cohort in the multivariable Cox regression, suggesting that baseline hazards differed between studies. Therefore cohort was used as a stratification variable in the Kaplan-Meier analysis and in the univariable and multivariable Cox regression models. Supplemental Figure 1 shows the Kaplan-Meier survival curves with cohort as a stratification variable.

Univariable Cox regression models

In the univariable Cox regression models, *CTNNB1* mutation type, tumour site, and tumour size (log-transformed) were significant prognostic factors for local tumour recurrence after surgical treatment (Table 3).

Multivariable Cox regression models

In the first multivariable analysis, resection margin status was left out to reduce model complexity. Since none of the included cohorts indicated tumour size as a prognostic factor, the first multivariable model was only adjusted for sex, age, tumour site, and *CTNNB1* mutation, stratified by cohort. In this multivariable analysis, the S45P mutation and WT DTF were significantly less likely to recur compared to S45F mutated DTF with a hazard ratio (HR) of 0.32 (95% CI: 0.11-0.97), $p = 0.043$ and a HR of 0.34 (95% CI: 0.17-0.69), $p = 0.003$, respectively. A tumour located in the extremities was shown to be an adverse prognostic factor with a HR of 4.09 (95% CI: 2.11-7.92), $p < 0.001$ compared to trunk/back. The results are displayed in Table 4.

Table 3. Univariable Cox proportional hazards regression model for recurrence

		Univariable analysis [a]	
Variable	**n**	**HR (95% CI)**	**p-value**
Sex			0.290
Female	247	*1.00 (reference)*	-
Men	82	1.31 (0.80-2.14)	0.290
Age	288	0.99 (0.97-1.01)	0.190
Mutation type			**0.006**
S45F	66	*1.00 (reference)*	-
T41A	154	0.64 (0.39-1.06)	0.081
S45P	24	0.30 (0.10-0.86)	**0.026**
WT	85	0.34 (0.17-0.68)	**0.002**
Tumour site			**<0.001**
Trunk/back	194	*1.00 (reference)*	-
Head/neck	9	2.53 (0.54-11.8)	0.236
Extremity	77	3.81 (1.98-7.35)	**<0.001**
EA NS	49	2.70 (1.33-5.46)	**0.006**
Tumour size in mm [b]	283	1.69 (1.16-2.47)	**0.007**
Resection margin status			0.170
R0	185	*1.00 (reference)*	-
R1	126	1.38 (0.87-2.18)	0.170

HR, hazard ratio; CI, confidence interval; WT, wild-type; EA NS, extra-abdominal not specified
[a] Cohort was used as a stratification variable due to a violation of the proportional hazards assumption (p = 0.019)
[b] Used as a log transformed value

Tumour size

Despite the fact that none of the included studies described tumour size as a prognostic factor, a significant p-value for tumour size (log-transformed) was found in the univariable analysis. To gain more insight into this variable, we tested with an analysis of variance whether there were differences in tumour size (as a log transformed variable) between the cohorts and we found a statistically significant difference ($p < 0.001$). The cohort from Van Broekhoven et al. and Mussi et al. contained smaller tumours. Next, we investigated whether the tumour sizes differed between the various mutation groups and found a statistically significant difference ($p = 0.001$) in tumour size between mutation groups. From this analysis we concluded that tumours harbouring a S45F and S45P mutation were larger compared to T41A and WT tumours. Additionally, we looked whether the tumour size differed between the tumour sites, but this difference was not statistically significant ($p = 0.392$). No significant association could be found between tumour site and *CTNNB1* mutation type ($p = 0.261$), and between mutation type and sex ($p = 0.643$), using the chi-

square test. Taken together these findings indicated an important association between tumour size and *CTNNB1* mutation and led to the development of a second multivariable model (II) including tumour size as a continuous, log transformed variable. This model led to non-significant results for the association between *CTNNB1* mutation and recurrence (p = 0.082). The results of both multivariable analyses are displayed in Table 4.

Table 4. Multivariable Cox proportional hazards regression model for recurrence

		Multivariable analysis I [a]		**Multivariable analysis II [a] with additional correction for tumour size**		
Variable	**n**	**HR (95% CI)**	**p-value**	**n**	**HR (95% CI)**	**p-value**
Sex			0.434			0.482
Female	247	*1.00 (reference)*	-	242	*1.00 (reference)*	
Men	82	1.23 (0.73-2.06)	0.434	80	1.21 (0.71-2.04)	0.482
Age	288	0.99 (0.98-1.01)	0.272	283	0.99 (0.98-1.01)	0.405
Mutation type			**0.011**			0.082
S45F	66	*1.00 (reference)*	-	61	*1.00 (reference)*	-
T41A	154	0.70 (0.42-1.17)	0.178	153	0.83 (0.48-1.42)	0.490
S45P	24	0.32 (0.11-0.97)	**0.043**	24	0.37 (0.12-1.14)	0.084
WT	85	0.34 (0.17-0.69)	**0.003**	84	0.44 (0.21-0.92)	**0.029**
Tumour site			**<0.001**			**<0.001**
Trunk/back	194	*1.00 (reference)*	-	192	*1.00 (reference)*	-
Head/neck	9	1.67 (0.33-8.45)	0.534	8	1.60 (0.31-8.34)	0.581
Extremity	77	4.09 (2.11-7.92)	**<0.001**	77	4.15 (2.14-8.05)	**<0.001**
EA NS	49	2.42 (1.18-4.95)	**0.016**	45	2.04 (0.97-4.31)	0.061
Tumour size in mm [b]	-	-	-	283	1.53 (1.03-2.280)	**0.034**

HR, hazard ratio; CI, confidence interval; WT, wild-type; EA NS, extra-abdominal not specified
[a] Cohort was used as a stratification variable due to a violation of the proportional hazards assumption(p = 0.019)
[b] Used as a log transformed value

To examine the impact of tumour size on recurrence, we tested whether there was an effect modification of tumour size and mutation type and found a significant effect modification ($p_{interaction} = 0.09$) between those variables, adjusting for mutation, tumour size, tumour site, sex and age. To account for potential effect modification, we used the median tumour size (55 mm) as a cut-off value to perform a subgroup analysis of multivariable analysis (Supplemental Table 6). Supplemental Figure 2 presents the combined estimated effect (in terms of HR) of tumour size and *CTNNB1* mutation for the multivariable model for all patients with the interaction between tumour size (log-transformed) and *CTNNB1* mutation.

Discussion

This meta-analysis uniquely combined the IPD of seven studies to determine the effect of the *CTNNB1* mutation type on recurrence rate in a cohort of surgically-treated DTF patients, who did not receive any additional perioperative therapy for their primary tumour. Although active surveillance is advocated in asymptomatic patients, a substantial number of patients still receive surgical treatment for their DTF during their course of disease [10, 36]. Reported recurrence rates after surgical treatment remain high, between 20% and 68%, and highlight the need for prognostic factors to predict recurrence, to inform patients and to explore strategies to reduce recurrence risk in high risk groups [14, 24, 30, 37-39]. Tumour site, age at onset, and *CTNNB1* mutation type are most frequently mentioned as important prognostic factors [14, 40]. Although several studies have reported that *CTNNB1* mutation type had no prognostic value for recurrence compared to WT DTF, others have identified a correlation between the *CTNNB1* S45F mutation and a higher risk of recurrence [15, 20, 24, 25, 41, 42]. This study showed that *CTNNB1* mutation type is indeed relevant for recurrence, and the observed risk of recurrence was highest for S45F tumours, although the association was not statistically significant ($p = 0.082$). We also found that tumour size is a mediator for recurrence rate. The findings of this study are remarkable since none of the included studies found a significant correlation between recurrence and tumour size, and did not identify size as a prognostic marker recurrence [20, 22, 41-43]. However, others did report that tumour size is an independent predictor of recurrence (Crago et al., $p = 0.004$) or event free survival (Huang et al., $p = 0.006$), but they did not adjust for *CTNNB1* mutation type[44, 45]. Other larger series, not included in this meta-analysis because they did not meet our inclusion criteria or used overlapping patient cohorts, did not find a correlation between tumour size and progression free survival [31], time to recurrence [19], mutation type [34], and progression or recurrence [30]. Although the variable tumour size is prone to inter-observer and intra-observer variability, the current multivariable model suggests that both tumour size and mutation type should be considered as predictors for recurrence in patients with primary, extra-abdominal, surgically treated DTF.

Tumour site, especially the extremity, is reported as a significant prognostic factor for recurrence in the univariable analysis of multiple studies. However, this statement often does not hold in the multivariable analysis [19, 24, 37, 45]. These conflicting results could be explained by the fact that most of these studies comprise relatively small case series or cohort studies and include patients who received neoadjuvant/adjuvant chemotherapy, or radiotherapy, which influences the recurrence rates and impairs the assessments of the true prognostic value of these parameters [46]. The current study shows that a tumour located in one of the

extremities, is an adverse prognostic factor ($p < 0.001$) for recurrence compared to tumours located on the trunk/back.

Females represented the majority of patients in the current cohort (75%). This is in line with previous reports describing nationwide cohorts by Broekhoven et al.[47] (71.3% females) and Penel et al. [36] (72.6% females). In the current study, no statistically significant association between sex and mutation type ($p = 0.643$) could be found. Additionally univariable analysis did not identify sex as a prognostic marker for recurrence ($p = 0.290$).

The added value and the strength of the current study is the use of individual patient data, which created a relatively large cohort of patients that receive the same treatment for their primary DTF, but there are some limitations to take into account. The use of IPD demanded the formation of new variables, for example, T0 which was defined as date of surgery. Unfortunately, this variable was not available for all patients and was solved by the use of "date of diagnosis" in a small part of patients. Another example of a new variable which had to be created was tumour site. Although a statistically significant result was found in both univariable and multivariable analyses, one should keep in mind that there is a relatively large group of patients having an extra-abdominally located tumour without any further specification (extra-abdominal NS). A major limitation, considering the present knowledge, is the relatively large number of patients with WT tumours in the current cohort. Multiple studies report that *CTNNB1* mutations are not always detected by Sanger sequencing due to the low frequency of mutant alleles and the relatively low sensitivity of the technique. This can lead to incorrect allocation to the WT group while having *CTNNB1* or other, novel, mutations [15, 48, 49]. The *CTNNB1* genotyping protocols, used in the included studies (Supplemental Table 5), focused on the presence of a *CTNNB1* mutation in exon 3 and provided no information about the presence of other mutations that may be present in the tumours. Another limitation is the heterogeneity of included studies which differed in primary outcome and differences in follow-up procedures, possibly leading to missing a certain number of recurrent tumours. This is also reflected by the variable recurrence rates reported by the included studies. Moreover, the rarity of DTF is reflected by the limited number of available studies with available *CTNNB1* type assessing recurrence, and led to the inclusion of studies with a high risk of bias. As the aim of the current study was to gain insight into the prognostic values of the *CTNNB1* mutation, every study, regardless of their sample size, was included in this meta-analysis. Several studies could not be included in this meta-analysis due to potential cohort overlap and due to the impossibility to acquire the

IPD [15, 19, 23-25, 29-35]. Although there might be a selection, this is the largest cohort describing the correlation between *CTNNB1* mutation type and recurrence risk to date.

Despite these limitations, using the IPD of several international cohorts created a large pooled cohort of patient that received the same treatment, unique for a rare disease like DTF. The pooled data provided new insights into the prognostic value of the *CTNNB1* mutation type in predicting local recurrence in surgically treated DTF patients, with tumour size as an important mediator. Currently, there are no prospective studies examining the value of adjuvant therapy after surgical resection of DTF. Future studies investigating the role of adjuvant therapy in patients with an S45F mutation, in whom re-resection would result in unacceptable morbidity, are recommended. Additionally, mechanistic studies, exploring how the different *CTNNB1* mutations affect DTF tumour biology are warranted, especially since an increasing number of patients is being treated with active surveillance. Data from the three studies, that are currently investigating the active surveillance approach, will give valuable information regarding the association between *CTNNB1* mutation type and DTF tumour behaviour.

Conclusions

Tumour size and mutation type should be considered as predictors for recurrence in patients with extra-abdominal, surgically treated primary sporadic DTF. Ongoing studies about upfront active surveillance, are evaluating whether mutation type can predict the risk of progression, to anticipate the need for treatment in patients who received an initial active surveillance approach. If the predictive value of mutation type for the risk of progression is proven, mutation type should be included in the algorithm for managing DTF.

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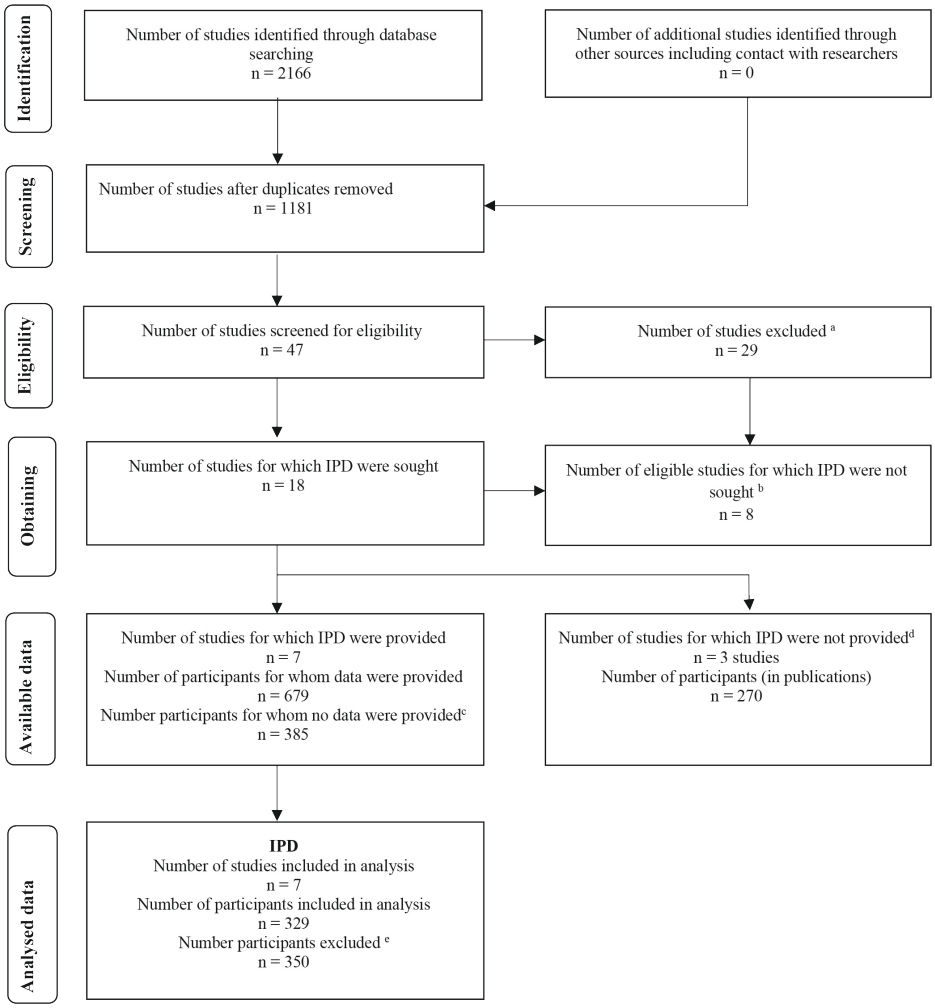
Supplementary Table 1. Electronic search, Embase.com, conducted on June 6, 2018

('desmoid tumor'/de OR 'desmoid type fibromatosis'/de OR 'desmoid fibromatosis'/de OR 'abdominal fibromatosis'/de OR (desmoid* OR ((fibromatos*) NEAR/3 (aggressive OR abdom* OR extra*))):ab,ti**) AND (**'mutation'/exp OR 'beta catenin'/de OR 'ctnnb1 gene'/de OR 'ctnnb1 protein human'/de OR 'ctnnb1 protein'/de OR 'gene frequency'/de OR 'molecular genetics'/exp OR 'molecular diagnosis'/de OR 'DNA microarray'/de OR 'tissue microarray'/de OR 'microarray analysis'/de OR 'nucleotide sequence'/de OR 'DNA sequence'/de OR 'tumor marker'/de OR 'biological marker'/de OR 'genetic analysis'/exp OR 'threonine'/de OR 'phenylalanine'/de OR 'alanine'/de OR 'proline'/de OR 'serine'/de OR (mutation* OR gene OR genes OR genetic* OR genotyp* OR molecular OR biomarker* OR ((tumor OR tumour OR biologic*) NEXT/1 (marker*)) OR 'beta catenin' OR 'b-catenin' OR 'β-catenin' OR ctnnb1 OR ((tissue) NEXT/1 (array OR analys*)) OR microarray* OR DNA OR 'nucleic acid' OR RNA OR S45f OR T41A OR S45P OR threonin* OR phenylalanin* OR alanine* OR proline* OR serine*):ab,ti**) NOT (**[animals]/lim NOT [humans]/lim**) NOT (**'Conference Abstract'**)**

Supplementary Table 2. Flowchart of the systematic literature search conducted on June 6th, 2018 and study selection



The PRISMA IPD flow diagram

[a] **Reason for exclusion n = 29**
n = 1 paediatric cohort
n = 14 no *CTNNB1* mutation type data available
n = 3 no full text available
n = 1 conference abstract
n = 6 no recurrence data described
n = 1 no DTF as a main subject
n = 3 review, no original article

[b] **Number of eligible studies for which IPD were not sought (give reasons) n=8**
n = 8 overlapping databases (defined as using a cohort from the same institute(s))

[c] **Number participants for whom no data were provided**
n = 385 patientsw
- n = 270 corresponding authors not willing to participate
- n = 115 unknown reasons, possible exclusion by the corresponding authors due to our inclusion criteria

[d] **Number of studies for which IPD were not provided**
n = 3 corresponding authors not willing to participate

[e] **Number participants excluded n = 350**

Colombo et al.[23] (n = 179)
Total excluded patients: n = 68
Total included patients: n = 111
n = 32 intra-abdominal DTF
n = 29 other treatment than surgery alone
n = 7 age <18 years

Mussi et al.[41] (n=25)
Total excluded patients: n = 8
Total included patients: n = 17
n = 1 other treatment than surgery alone
n = 4 wait and see only
n = 1 R2 resection
n = 2 recurrent tumours

van Broekhoven et al.[22] (n = 101)
Total excluded patients: n = 54
Total included patients: n = 47
n = 40 adjuvant radiotherapy
n = 4 other treatment than surgery alone
n = 6 age <18 years
n = 1 other mutation
n = 3 intra-abdominal DTF

Mullen et al.[20] (n = 115)
Total excluded patients: n = 52
Total included patients: n = 63
n = 8 pre-/ post-operative radiotherapy
n = 2 pre-/ post-operative photon therapy
n = 13 intra-abdominal DTF
n = 10 systemic treatment
n = 2 second gross resection
n = 1 other mutation
n = 6 age <18 years
n = 10 recurrent tumours

Nishida et al.[42] (n = 11)
Total excluded patients: n = 8
Total included patients: n = 3
n = 8 other treatment than surgery alone

Salas et al.[49] (n = 89)
Total excluded patients n = 50
Total included patients: n = 39
n = 1 other mutation
n = 14 age <18 years
n = 1 recurrence unknown
n = 31 recurrent tumours
n = 3 unknown tumour status

Kim et al.[43] (n = 159)
Total excluded patients: n = 110
Total included patients: n = 49
n = 20 other treatment than surgery alone
n = 10 age <18 years
n = 6 other mutation
n = 15 intra-abdominal DTF
n = 1 unknown tumour status
n = 18 recurrent tumours
n = 40 other diagnosis (Dupuytren's contracture, plantar fibromatosis)

Supplementary Table 3. Inclusion and exclusion criteria of the literature search, applied at study level

Inclusion criteria	Exclusion criteria
• Human subjects • Studies only reporting data on patients with a histologically proven primary DTF • Studies with at least one participant with age ≥ 18 years. • Studies with at least one participant having extra-abdominal or abdominal-wall DTF • Studies with at least one participant who received only surgical treatment for their DTF • Studies with at least one participant from which the *CTNNB1* (β-catenin) mutation type is known. • Studies that report recurrence, recurrence rate, follow-up time, recurrence free survival, event-free survival, disease free-survival, report of a local recurrence rate, progression free survival, local recurrence rate, overall recurrence rate, and overall survival time.	• Non-human models • Studies reporting about familial adenomatous polyposis exclusively • Studies reporting about intra-abdominal (mesentery) fibromatosis exclusively • Studies reporting about surgery in combination with other treatments exclusively • Studies describing a pediatric cohort (solely patients of 18 years and younger)

DTF, desmoid-type fibromatosis

Supplementary Table 4. Risk of bias assessed using the Quality In Prognostic Studies (QUIPS) tool[28]

	Study participation	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Study confounding	Statistical analysis & reporting	Overall risk of bias
Kim et al. [43]	Moderate	Moderate	Moderate	Low	Low	Low	**Low risk**
Salas et al. [49]	High	High	Low	Low	High	Low	**High risk**
Nishida et al. [42]	High	Moderate	Moderate	Low	Low	Low	**High risk**
Mullen et al. [20]	Low	Low	Low	Low	Low	Low	**Low risk**
Mussi et al. [41]	Low	Moderate	Moderate	Low	High	Low	**High risk**
Van Broekhoven et al. [22]	Low	Moderate	High	Low	Low	Low	**High risk**
Colombo et al. [23]	Low	Moderate	Moderate	Low	Moderate	Low	**Low risk**

When all domains were rated as low or moderate risk, a study was considered to be of low risk of bias
When at least 1 domain was rated as high risk, a study was considered to be of high risk of bias

Supplementary Table 5. Published patient characteristics of the included studies

		Kim et al.[43] (2016), Oncology Letters	Salas et al.[49] (2010), Genes, Chromosomes & Cancer	Nishida et al.[42] (2016), Oncology Letters	Mullen et al.[20] (2013), The Oncologist	Mussi et al.[41] (2016), Tumori	Broekhoven et al.[22] (2015), Annals of Surgical Oncology	Colombo et al.[23] (2013), Cancer
Main study outcome		To assess the predictive value *CTNNB1* mutation, midkine, B-catenin, TCF-4 and menin in the risk of tumour recurrence	Molecular characterization by array CGH	To analyse the surgical outcome of patients with extra-peritoneal DTF treated with ST ± surgery	To analyse prevalence of *CTNNB1* mutations and to determine the correlation between *CTNNB1* mutation type and disease outcome	To analyse prognostic factors and local disease free survival of abdominal DTF	To analyse clinico-pathologic factors and their prognostic significance	To test β-catenin as a prognostic marker of aggressiveness
Total number of patients		**159**	**194**	**13**	**115**	**33**	**101**	**179**
Sex	**Male**	77 (48%)	60 (31%)	2	30 (26%)	5 (15%)	35 (35%)	62 (35%)
	Female	82 (52%)	132 (68%)	11	85 (74%)	28 (5%)	66 (65%)	117 (65%)
	Unknown	-	2 (1%)	-	-	-	-	-
Median age in years / mean age in years [IQR] / (range)		41.2 (7-83) [a]	32 (0.04–80) [b]	36 (19-70) [b]	36 (3-76) [b]	37 (28-61) [b]	36 [28-44] [c]	39 (5-76) [b]

Supplementary Table 5. *(continued)*

		Kim et al.[43] (2016), Oncology Letters	Salas et al.[49] (2010), Genes, Chromosomes & Cancer	Nishida et al.[42] (2016), Oncology Letters	Mullen et al.[20] (2013), The Oncologist	Mussi et al.[41] (2016), Tumori	Broekhoven et al.[22] (2015), Annals of Surgical Oncology	Colombo et al.[23] (2013), Cancer
Tumour site	**Head/neck**	NS	NS	3 (23%)	4 (3%)	NS	10 (10%)	NS
	Chest wall / back	NS	NS	4 (31%)	NS	NS	23 (23%)	NS
	Extremity	NS	NS	NS	36 (31%)	NS	26 (26%)	NS
	Groin	NS	NS	NS	NS	NS	4 (4%)	NS
	Retroperitoneal	NS	NS	NS	NS	NS	3 (3%)	NS
	Abdominal wall	24 (15%)[d]	39 (20%)	6 (46%)	NS	33 (100%)	35 (35%)	75 (42%)[j]
	Trunk-superficial	NS	NS	NS	43 (37%)	NS	NS	NS
	Girdle	NS	NS	NS	18 (16%)	NS	NS	NS
	Intra-abdominal	19 (12%)	14 (7%)	NS	14 (12%)	NS	0 (0%)	32 (18%)
	Extra-abdominal (NS)	71 (45%)	132 (68%)	NS	NS	NS	NS	72 (40%)
	Superficial (fascial)	45 (28%)	NS	NS	NS	NS	NS	NS
	Unknown	NS	9 (5%)	NS	NS	NS	NS	NS
Presentation	**Initial tumour**	159 (100%)	112 (58%)	NS	95 (83%)	31 (94%)	101 (100%)	179 (100%)
	Recurrent tumour	0	67 (35%)	NS	20 (17%)	2 (6%)	0	0
	Unknown	-	15 (8%)	13 (100%)	-	-	-	-
Mutation type	**S45F**	13 (8.2%)	60 (33%) [e]	1 (8%)	29 (25%)	3 (12%)	18 (18%)	39 (22%)
	T41A	89 (56%)	70 (38%) [e]	6 (46%)	53 (46%)	9 (34%)	49 (49%)	81 (45%)
	Other	8 (5%)	20 (11%) [e]	0 (0%)	4 (4%)	1 (4%)	9 (9%)	11 (6%)
	Wild-type	48 (30.2%)	32 (18%) [e]	6 (46%)	29 (25%)	13 (50%)	25 (25%)	48 (27%)

Supplementary Table 5. *(continued)*

		Kim et al.[43] (2016), Oncology Letters	Salas et al.[49] (2010), Genes, Chromosomes & Cancer	Nishida et al.[42] (2016), Oncology Letters	Mullen et al.[20] (2013), The Oncologist	Mussi et al.[41] (2016), Tumori	Broekhoven et al.[22] (2015), Annals of Surgical Oncology	Colombo et al.[23] (2013), Cancer
***CTNNB1* genotyping**		• FFPE • 2-step PCR using nested primers • PCR amplification of exon 3 • Direct sequencing of amplified PCR product	• FT • PCR amplification of exon 3 • Direct sequencing of amplified PCR product • Lymphocytes as non-mutated controls	• FFPE/FT • PCR amplification of exon 3 • Direct sequencing of amplified PCR product	• FFPE • SNaPshot assay • PCR amplification of exon 3 • Direct sequencing of amplified PCR product	• FFPE • PCR amplification of exon 3 • Direct sequencing of amplified PCR product	• FFPE • PCR amplification of exon 3 • Direct sequencing of amplified PCR product	• FFPE • PCR amplification of exon 3 • Direct sequencing of amplified PCR product
Tumour size in centimetres, median (range)		NS	7 (1-30)	8.7 (4.5-18)	7.0 (0.8-29)	4.6 (1-11)	NS	7 (1-32)
Tumour size	**0-50 mm**	NS	NS	4 (31%)	NS	NS	61 (61%)	NS
	51-100 mm	NS	NS	3 (23%)	NS	NS	32 (32%)	NS
	> 100 mm	NS	NS	6 (46%)	NS	NS	8 (8%)	NS
	<50 mm	NS	NS	NS	29 (25%)	NS	NS	NS
	≥ 50 mm	NS	NS	NS	86 (75%)	NS	NS	NS
	< 60 mm	96 (61%)	NS	NS	NS	NS	NS	NS
	> 60 mm	61 (39%)	NS	NS	NS	NS	NS	NS
Depth	**Superficial**	45 (28%)	NS	NS	NS	NS	21 (21%)	NS
	Deep	114 (72%)	NS	NS	NS	NS	80 (80%)	NS
Gardner's syndrome		0	10 (5%)	NS	NS	NS	0	0

Supplementary Table 5. *(continued)*

		Kim et al.[43] (2016), Oncology Letters	Salas et al.[49] (2010), Genes, Chromosomes & Cancer	Nishida et al.[42] (2016), Oncology Letters	Mullen et al.[20] (2013), The Oncologist	Mussi et al.[41] (2016), Tumori	Broekhoven et al.[22] (2015), Annals of Surgical Oncology	Colombo et al.[23] (2013), Cancer
Recurrence	**Yes**	67 (43%)	69 (36%)	1 (8%)	31 (27%)	3 (9%)	17 (17%)	48 (27%)
	No	90 (57%)	83 (43%)	12 (92%)	84 (73%)	30 (91%)	84 (84%)	131 (73%)
	Not applicable	NS	23 (12%)	NS	NS	NS	NS	NS
	Unknown	2 (1%)	19 (10%)	NS	NS	NS	NS	NS
Median/Mean follow-up in months [IQR]/(range)		NS	58 (0.13-454) [f]	26 (6-63) [f]	31 [g]	42 (1-49) [h]	41 [18-71] [i]	50 [28-64] [i]
Treatment [k]	**Wait and see**	NS	NS	NS	NS	NS	NS	NS
	Surgery	98 (62%)	125 (64%)	4 (31%)	105 (91%)	30 (91%)	56 (55%)	145 (81%)
	ST	NS	NS	NS	NS	NS	NS	NS
	Surgery + (neo) A-ST	45 (28%)	20 (10%)	9 (69%)	NS	NS	5 (5%)	18 (10%)
	Rtx	NS	NS	NS	NS	NS	NS	NS
	Surgery + Rtx (pre-or postoperative)	12 (8%)	21 (11%)	NS	10 (9%)	NS	40 (40%)	16 (9%)
	Surgery + (neo) A-ST + Rtx (pre- or postoperative)	4 (3%)	NS	NS	NS	NS	NS	NS
	Unknown	NS	28 (14.4%)	NS	NS	3 (9%)	NS	NS

Supplementary Table 5. *(continued)*

		Kim et al.[43] (2016), Oncology Letters	Salas et al.[49] (2010), Genes, Chromosomes & Cancer	Nishida et al.[42] (2016), Oncology Letters	Mullen et al.[20] (2013), The Oncologist	Mussi et al.[41] (2016), Tumori	Broekhoven et al.[22] (2015), Annals of Surgical Oncology	Colombo et al.[23] (2013), Cancer
Resection margin status	**R0**	65 (54%)	NS	NS	52 (45%)	24 (80%)	64 (63%)	98 (55%)
	R1	56 (46%)	NS	NS	63 (55%)	3 (10%)	32 (32%)	68 (41%)
	Unknown	-	NS	13 (100%)	0	3 (10%)	5 (5%)	13 (4%)

NS, not specified; ST, systemic treatment; A ST, adjuvant ST; Rtx, radiotherapy; CGH, comparative genomic hybridization; FFPE, formalin fixed, paraffin embedded, FT, frozen tissue
[a] mean age (range); [b] median age (range); [c] median age [IQR]; [d] stated as 'abdominal'; [e] out of 182 samples; [f] median follow-up (range); [g] median follow-up, no range provided; [h] mean follow-up (range); [i] median follow-up [IQR]; [j] abdominal and chest wall; [k] treatment data was extracted from the publication. S45P CTNNB1 mutation was categorized as 'other' in this table
Percentages may not add up to 100% due to rounding up of decimals

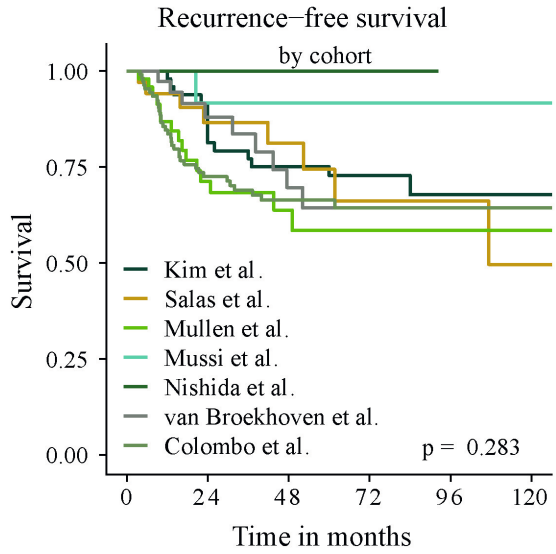
Supplementary Table 6. Cox proportional hazards regression multivariable analysis for recurrence, categorized according to tumour size

n = 136	Variable	HR (95% CI)	p-value
Tumour size 0- 55 mm	**Sex**		0.203
	Female	*1.00 (reference)*	-
	Male	1.81 (0.72-4.48)	0.203
	Age	0.98 (0.96-1.01)	0.177
	Mutation type		**0.020**
	S45F	*1.00 (reference)*	-
	T41A	0.32 (0.12-0.87)	**0.026**
	S45P	0.07 (0.01-0.77)	**0.030**
	WT	0.31 (0.11-0.92)	**0.035**
	Tumour site		0.107
	Trunk / back	*1.00 (reference)*	-
	Head / neck	11 (1.44-86.91)	**0.021**
	Extremity	1.64 (0.55-4.92)	0.375
	EA NS	1.9 (0.53-6.89)	0.324
	Tumour size [b]	4.37 (1.39-13.8)	**0.012**
n = 139 Tumour size >55 mm	**Sex**		0.853
	Female	*1.00 (reference)*	
	Male	1.08 (0.50-2.33)	0.853
	Age	0.99 (0.97-1.01)	0.390
	Mutation type		0.294
	S45F	*1.00 (reference)*	
	T41A	1.46 (0.69-3.13)	0.326
	S45P	0.76 (0.20-2.87)	0.687
	WT	0.56 (0.18-1.73)	0.316
	Tumour site		**0.001**
	Trunk / back	*1.00 (reference)*	
	Head / neck	0 (0)	0.982
	Extremity	6.25 (2.43-16.07)	**<0.001**
	EA NS	2.06 (0.81-5.29)	0.132
	Tumour size [b]	1.80 (0.77-4.20)	0.173

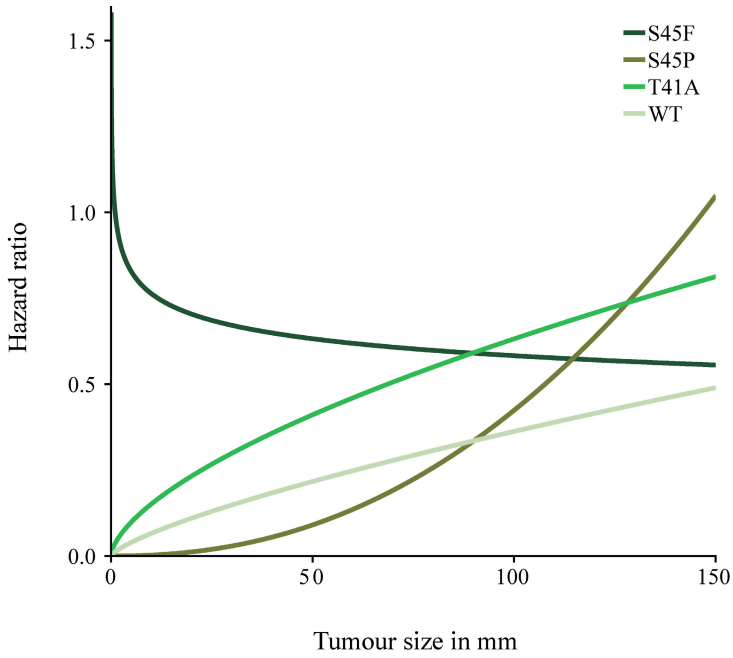
HR, hazard ratio; CI, confidence interval; WT, wild-type; EA NS, extra-abdominal not specified

[a] Cohort was used as a stratification variable due to a violation of the proportional hazards assumption (p=0.019)

[b] Used as a log transformed value



Supplementary Figure 1. Recurrence-free survival curve per included cohort calculated with the log rank test.



Supplementary Figure 2. Estimated Hazard Ratios per CTNNB1 mutation type for the total effect of tumour size in mm based on the multivariable model including the interaction between tumour size (log transformed) and CTNNB1 mutation.

III

Health-related Quality of Life

8

Identification and assessment of health related quality of life issues in patients with sporadic desmoid-type fibromatosis: A literature review and focus group study

Milea J.M. Timbergen, Lonneke V. van de Poll-Franse, Dirk J. Grünhagen, Winette T. van der Graaf, Stefan Sleijfer, Cornelis Verhoef, Olga Husson

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Abstract

Background

Sporadic desmoid-type fibromatosis (DTF) is a rare, chronic, non-metastasizing, disease of the soft tissues. It is characterised by local invasive and unpredictable growth behaviour and a high propensity of local recurrence after surgery thereby often having a great impact on health related quality of life (HRQoL). This study aims to review currently used HRQoL-measures and to asses HRQoL-issues among DTF patients.

Materials and methods

A mixed methods methodology was used consisting of (1) a systematic literature review, according to the PRISMA guidelines (2009), using search terms related to sporadic DTF and HRQoL in commonly used databases (e.g., Embase, Medline Ovid, Web of science, Cochrane Central, Psyc Info, and Google scholar), to provide an overview of measures previously used to evaluate HRQoL among DTF patients; (2) focus groups to gain insight into HRQoL issues experienced by DTF patients.

Results

The search strategy identified thirteen articles reporting HRQoL-measures using a wide variety of cancer-specific HRQL tools, functional scores, symptom scales (e.g., NRS), and single-item outcomes (e.g., pain and functional impairment). No DTF specific HRQoL-tool was found. Qualitative analysis of three focus groups (6 males, 9 females) showed that participants emphasised the negative impact of DTF and/or its treatment on several HRQoL-domains. Six themes were identified: 1) diagnosis, 2) treatment, 3) follow-up and recurrence, 4) physical domain, 5) psychological and emotional domain and 6) social domain.

Conclusions

A DTF-specific HRQoL-tool and consensus regarding the preferred measurement tool among DTF patients is lacking. Our study indicates that HRQoL of DTF patients was negatively affected in several domains. A DTF-specific HRQoL-measure could improve our understanding of short- and long-term effects and, ideally, can be used in both clinic and for research purposes.

Introduction

Desmoid-type fibromatosis (DTF) is a soft tissue tumour that arises from musculoaponeurotic structures. It is incapable of metastasizing and is often described as a benign tumour in clinical practice. However, due to its local aggressive behaviour and its known tendency of local recurrence after initial surgical resection, it is categorized as a borderline tumour [1]. Desmoid-type fibromatosis is rare, with a reported incidence of 5.4 new cases per million persons per year in the Dutch population [2]. Symptoms vary, depending on tumour location and size, and can be very severe. Roughly two types can be distinguished: sporadic DTF with extra-abdominal or abdominal wall tumour formation and familial adenomatous polyposis (FAP) related DTF with intra-abdominal tumour formation [3, 4].

The aetiology of sporadic DTF remains doubtful although a history of trauma has been reported, as well as specific hormonal status (such as pregnancy) and genetic predisposition [5-8]. With local recurrence rates up to 50%, potential treatment benefits and adverse effects of treatment should be considered carefully [9-11]. Nowadays, active surveillance is recommended in asymptomatic patients, while treatment options for symptomatic patients include surgical resection, radiation therapy, and systemic therapy [12-16]. Determination of treatment effectiveness is currently mainly evaluated by tumour size or recurrence free survival [11, 17, 18]. Although such end-points can be appropriate in malignant diseases, the unpredictable growth behaviour including spontaneous regression and the low mortality rate of sporadic DTF renders such outcomes less appropriate for this borderline disease [16]. Consequently, the question rises whether health related quality of life (HRQoL) assessment could be a more appropriate outcome measure in DTF [10, 14, 19, 20]. The definition of HRQoL is 'a patients' evaluation of the impact of a health condition and its treatment on all relevant aspects of life'. Patient-reported outcome measures (PROMS) can be used to measure HRQoL with various purposes: as screening tools, as a method for identifying patient preferences, to guide clinicians for informed decision making, to improve patient-provider communication, and to assess the efficacy of treatments in the context of clinical trials [21]. In DTF, few researchers have sought to understand patient's perceptions on the disease, and HRQoL is not (yet) widely accepted as an appropriate outcome measure. The aim of this mixed-method study is to explore currently used HRQoL-tools and identify HRQoL-issues of DTF patients.

Materials and methods

Literature review

The literature review was conducted in accordance with the PRISMA guidelines [22]. A systematic literature search with terms related to sporadic DTF and HRQoL (Supplemental Materials 1) was conducted by an expert research librarian on November 6th, 2017 to identify HRQoL-tools currently used among DTF patients. No language or publication limitations were applied. Used databases were Embase, Medline Ovid, Web of science, Cochrane Central, Psyc Info, and Google Scholar. The resulting publications were analysed using inclusion and exclusion criteria at two levels: title/ abstract (1) and full text (2) by two reviewers (MJMT and OH). Data from papers that met the inclusion criteria at full text level were extracted for final inclusion by one reviewer (MJMT) (Supplemental Materials 2). Corresponding authors were contacted in case of lack of availability of full text, three authors granted our request. Variables that were identified in included papers were number of patients, number of patients for which PROMs were available, tumour location, treatment, PROM outcome pre-treatment, and PROM outcome post-treatment. The outcome of each study was reported according to the specific PROM used in the study.

Patient recruitment

To identify the HRQoL issues of DTF patients, focus group sessions were organised. Patients diagnosed with sporadic DTF were recruited from the Erasmus Medical Centre (MC) in Rotterdam, the Netherlands. As FAP-associated DTF patients are also confronted with many other issues compared to patients with sporadic DTF, these patients were excluded. Eligible patients were diagnosed with DTF, regardless of their stage of disease (e.g., pre-treatment or during follow-up), previous or current treatments and site of disease. Additionally, they had to be above the age of 18 at the time of the focus group and participation required sufficient Dutch language skills. Patients with a recent diagnosis of cancer were excluded since this diagnosis might influence their HRQoL. Potential participants were approached by telephone, with a maximum of four attempts to explain the study objectives and received a written invitation and information letter. In total, three focus groups were organised in July and August 2017; one with male participants, one with female participants and one mixed sex group. The decision to organize separate sessions for both sexes was based on the assumption that patients would be more likely to share personal experiences or feelings with the same sex. The third, mixed sex group was organized separately because of logistic reasons. The focus group sessions took place in the Erasmus MC. Written informed consent (including permission for making field notes and audio recording for anonymous processing) and background information was obtained at the start.

Data collection

The focus group sessions were supervised by the first author (MJMT), a second independent researcher kept written records and was not actively involved in the discussions. A pre-prepared protocol, based on the protocol of Husson et al. [23] was used for guidance (Figure 1).

Figure 1. Focus group guideline

Opening question

- Can you introduce yourself by telling your name, age and place of residence and tell us shortly about the location of your DTF tumour, which treatments you had and what the current status is. (This question was asked in to every participant at the start of the focus group)

Transition questions

Moment of diagnosis

- Can you share your experience around the moment of diagnosis?
- Can you share your experience about the referral to this specialised centre?
- What kind of feelings did you experience when you were first diagnosed?
- How was the information about DTF in the first period?
- Can you remember the expectations that you had when you visited the specialist for the first time?
- How did your family and friends react? Did you need any support? Did you receive this support?
- Can you explain how you were informed about the treatment?

Symptoms

- Did you experience any problems on physical, emotional, social and/or financial level?
- How do / did you cope with your symptoms?
- Did you have to adjust your way of life? I yes, in what manner?
- Did you receive any support from your family and friends?
- How did you experience your contact with your treating specialist?
- How did you experience the professional support during the course of disease?
- How did you experience the amount of information about the disease and the treatment(s)?

Treatment

- Can you tell us what kind of treatment(s) you received?
- Can you tell us how the treatment(s) affected your life?

Work/ finances

- How did the disease effect your work and financial situation?

Key questions

- Which symptoms do you associate with your primary treatment(s)?
- Which symptoms do you experience during your follow-up?
- Which symptoms, caused by the desmoid tumour, do you experience on the long term?
- Which symptoms, caused by the desmoid tumour, have the most impact on your life?

Exit questions

- Can you describe your feelings when we are discussing your disease?
- Can you describe your feelings about this discussion?

Participants received a brief introduction with the explanation of the study objectives and an opening question for introduction was answered by each participant. Next, pre-prepared exploratory questions developed specific for the objectives of this study were asked to encourage conversation and discussion. Transition questions were asked to explore several aspects of HRQoL-issues around the time of diagnosis, treatment and follow-up. The focus groups lasted 1.5-2 hours, and an exit question was used to terminate the focus group. Participants received an evaluation form and 15 euro gift certificate in order to express our appreciation for their participation Focus group sessions were audio recorded, and transcribed by the first author (MJMT) ATLAS.ti 8.0© (Scientific Software Development GmbH, Berlin, Germany) was used for generating codes for themes and subthemes. The field notes were used as complementary data to transcripts as they described non-verbal communication of participants. Data was ordered into relevant code terms and then categorised into themes by two researchers (MJMT and OH) and analysed independently. Consensus was reached through continuous discussion. Relevant quotes of focus group participants were selected to support findings.

Approval from the Medical Ethics Committee of Erasmus Medical Centre (MC) in Rotterdam, the Netherlands was obtained for this study (file number MEC-2017-269). All patients gave written informed consent before the start of the focus groups and patient's anonymity and confidentiality were ensured throughout the study by the use of study codes replacing identifying information. Only the first author had direct access to the digital record of study codes and patient information.

Results

Literature review

A systematic literature search (Supplemental Materials 1) showed 3114 articles after deduplication. In total, 3067 articles were excluded based on title or abstract. Full-text reviewing, took place for the remaining 47 articles excluding another 34 articles (flow chart Supplemental Material 2). Thirteen articles, describing seven validated scoring systems were identified. No DTF-specific questionnaires were identified in this literature review (Table 1).

Table 1. Review of literature Patient Reported Outcome Measurements (PROM)

Score 1.	Score 2. / additional interests	Ref.	N (N-PROM)	Location	T	Outcome Pre-treatment	Outcome Post-treatment
DASH	-	[24]	14 (1)[a]	UE	SG	NA	[a] n = 1 DASH 38 (after interthoracoscapular amputation)
	MSTS score	[25]	12 (1)[a]	UE	SG	NA	[a] n = 1 MSTS 50%[b] (pain 2, function 1, acceptance 2, hand positioning 4, dexterity 3, lifting 3), DASH 62.5[b]
Enneking score / MSTS score	-	[26]	21	UE	SG BT	NA	mean MSTS 79% [b] (range 57% - 97%) n = 10 (48%) excellent; n =1 0 (48%) good; n = 1 (4%) medium
	pain functional impairment	[27]	7	UE	SG, ARTx CT	NA	mean MSTS 73% (range: 36-90%), n = 5 moderate functional impairment of the shoulder, n = 1 deficit flexion and extension of the elbow. n = 2 pain with use of medication
	TESS	[28]	14 (1)[a]	UE	SG	NA	[a] n = 1 MSTS 50 (pain 3, function 2, acceptance 5, hand positioning 0, dexterity 5, lifting 0), TESS 62
EORTC QLQ-C30		[19]	14	AW	SG	NA	mean global health status [b]: 97 (± 5.9); physical functioning 93 (± 11.1); role functioning 89 (± 16.7); emotional functioning 87 (± 19.1); cognitive functioning 94 (± 8.3); social functioning 93 (± 14.7)
MDASI	-	[29]	17	AS	GSI	NA	mean symptom severity: partial responders (n = 5): 1.65 point improvement, stable disease (n = 5): 0.8 point improvement, no response/ dropout (n = 7) 0.04 point improvement

Table 1. *(continued)*

Score 1.	Score 2. / additional interests	Ref.	N (N-PROM)	Location	T	Outcome Pre-treatment	Outcome Post-treatment
Modified Johnstone scale	-	[30]	40 (24)	AS	SG ARTx CT	n= 24 amputation required (grade 0): n = 1 (4%), severe functional deficit (grade 1): n=1 (4%), major functional limitations (grade 2): n = 4 (17%), Mild functional limitations (grade 3): n = 7 (29%), no functional limitations (grade 4): n = 9 (38%), NA: n = 2 (8%)	n = 24 grade 0: n = 5 (21%); grade 1: n = 0; grade 2: n = 6 (25%); grade 3: n = 3 (13%); grade 4: n = 8 (33%) not recorded: n = 2 (8%)
NRS	-	[31]	15 (6)	EA	MRg-FUS CT CA	n=6 NRS 7.5 (±1.9) (worst daily NRS); n=6 NRS 6 (±2.3) (average daily NRS)	n = 6 NRS 2.7 (± 2.6) (worst daily NRS); n = 6 NRS 1.3 (± 2) (average daily NRS)
	-	[32]	44	AS	ST	[c] median NRS 6 (IQR: 2-7) group B: median NRS 7/10; n=7 moderate pain, n=17 severe pain; group C: n=8 severe pain	[c] group B n = 12 (75%) pain improvement, n = 1 (6%) pain worsening, n = 3 (19%) stable symptoms; group C: n = 8 (100%) pain relief
Other scores	functional outcome	[33]	21	HN	SG ARTx	asymptomatic n=14 (62%), neurologic symptoms n=8 (38%)	N = 8 (38%) good, n = 13 (62%) persistent functional problems (motor (n = 7), paraesthesia (n = 4))

Table 1. *(continued)*

Score 1.	Score 2. / additional interests	Ref.	N (N-PROM)	Location	T	Outcome Pre-treatment	Outcome Post-treatment
	functional impairment	[34]	106	AS	SG ARTx RTx ST	NA	0-1 T: 23% functional impairment: moderate n = 13, major n = 2; 2 T: 56% functional impairment: moderate n=8, major n = 7; ≥ 3 T: 74% functional impairment: moderate n = 7, major n = 10
	pain; functional; impairment cosmetic outcome	[35]	12 (7)	EA	CT	n=7 pain; n=7 functional limitation; n=3 cosmesis	n = 6 pain relief; n = 3 partial improvement of function, n = 4 restore of normal function; N = 2 improvement of cosmetic outcome.

[a] Desmoid-type fibromatosis and soft tissue sarcoma combined, reported outcome for subgroup of DTF, [b] At most recent reported follow-up, [c] Group A: radiological progressive disease (PD), Group B: symptomatic deterioration and Group C: radiologically PD and symptomatic deterioration

AS: all sites; EA: extra-abdominal; AW: abdominal wall; IA: intra-abdominal; LE: lower extremities; HN: head and neck; UE: upper extremities

ACT: adjuvant chemo therapy; ARTx: adjuvant radiotherapy; BT: brachytherapy; CA: cryoablation; CT: chemotherapy; DASH, Disabilities of the Arm, Shoulder and Hand; Enneking / MSTS score, Enneking score adopted by the Musculoskeletal Tumour Society; EORTC QLQ-C30, The European Organisation for Research and Treatment of Cancer quality of life questionnaire C30; GSI: γ-secretase inhibitor; MDAS, MD Anderson symptom Inventory; MRgFUS: magnetic resonance-guided focused ultrasound; N, number of patients; NA, Not applicable; N-PROM, Number of patients with DTF and available patient reported outcomes; NRS, Numerical Rating Scale; NSG: no-surgery; PD, Progressive disease; PROM, Patient Reported Outcome Measurement; SG: surgery; Refs, reference; RTx: radiotherapy; T, treatment; TESS, Toronto Extremity Salvage score

The Disabilities of the Arm, Shoulder and Hand (DASH) score is a 30-item questionnaire designed to evaluate disability of the upper limb region by measuring symptoms and physical functions with 5 response options and higher scores reflecting greater disability [24, 25, 36, 37]. The Enneking/ Musculoskeletal Tumor Society (MSTS) score comprises six categories: pain, function and emotional acceptance of both lower and upper extremities, support, walking and gait of the lower extremities and hand positioning, dexterity and lifting ability in the upper extremity, for which patients have to assign values ranging from 0 to 5 points. Higher values indicate better functioning [25-27, 38, 39]. The European Organisation for Research and Treatment of Cancer quality of life questionnaire C30 (EORTC QLQ-C30) is a 30-item, cancer-specific questionnaire designed for evaluating quality of life incorporating five functional scales, symptom scales and global health and quality of life scales [19, 40]. The MD Anderson Symptom Inventory (MDASI) measures the severity of 13 cancer-related symptoms experienced by the patient during the previous 24 hours. The score rates symptoms on an 11-point scale; higher scores reflect more severe symptoms [29, 41]. The (modified) Johnstone scale provides a functional grading system with grades ranging from 0 to 4, higher scores reflect fewer limitations [30, 42]. The Numerical Rating Scale (NRS) is used for self-reporting subjective conditions, currently in use for several symptoms. Symptoms are rated on a 0 to 10 scale; higher scores reflect more severe symptoms [31, 32, 43]. The Toronto Extremity Salvage score (TESS) is internationally used for measuring functional outcome and physical disability in patients with extremity tumours undergoing limb preservation surgery. This questionnaire consists of 29 (upper extremity) or 30 (lower extremity) questions regarding daily activities. Each item is rated on a scale from 1-5, higher values represent better function [38, 44, 45].

Other identified measures and questionnaires included items related to functional impairment, pain and cosmetic outcome (Table 1.) [33-35].

Focus group

In total, 45 patients were approached to participate; 22 patients agreed to receive written information, 15 patients could not be reached by telephone. Reasons for refusal included not willing to participate in a group experience but willing to do a personal interview, not available at pre-set dates, language barrier or not willing to participate because of minimal symptoms. A total of 15 patients participated in the focus groups. The first group consisted of five female participants with a median age of 37 years (range: 25-60 years), the second group consisted of five male participants with a median age of 62 (range: 37-75 years), the third group was a mixed sex group with a median age of 37 years (range: 36-53 years).

Participants differed in age at diagnosis, education level and treatment (Table 2.). None of the participants knew another person with the same condition before the focus group. Most participants were treated surgically (n = 8) or received a conservative management (n = 4). Three participants received a combination of therapies. A minority of the participants sought support in the paramedic field (e.g., physiotherapist, occupational therapist, social worker, and dietician).

Table 2. Characteristics of fifteen focus group participants

Age in years at time of focus group	Median (range) years	46 (25-75)
Age in years at time of diagnosis	Median (range) years	43 (16-75)
		Number of patients (%)
Sex	Male	9 (60%)
	Female	6 (40%)
Marital status	Single	3 (20%)
	Married	9 (60%)
	Partnership	2 (13%)
	Windowed	0 (0%)
	Divorced	1 (7%)
Nationality	Dutch	14 (93%)
	Other	1 (7%)
Highest completed education	Elementary education	1 (7%)
	Secondary education	2 (13%)
	Middle-level applied education.	3 (20%)
	Higher professional education	6 (40%)
	Scientific education (university)	1 (7%)
	Missing value	2 (13%)
Current paid employment	Yes	8 (53%)
	No	5 (33%)
	Retired	2 (13%)
Familiar with DTF before diagnosis	Yes	0 (0%)
	No	15 (100%)
Location of DTF	Head / neck	1 (7%)
	Upper extremity / shoulder	2 (13%)
	Thoracic wall	0 (0%)
	Abdominal wall	4 (27%)
	Back	1 (7%)
	Retroperitoneal / intra-abdominal	2 (13%)
	Hip / pelvis/ gluteal region	2 (13%)
	Lower extremity	3 (20%)
Received treatment(s)	Conservative management	4 (27%)
	Surgery	8 (53%)
	Radiation therapy	0 (0%)
	Systemic therapy	0 (0%)
	Combination of therapies [a]	3 (20%)

Table 2. *(continued)*

Contact with health care professionals	Physiotherapist / Occupational therapist	5
	Dietician	1
	Social worker	2
	Psychologist	1
	Pain specialist	1
	Home care / nursing care	1
	Other [b]	
Self-reported symptoms [c]	Lump with obvious growth	10
	Pain	3
	Tumour complains during daily activities	8
	Functional limitations (before treatment)	3
Self-reported medical history [c]	Surgery related desmoid	6
	Desmoid related to hormonal status	3

[a] *n = 1: surgical resection with post-operative radiotherapy, n = 1 surgical resection, radiotherapy and isolated limb perfusion (ILP) ; n = 1 surgical resection (with final amputation of the lower leg, radiotherapy, isolated limb perfusion, hormonal therapy, experimental chemotherapy.*
[b] *lymphatic therapy;* [c] *obtained during the focus group sessions as reported by the patients*

Qualitative analysis

HRQoL-issues were categorised into six themes 1) diagnosis, 2) treatment, 3) follow-up and recurrence, 4) physical domain, 5) psychological and emotional domain and 6) social domain. The themes were further categorised into subthemes. An overview of themes, subthemes, key issues and quotes is provided in Table 3.

Diagnosis

Almost all participants reported feelings of uncertainty and anxiety of having cancer during the period of waiting on their final diagnosis. They described this as having a great impact on their overall life. Upon diagnosis, feelings of relief are described due to the borderline nature of this disease. Participants with more symptoms and a more aggressive clinical course of DTF mentioned being frustrated about underestimation of the consequences since the disease is categorised as a borderline tumour and can act in a more malignant way with sometimes severe sequelae compared to benign tumours. The opinion on receiving information about DTF varied amongst participants. Some participants felt they did not receive enough information from their treating physician, some participants searched for more information on internet or asked their general practitioner, and some deliberately did not search on the internet because of fear to find unpleasant information. Most participants agreed that the amount and depth of information they found in general was

Table 3. Themes, subthemes, key issues and quotes of three focus group sessions

Themes	Subthemes	Key issues	Quotes
Diagnosis	Uncertainties about diagnosis Diagnosis Information about DTF Need for information about DTF	• Broad differential diagnosis, lack of knowledge about DTF creating feelings of uncertainty and anxiety • Referral to specialized centre is considered to be time consuming • Borderline entity, presented as a "benign tumour" with an aggressive clinical course in some cases • Lack of knowledge about DTF of treating physicians in regional hospitals • Lack of up to date information for DTF patients and their relatives	*"sent from one specialist to another"* *"the feeling of insecurity, the fear of dying"* *"'it's a tumour and that is a disastrous scenario"* *"I took a whole different scenario into account"* *"you have cancer"* *"we can't help you"* *"I think we have to amputate your arm"* *"to me it is frustrating, this is a benign disease, but the more you read, the more information you receive, the more you find out about its aggressiveness and invasiveness, so for me this is a malignancy"*
Treatment	Treatment	• Lack of uniformity in treatment between hospitals • Shared decision making, patient autonomy	*"I am glad that the surgeon took it out"* *"the surgeon said: I don't want to operate because if I do, I'm not sure what I'm going to find"*
Follow-up & recurrence	Follow-up (concerns about) recurrence / concerns about the future	• Lack of clear information about recurrences rates specific for personal situation • Concerns about recurrence or concerns about future problems due to DTF	*"you know it is possible that you might need surgery another time, but if it happens, it happens"* *"I would love to have assurance that I am done with it"* *"you have a diagnosis, no prognosis"*
Physical domain	Symptoms (pre-treatment / post-treatment) Localization Medical history / co-morbidity Support physical therapy Self-image / cosmetic	• Awareness for functional problems and anticipate by offering physical therapy	*"the size of a tennis ball"* *"taking off my t-shirt is not easy, absolutely not"* *"I am asymmetrical after the surgery"* *"It took 4-6 months to be ready to practice with a prosthesis, but this leg was pretty messed up because of all the treatments"*

Table 3. *(continued)*

Themes	Subthemes	Key issues	Quotes
Psychological/ emotional domain	Coping strategy Lifestyle changes Emotional& psychological consequences Psychological support	• Awareness for psychological or emotional issues and anticipate by offering psychologic therapy	*"this is part of my pathway in life"* *"you learn to deal with this functional limitation; you just have to changes things"* *"if this is the worst scenario, I am okay with it"* *"as long as you don't know, you can worry about it, but it will do no good"*
Social domain	Education / financial / employment Social support / support of family	• Interest for impact of situation on family members of DTF patients	*"my family had more difficulty with the surgery than I did"* *"everyone is relieved because it's benign; yes that's what I thought the first time. Sometimes I find that difficult, because that is easy to say for people not living with a tumour in their abdomen"* *"the social pictures has changed, people I went to college with are more advanced in life, I'm standing still in life"* *"I had to move to a ground floor apartment"*

not satisfying. This observation was substantiated by multiple questions from participants about DTF during the group sessions.

Treatment

Participants with minor symptoms and solely treated with surgery reported being glad or relieved that the tumour was removed as they had the feeling that it 'did not belong to their body'. One participant with major symptoms from an intra-abdominal tumour felt that surgery was the only treatment option, but feared for a stoma or dying during surgery. Participants with a conservative management reported to be satisfied since they had minor symptoms and potentially mutilating surgery could be avoided.

Follow-up and recurrence

A common theme in the qualitative study was fear of recurrence or worries about the future and future health. Not all participants were correctly informed about the risk on local recurrence. Feelings of uncertainty remained present during follow-up because of the knowledge that the tumour may be able to recur. One participant with DTF localised in the lower extremity, reported struggle with weakness in the leg due to previous treatments, which made her fearful of the future.

Physical domain

The most common symptoms before diagnosis are described in Table 2. Complications of treatment included infection of the surgical wound, and severe neuropathic pain due to nerve damage. Residual issues after treatment regarded scars, being asymmetrical, having function restrictions, oedema, stiffness, lack of sensibility and muscle weakness. One participant used a wheelchair and crutches due to a lower leg amputation, another patient used an electric wheelchair due to severe neuropathic pain after being treated surgically. One participant reported that physical therapy was not offered to her, but in retrospect she would have appreciated it since she experiences weakness of the affected limb.

Emotional / psychological domain

Participants expressed that 'they felt they did not have a choice' and 'they will face the situation as it comes' and learned how to deal with their problems over time. Life-style changes included minor adjustments because of functional limitations and major adjustments including movement to a ground floor apartment. One participant reported that DTF restricted her from having another child, which had a major impact on her family. One participant reported a low self-esteem and problems with body image due to scars. Another

participant reported the feeling that he missed out on starting a family because of extensive treatments which started at a young age. One participant was treated by a psychologist. Several participants stressed that they felt differently about life after diagnosis and stated to be more grateful for their life compared to the time before the diagnosis.

Social domain

Participants reported that DTF had influenced their working life, as they had to stop working temporarily after treatment. This period ranges from a couple of weeks to two years and in one case not being able to work at all. Participants reported that the uncertainty during the time of diagnosis and the fear of cancer influenced their family life. Several participants mentioned to downstage their problems since they did not want to be a burden to their families or they wanted to protect their loved ones. One participant reported that social relationships changed after the diagnosis. Some friendships became closer and some friendships had ended due to lack of support. She specifically mentioned that her friends paid less attention to her disease and health status because of the term 'benign disease' which implies minor disease related issues or short course of disease.

Discussion

With this study, we aimed to gain more insight in HRQoL-issues and currently used HRQoL-tools in the setting of DTF. The results of this study can be seen as the first step towards developing a disease specific HRQoL-tool that can be used in clinical practice or research. The literature review identified several non-disease specific HRQoL-tools; no tool currently exists that assesses all issues relevant for DTF patients. Functional scores like the DASH score [24], the Enneking score / MSTS [25-27, 38], the TESS[38], and the Johnstone scale [30] are used for extremity diseases but are not suitable for patients who have sites of disease other than the extremities. Symptoms scores including the MDASI score [29] and the NRS [31, 32] are quite specific for measuring the severity of symptoms, and could be useful in combination with HRQoL-tools measuring issues like emotional or social wellbeing. The EORTC QLQ-C30 [19] is designed to cover issues relevant for cancer patients and may be a good generic measure to be completed by an item list consisting of the key DTF-specific issues identified in our focus groups, in order to create a more holistic perspective of HRQoL-issues in patients with DTF.

The results of the literature review show that researchers are interested in measuring the effect of DTF and its treatment on functioning or pain, but no consensus exists with respect

to the preferred tool, as a DTF specific tool has not been developed yet. One could argue that a combination of the aforementioned scores could be sufficient to get a clear view of relevant issues of DTF patients. A downside to this might be that patients are exposed to a large number of questions, which could be non-relevant and give patients an additional burden. A carefully developed DTF-specific tool could be effective in measuring HRQoL.

There are limitations to the current systematic literature review. Since DTF is a rare soft tissue tumour, included studies comprise retrospective, small sized studies with low methodologic quality. Additionally, risk of bias could not be assessed properly.

To create a HRQoL-tool which is suitable for DTF patients and to achieve at least satisfying content validity, focus groups were used which encouraged participants to discuss their views on HRQoL-issues [46]. Our focus group results suggest that patients with DTF often face problems with recognition and management because of the lack of diagnostic awareness, as a result of its rarity, and because of the striking discrepancy between its benign histological appearance and its local aggressive behaviour. This study identified key issues in six themes; 1) diagnosis, 2) treatment, 3) follow-up and recurrence, 4) physical domain, 5) psychological and emotional domain and 6) social domain, which will be the basis of a future DTF-specific tool. The first three themes (diagnosis, treatment and follow-up) can be clustered as 'the process of healthcare' and the last three themes (physical domain, psychological and emotional domain and social domain) can be clustered as 'symptoms and function'. We do acknowledge the overlap that can occur between themes.

The need to gain more insight into HRQoL of DTF patients is reflected by several attempts made around the world. In the USA, the Desmoid Tumor Research Foundation (DTRF) patient registry opened recently (September 2017) to register clinical, pathological and geographical variables of DTF patients. Additionally, a survey, based on both validated and non-validated HRQoL-questionnaires, was put together to gain more insight in HRQoL of DTF patients [47]. The latter, a PRO specific DTF-tool, was presented on the Annual Meeting of the American Society of Clinical Oncology of 2017 [48]. In the Royal Marsden UK, two focus group sessions took place in March 2017 [23]. This resulted in four key themes (diagnostic pathway; treatment pathway; living with DTF; supportive care). We found an interesting difference in the impact of DTF between the Dutch and UK focus group participants. Apart from the selection bias, which could be explained by the selection of patients and the willingness of patients to participate in such a study, and differences in the way patients had been treated with more often chemotherapy (Caelyx) in the UK focus

group, other factors may play a role, which are beyond the individual patient level of these focus groups participants. An international desmoid population-based questionnaire study could ideally give more detailed information. Such a study could also examine which patients are particularly at risk for poor disease related outcomes on their quality of life.

Our focus group study has several limitations. First; the recruitment of participants for focus group sessions might have led to selection bias. Patients who are introvert, or who have minor symptoms, or received successful treatment might have been less likely to agree to participate in a focus group session and vice versa. A frequently heard response, when being approached for participation, was the worry about being influenced by negative experiences of other patients. However, in that case, most patients were willing to do a private face-to-face interview with the author to share their experiences. This suggests that not all patients feel comfortable to join a group session. The second limitation involves the small number of DTF patients. Due to the rarity of DTF larger sample sizes are difficult to obtain in a single centre study. Nevertheless the small sample size gave all participants enough time to share their experiences [46]. The third limitation comprises the heterogeneity of the focus group participants, since we did not select participants based on their stage of disease or their treatment. Only one out of fifteen participants received previous systemic treatment, which might be an underestimation of the total percentage of patients in the DTF population receiving medication. We do acknowledge that every treatment modality (e.g., surgery, radiotherapy, chemotherapy) could impact HRQoL on the short- and the long-term. However, regardless of previous treatments, patients, included in the focus groups, shared a wide variety of experiences coinciding with the chronic nature of the disease. This resulted in the report of various HRQoL-issues, which we believe do represent the entire spectrum of HRQoL-issues experienced by the DTF population.

To our knowledge this is one of the few studies that explored currently used HRQoL-tools and the experience of HRQoL-issues in the setting of sporadic DTF. The strength of our study is the approach according to the EORTC guidelines for developing questionnaire modules [49]. By conducting the systematic literature review we revealed the necessity for measuring HRQoL-outcomes in clinical practice and exposed a deficit in suitable HRQoL-tools for this patient group. The focus group approach elicits patients to explore and to clarify individual and shared perspectives. This resulted in the identification of key issues experienced by DTF patients and ensures the achievement of high content validity.

The results of the systematic literature review and the focus group sessions will be used to create a provisional list of issues which will be ranked by both patients and healthcare professionals for their relevance. Next, an item list will be created which will form the basis of the DTF specific tool. This tool could complement the EORTC QLQ-C30 questionnaire with questions capturing issues raised from the focus groups such as concerns about recurrences and emotional or psychological problems and, site specific issues (i.e., extremity, abdominal wall etc.). This questionnaire is much needed in order to understand effects of DTF and its treatment on patient reported outcomes and provide support for patients who experience problems regarding physical, emotional, social and psychological well-being. Also, knowledge about HRQoL-outcomes can be used for informed decision making during the diagnosis and treatment trajectory of this patient group.

Conclusions

A DTF-specific tool and consensus regarding the preferred measurement tool for measuring HRQoL in DTF patients is lacking in the literature. Used questionnaires either focus on single items, excluding possible items of significance, or are too generic. Existing questionnaires could be complemented with questions regarding key HRQoL-issues, identified during the focus group sessions, which DTF patients experience in various HRQoL-domains. This DTF-specific tool, validated in a large population study, would provide guidance for clinical practice, can compare treatment effects on HRQoL and raise awareness of the impact of DTF on patients' life.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval from the Medical Ethics Committee of Erasmus MC in Rotterdam, the Netherlands was obtained for this study (file number MEC-2017-269).

Informed consent

Informed consent was obtained from all individual participants included in the study.

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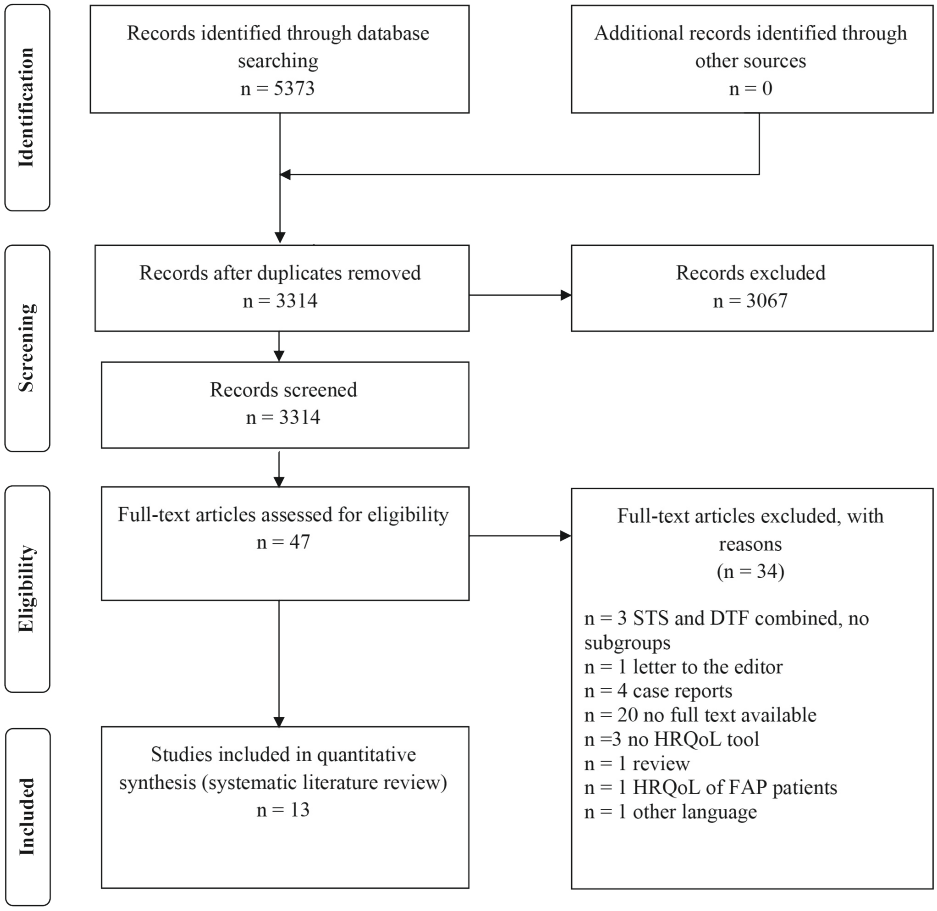
Supplemental Materials 1. Literature search, Embase.com, November 6th, 2017

('desmoid tumor'/exp OR Fibromatosis/exp OR 'familial colon polyposis'/exp OR (desmoid* OR Fibromatos* OR ((familial* OR heredit* OR genetic* OR Adenomatous*) NEAR/6 polypos*)):ab,ti) AND ('quality of life'/exp OR 'quality of life assessment'/exp OR 'functional assessment'/exp OR 'general health status assessment'/exp OR 'health status'/exp OR 'health impact assessment'/de OR 'daily life activity'/exp OR 'ADL disability'/exp OR 'patient satisfaction'/exp OR 'distress syndrome'/exp OR 'stress'/exp OR emotion/exp OR 'sexuality'/exp OR 'self concept'/exp OR 'family relation'/exp OR 'family life'/exp OR 'coping behavior'/exp OR 'disability'/de OR invalidity/de OR 'immobility'/de OR 'esthetics'/de OR 'pain assessment'/exp OR 'pain measurement'/de OR 'social interaction'/exp OR 'social life'/exp OR 'social environment'/de OR 'psychosocial environment'/de OR 'social support'/de OR 'social stress'/de OR 'social rejection'/de OR 'mental health'/exp OR 'wellbeing'/exp OR 'interview'/exp OR 'questionnaire'/exp OR 'assessment of humans'/exp OR 'psychological aspect'/exp OR 'psychology'/exp OR 'marriage'/exp OR ((quality NEAR/3 life) OR hrql OR qol OR (Functional* NEAR/3 (outcome* OR asses*)) OR (daily NEAR/3 (life OR living)) OR ADL OR (patient NEAR/3 satisf*) OR ((health OR function*) NEAR/3 status*) OR eortc OR ((short-form OR sf) NEXT/1 (12 OR 20 OR 36)) OR sf12 OR sf20 OR sf36 OR distress OR (stress NEAR/3 (patient* OR personal* OR psycho* OR mental* OR life)) OR emotion* OR anxi* OR sexual* OR (self NEXT/1 (concept* OR esteem OR satisf* OR percept*)) OR body-image* OR burden* OR ((impact* OR problem* OR issue*) NEAR/6 (function* OR disease* OR personal* OR psycholog* OR body OR clinical* OR health* OR life OR daily OR tumor* OR tumour* OR social*)) OR psychosocial* OR worry* OR worrie* OR ((family OR interpersonal OR partner* OR spous*) NEAR/6 (relation* OR communicat* OR life OR involve*)) OR coping OR ((adaptive* OR adjustment*) NEAR/6 (behav* OR psycho*)) OR impairment* OR disabilit* OR invalidit* OR esthetic* OR aesthetic* OR cosmetic* OR beauty OR fitness OR (physical* NEAR/3 (condition* OR mobility)) OR immobility OR (pain* NEAR/6 (assess* OR inventor* OR measure*)) OR attractiveness* OR (social* NEAR/3 (isolat* OR distan* OR interact* OR life* OR support OR reject* OR participat* OR environment*)) OR feeling* OR (mental NEAR/3 (health OR status OR suffer*)) OR wellbeing OR well-being OR insecur* OR resilien* OR (symptom* NEAR/6 (assess* OR inventor* OR check*)) OR karnofsk* OR (karno* NEXT/3 (score* OR scale* OR perform* OR function* OR stat* OR index* OR rating)) OR (focus NEAR/3 group*) OR interview* OR questionnaire* OR (assessment* NEAR/3 human*) OR hopeless* OR fear OR frustrat* OR hopeless* OR helpless* OR unhapp* OR mood OR uncertaint* OR (lack NEAR/3 informat*) OR disturb* OR concerned OR deficit* OR ((self OR patient*) NEXT/1 report*) OR marriage*):ab,ti) NOT ([animals]/lim NOT [humans]/lim)

Supplemental Material 2. Flow chart showing the selection of studies for the inclusion in the literature review



Inclusion criteria:

- patients with sporadic DTF
- original articles
- availability of full text
- the use of (non)validated tool or measurement to measure HRQoL
- language: Dutch, English, German or French.

Exclusion criteria:

- case reports, reviews
- FAP patients

DTF, desmoid-type fibromatosis; FAP, familial adenomatous polyposis HRQoL: health related quality of life; n, number of studies; STS, soft tissue sarcoma

9

Assessing the desmoid-type fibromatosis patients' voice: Comparison of health-related quality of life experiences from patients of two countries

Milea J.M. Timbergen, Winette T.A. van der Graaf, Dirk J. Grünhagen, Eugenie Younger, Stefan Sleijfer, Alison Dunlop, Lucy Dean, Cornelis Verhoef, Lonneke V. van de Poll-Franse, Olga Husson

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Abstract

Background

Desmoid-type fibromatosis (DTF) is a rare, non-metastasising soft tissue tumour. Symptoms, unpredictable growth, lack of definitive treatments, and the chronic character of the disease can significantly impact health-related quality of life (HRQoL). We aimed to identify the most important HRQoL-issues according to DTF patients in two countries, in order to devise a specific HRQoL-questionnaire for this patient group.

Methods

DTF patients and health care providers (HCPs) from the Netherlands and the United Kingdom individually ranked 124 issues regarding diagnosis, treatment, follow-up, recurrence, living with DTF, healthcare and supportive care experiences, according to their relevance. Descriptive statistics were used to calculate priority scores.

Results

The most highly ranked issues by patients (n = 29) were issues concerning 'tumour growth', 'feeling that there is something in the body that does not belong there', and 'fear of tumour growth into adjacent tissues or organs' with mean (M) scores of 3.0, 2.9 and 2.8 respectively (Likert scale 1-4). British patients scored higher on most issues compared to Dutch patients (M 2.2 vs. M 1.5). HCPs (n = 31) gave higher scores on most issues compared to patients (M 2.3 vs. M 1.8).

Conclusions

This study identified the most relevant issues for DTF patients, which should be included in a DTF-specific HRQoL-questionnaire. Additionally, we identified differences in priority scores between British and Dutch participating patients. Field-testing in a large, international cohort is needed to confirm these findings and to devise a comprehensive and specific HRQoL-questionnaire for DTF patients.

Introduction

Sporadic desmoid-type fibromatosis (DTF) is a rare, borderline tumour of the soft tissues [1-3]. Most patients are females, aged between 20 and 40 years at primary diagnosis [3]. Sporadic DTF arises in musculoaponeurotic structures with the most common sites being the abdominal wall and the extremities [4]. Symptoms vary, depending on tumour site, size and infiltration of adjacent structures, resulting in pain and/or functional impairment. DTF does not metastasize, rarely has fatal outcomes, often displays long periods of spontaneous stabilisation, and can undergo spontaneous regression [5]. Surgical resection, radiotherapy, non-cytotoxic and cytotoxic systemic therapies may be considered in patients with symptomatic disease but unfortunately, these "traditional" treatment options do not guarantee tumour reduction and/or clinical response [6]. Local recurrence after surgery remains high [7, 8], leading to a reduction in surgical treatments for DTF over recent decades [3, 4]. Additionally, "active" forms of treatment can be debilitating, causing greater morbidity than the tumour itself. For these reasons, active surveillance is now recommended as a first line management for most patients with DTF [6, 9]. Therefore, DTF has obtained a "chronic" status and its impact on patients should be evaluated accordingly.

Health-related quality of life (HRQoL) provides information beyond traditional measures of efficacy in oncology such as overall survival, and is increasingly used as an endpoint in clinical trials [10, 11]. We previously performed a systematic literature review to evaluate which HRQoL-measures were used in research to assess HRQoL in DTF [12]. Generic HRQoL-measures (e.g., the cancer specific core questionnaire from European Organisation for Research and Treatment of Cancer; the EORTC quality of life core questionnaire (EORTC QLQ-C30)) may not consider disease-specific issues in DTF patients. Site-specific tools (e.g., Toronto Extremity Salvage Score), may not be relevant to certain groups (e.g., those with an abdominal wall, or head and neck tumours).

At present, there is no validated DTF-specific HRQoL-tool, and this was illustrated by a systematic literature review published by our group [12, 13]. In order to gain greater insight into the issues that patients with DTF experience in their daily lives, and to evaluate their experiences of health care including the supportive care system, we previously organised focus groups and semi-structured interviews, in the United Kingdom (UK) and in the Netherlands (NL) [12, 13]. These studies identified issues covering various domains including the diagnostic pathway, the treatment pathway, daily limitations (e.g., physical and psychological symptoms), and experiences with the current health care system.

The main goal of this study was to determine the relative importance of each issue and receiving feedback on appropriateness of content and breadth of coverage. In the present study, we used the previously identified issues to 1) identify the most relevant issues to patients with DTF in two health care settings (UK and NL) and to 2) identify differences in scores between both countries.

Materials and Methods

Identification of issues

The EORTC Quality of Life group methodology for developing a questionnaire was used for the selection of relevant issues based on previous focus groups and patients interviews [14]. Issues, that had previously been identified to be of concern to DTF patients, were listed per country (UK and NL). A total of 188 issues were identified in the UK group and 110 issues were identified in the Dutch group. Next, issues were grouped into categories and duplicate issues, covering the same topics were removed. A total of 124 issues were converted into a provisional list of issues. All issues were reviewed by two authors (M.J.M.T. and O.H.). All issues were translated by native English and Dutch speakers.

Patient selection

Patients with DTF were approached for participation by their treating physician. Inclusion criteria were; histologically proven DTF, age ≥18 years, Dutch or English language skills and a "recent" visit (<2 years) to the hospital. Exclusion criteria were; participation in one of the previous focus groups or patient interviews, and patients with a diagnosis of cancer or familial adenomatous polyposis (FAP). Patients received an information letter which explained study objectives. Baseline characteristics and details about the individual disease trajectory of participants were obtained. Patients were only invited to participate once and did not have to provide a reason if they declined. No reminders were send. All data from patients were collected and processed anonymously.

Selection of health care providers

To examine whether HCPs, with expertise and experience in sarcomas and DTF have the same perspectives as patients with DTF about key HRQoL-issues, an e-survey of the same 124 issues was created using Lime Survey Servicebedrijf© software. The issue list was available in two languages (Dutch and English) and issues were presented in a random order. In the Netherlands, HCPs from the multidisciplinary team (e.g., surgeons, oncologists, radiologists, radiotherapists, sarcoma clinical nurse specialists, and physiotherapists) were

identified using the website Orphanet, which provides information on centres of expertise dedicated to the medical management for rare diseases (https://www.orpha.net/consor/cgi-bin/Clinics_Search.php?lng=EN). In the UK, HCPs of the aforementioned disciplines were identified using the sarcoma network group of the Royal Marsden Hospital, London, UK. Every HCP received an invitation email with a token and link to the e-survey. A reminder was sent after one week if the HCP had not responded.

Sociodemographic and clinical characteristics

Age at time of diagnosis was either stated by the patient or calculated using the date of birth and date of the first pathology report. Age at the time of questionnaire completion was either stated by the patient or calculated using the date of informed consent and the date of birth. Education levels were categorized into "high" (PhD, university, higher education postgraduate/undergraduate degree), "intermediate" (professional qualification, vocational work, work related qualification, general secondary education, further/intermediate education), and "low" (primary education [with a higher, but not completed education] and secondary education). Continuous variables were presented as a mean with a standard deviation (SD) or as a median with an interquartile range (IQR). Categorical variables were presented as numbers (*n*) using frequencies and percentages.

Presentation of issues to patients and health care providers

A total of 124 issues were presented to patients and health care providers (HCPs) in a random order (Supplemental Table 1). Patients and HCPs scored 124 issues by relevance on a Likert scale from 1 to 4 ((1) not at all, (2) a little, (3) quite a bit, and (4) very much) and ranked the top ten most important issues. The frequency that each issue appeared in the top ten most important issues was converted into the mean priority score (M-score) per issue. The frequency of top ten priority score of each issue was calculated and ranked in overall priority score. Where questions were left blank by the participant, they were coded as a "missing value" and not incorporated in the total score. Space for general remarks was available at the end of the questionnaire.

EORTC QLQ-C30 questionnaire

In addition to the issue list, patients were asked to fill out the 30-item EORTC QLQ-C30 questionnaire (version 3) to assess HRQoL [15]. Norm data was obtained from the EORTC, which recently collected data from the general population in Europe and North America [16]. Only data from the general population in the Netherlands and the UK were used for the current study. The EORTC QLQ-C30 questionnaire contains five functional

scales (physical, role, cognitive, emotional and social functioning), a global health status scale, three symptom scales (fatigue, nausea and vomiting, and pain), and six single items (appetite loss, diarrhoea, dyspnea, constipation, insomnia, and financial difficulties). The questionnaire has a 1-week time frame and uses a four-point response format ("not at all", "a little", "quite a bit", and "very much"), with the exception of the global health status scale, which has a seven-point response format. The scores were calculated using linear transformation to a score between 0 and 100. For the functional scales and the global health status, a high score represents a high (healthy) level of functioning. A high score for the symptom scales represents a high level of symptoms (greater symptom burden) [17]. The EORTC QLQ-C30 summary score was calculated using the mean scores of the function scales and the reversed mean scores of the symptom scales and single items (financial impact and global health status excluded), and are summarized as the mean of the combined 13 QLQ-C30 scale scores A higher summary score represented a better outcome [18, 19]. The summary score was only calculated when all of the required 13 scale and item scores were available. Data analysis and handling of missing items were done according to the scoring manual of the EORTC [17].

Statistical analysis

Patients were matched, using a 1:10 nearest-neighbour match method, with the general population based on nationality, age, and sex using Rstudio (RStudio, version 1.0.153, Boston, MA, package MatchIt). Patients with missing values (lacking information regarding age or sex) were excluded from the analysis. Differences in priority scores (Dutch versus British participating patients, and HCPs versus participating patients), and differences in scores of the EORTC QLQ-C30 scales between groups (Dutch versus British participating patients, and Dutch and British participating patients versus the Dutch and British general population) were tested for their significance using the Mann-Whitney *U* test. SPSS Statistics (version 24) was used for the Mann-Whitney *U* tests (IBM, Armonk, New York, USA). Two-sided $p < 0.05$ was considered statistically significant.

Results

Patient cohort

Forty-one patients from the Erasmus MC, Rotterdam, the Netherlands, and 32 patients from the Royal Marsden Hospital, London, UK, were approached during July and August 2018. Out of 73 patients, 29 patients (total response rate of 39.7%) gave written informed consent (Figure 1). The cohort comprised of 10 males and 19 females with DTF most

commonly localized in the extremities, flank and chest wall (n = 15, 52%). Nine participants had received active treatment at the time of the questionnaire. The median, self-reported age at diagnosis was 38 years (IQR: 30-48) (Table 1). Sociodemographic characteristics are summarized in Supplemental Table 1. All participants completed the issue list, and sixteen participants ranking their top 10 most relevant issues.

Figure 1. Flow diagram of participating patients and health care providers' responses to this survey

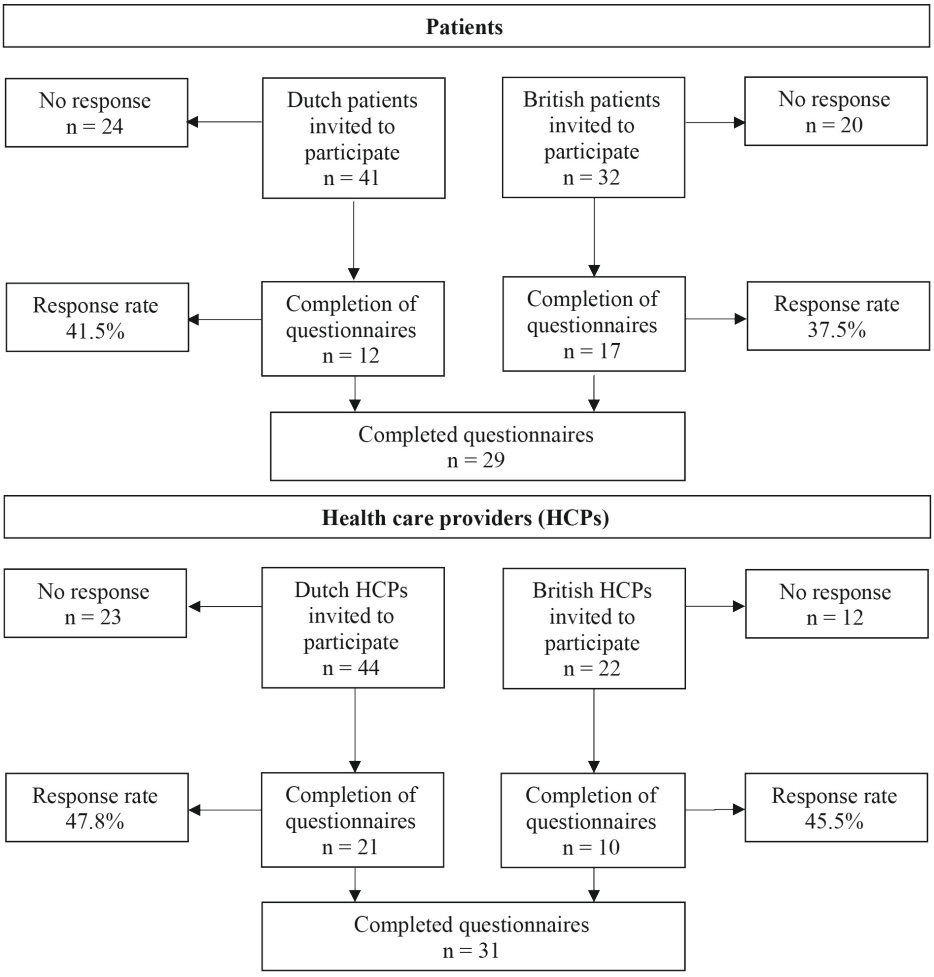

Table 1. Clinical characteristics of 29 participating patients

		Total group (%) [a]		Dutch patients (n = 17)		British patients (n = 12)	
Sex	Male	10	(35%)	5	(29%)	5	(42%)
	Female	19	(65%)	12	(71%)	7	(59%)
Median age in years at time of questionnaires (IQR) [b]		43	(36-55)	44 (36-55)		41 (32-56)	
Median age in years at time of diagnosis (IQR) [c]		38	(30-48)	38 (30-48)		37 (28-50)	
Tumour localisation	Abdominal wall	2	(7%)	2	(12%)	0	(0%)
	Intra-abdominal	10	(35%)	8	(47%)	2	(17%)
	Extremity/girdles/chest wall	15	(52%)	6	(35%)	9	(75%)
	Head/neck/intrathoracic	1	(3%)	1	(6%)	0	(0%)
	Missing value	1	(3%)	0	(0%)	1	(6%)
Recurrent disease	Yes	6	(21%)	2	(12%)	4	(33%)
	No	21	(72%)	15	(88%)	6	(50%)
	Missing value	2	(7%)	0	(0%)	2	(17%)
Received treatment(s) (some patients gave multiple answers)	Wait and see	21		12		9	
	Surgery	14		8		6	
	Radiotherapy	4		1		3	
	Chemotherapy	5		1		4	
	Non-steroidal anti-inflammatory drugs	8		1		7	
	Hormonal treatment	7		2		5	
	Pain management	9		0		9	
	Physiotherapy	7		3		4	
	Occupational therapy	2		1		1	
Currently receiving any active form of treatment	Yes	9	(31%)	0	(0%)	9	(75%)
	No	19	(66%)	17	(100%)	2	(17%)
	Missing value	1	(3%)	0	(0%)	1	(8%)
Comorbidity (some patients gave multiple answers)	No	11		6		5	
	Arthritis or long-term joint problem	3		2		1	
	Asthma or long-term chest problem	4		2		2	
	Diabetes	1		1		0	
	High blood pressure	1		0		1	
	Kidney or liver disease	1		1		0	
	Long-term back problem	6		3		3	
	Long-term mental health problem	2		2		0	
	Long-term neurological problem	1		1		0	
	Physical disability	2		1		1	
	Other d	3		2		1	
	Missing value	2		2		0	

[a] Percentages may not add up to 100% due to rounding up of ; [b] answered by n = 21 participating patients; [c] answered by n = 29 participating patients; [d] Including, digestive problems, coeliac disease lactose intolerance, and iron deficiency

Ranking of priority of the issues

Ranking of HRQoL-issues revealed that 13 out of 124 issues (10.5%) were chosen to be the most relevant (prevalence ratio of >30%) (Table 2). Patients considered the following issues as relevant and missing on the current issue list; "problems with health care insurances", "coverage of costs related to the disease such as traveling costs", "lack of adequate online information", "lack of knowledge about treatment options outside the region or country", "lack of information about pain management and referral to pain professionals" and "lack of advice regarding dietary restrictions or playing sports". A list of the missing items, general remarks and quotes is provided in Supplemental Table 2 and Supplemental Table 3.

British versus Dutch patients

Table 2. Top 10 most important issues according to number of participating patients (n)

Participating patients (total n = 16)[a]			**Health care providers (total n = 31)**		
	n	**Prevalence ratio**		**n**	**Prevalence ratio**
Worries about tumour growth	10	62.5%	Worries about tumour growth	17	54.9%
Fear of the tumour growth and/or tumour growing into adjacent tissues or organs	9	56.3%	Experience of uncertainty during the course of disease	12	38.7%
Feeling that there is something in your body that does not belong there	7	43.8%	Pain	11	35.5%
Stress around check-ups during the follow-up	6	37.5%	Lack of optimal treatment options and /or uncertainty about preferred treatment	10	32.3%
Pain	6	37.5%	Concerns about the future	10	32.3%
Reaching a definite diagnosis is time consuming	5	31.3%			
Not being able to sleep because of pain	5	31.3%			
Feeling frustrated about the "benign" diagnosis with malignant features	5	31.3%			
Desmoid-type fibromatosis is unknown among most doctors	5	31.3%			

[a] n = 13 participating patients failed to provide a top 10
The cut-off value for inclusion in the DTF-specific HRQoL-questionnaire is a prevalence ratio of > 30%

Overall, British patients gave higher scores for each issue compared to Dutch patients (M-score 2.2 (UK) vs M-score 1.5 (NL)) (Supplemental Table 4). Differences in score of more than 1 point between Dutch and British patients are displayed in Supplemental Figure 1. Additionally, priority scores of Dutch and British HCPs, and scores of participating patients and HCPs from the Netherlands and the UK were compared (Supplemental Table 4). The total cohort of patients was too small to identify any differences between subgroups (e.g., initial treatment type, tumour location, and age at diagnosis).

Health care providers

In the Netherlands, HCPs were invited at six sarcoma centres. All HCPs from the UK were employees at the Royal Marsden Hospital, London. Twenty-one Dutch and ten British HCPs responded. Professional backgrounds included; surgical oncologist (n = 12), medical oncologist (n = 6), radiation oncologist (n = 5), specialized sarcoma nurse (n = 5), and other professions including a radiologist, physiotherapist and pain specialist (all n = 1). Seventeen professionals had more than 10 years of experience, three had 6-10 years of experience, and eleven had 5 or less years of experience working with desmoid patients. Frequency of contact with DTF patients varied between once a week (n = 9, 29%) to rarely (less than once every 3 months) (n = 1, 3%).

Issues with the highest scores according to HCPs included; "worries about tumour growth" (M-score 3.4), "stress about the diagnosis" (M-score 3.2), "the experience of uncertainty during the course of the disease" (M-score 3.2), "pain" (M-score 3.2), "concerns about the future" (M-score 3.0), "stress around check-ups during the follow-up" (M-score 3.0), "fear of recurrence after treatment" (M-score 3.0), "fear of tumour growth/tumour growth into adjacent tissues or organs" (M-score 2.9) and "the feeling that patients do not have a clear prognosis" (M-score 2.9). Overall, HCPs from the UK gave higher scores, compared to Dutch HCPs with M-scores of 2.8 and 2.0 respectively (Supplemental Table 4).

Participating patients versus health care providers

There was considerable overlap between the highest ranked issues according to patients and HCPs, particularly regarding the unpredictable growth pattern of DTF tumours (Supplemental Table 4). HCPs scored significantly higher ($p < 0.05$) on 77 out of a total of 77 of 124 issues. HCPs also gave a higher mean overall score on the issues list (total M-score 2.3) compared to patients (total M-score 1.8) (Supplemental Table 4).

Table 3 Results of the EORTC QLQ-C30 questionnaire (version 3.0) of patients and the general population

	Total mean (SD) patients		**British participating patients, n = 12 mean (SD)**		**Dutch participating patients, n = 17 mean (SD)**		**p-value**	**Dutch general population, n = 170 mean (SD)**		**British general population, n = 80 [a] mean (SD)**	
Dyspnea	10.3	(23.7)	8.0	(15.1)	11.8	(28.7)	0.845	8.2	(19.1)	18.7	(11.8)
Insomnia	31	(38.8)	55.6	(38.4)	13.7	(29.0)	**0.004***	20.8	(25.1)	37.9	(39.6)
Appetite loss	14.9	(26.1)	22.2	(21.7)	9.8	(28.3)	0.073	2.9	(11.9)	16.2	(24.8)
Constipation [a]	16.7	(32.1)	21.2	(37.3)	13.7	(29.0)	0.781	4.7	(13.7)	14.6	(30.9)
Diarrhoea	17.2	(30.4)	27.8	(39.8)	9.8	(19.6)	0.325	7.3	(17.9)	14.2	(45.2)
Financial difficulties	11.5	(24.0)	22.2	(29.6)	3.9	(16.2)	0.059	5.7	(18.9)	23.3	(34.5)
Nausea/vomiting	7.5	(17.6)	13.9	(24.4)	2.9	(8.8)	0.180	4.7	(13.7)	14.6	(28.8)
Pain	33.9	(36.0)	58.3	(37.3)	16.7	(23.6)	**0.004***	16.2	(21.9)	29.4	(40.3)
Fatigue	31	(32.9)	49.1	(29.0)	18.3	(29.9)	**0.004***	22.5	(22.4)	33.9	(32.2)
Cognitive functioning	79.9	(30.3)	65.3	(32.1)	90.2	(25.0)	**0.030***	91.7	(15.7)	76.7	(29.5)
Emotional functioning	73.6	(32.4)	59.0	(33.6)	83.8	(28.2)	**0.021***	84.3	(18.9)	67.3	(36.7)
Social functioning	77.6	(29.6)	58.3	(33)	91.2	(17.8)	**0.001***	94.2	(14.9)	75.4	(38.8)
Physical functioning	75.2	(27.9)	67.8	(32.5)	80.3	(23.9)	0.394	92.5	(13.1)	80.2	(33.3)
Role functioning	71.8	(32.8)	52.8	(40.1)	85.3	(17.6)	**0.027***	91.8	(19.5)	76.5	(38.8)
Global health status	68.7	(27.7)	56.9	(29.5)	77.0	(23.9)	**0.043***	78.7	(18.2)	60.2	(34.6)
Summary score	**78.1**		**63.5**		**87.2**			**89.8**		**76.7**	

[a] *data missing from 1 British patient; * statistically significant difference*

Mean scores with standard deviation (SD) are displayed for all scales of the EORTC QLQ-C30. The p-value represents the comparison of the scores of the British participating patients versus the scores of the Dutch participating patients. Two-sided p<0.05 was considered statistically significant.

EORTC QLQ-C30 - Dutch versus British participating patients

Overall, the mean summary score for the EORTC QLQ-C30 for all DTF patients together was 78.1, with a mean global health score of 68.7 (Table 3). Statistically significant differences between scores of British and Dutch patients were found for "global health", "insomnia", for the symptom scales "pain" and "fatigue", and for the following functioning scales "cognitive functioning", "emotional functioning", "social functioning" and "role functioning" (Table 3).

EORTC QLQ-C30 - Participating patients versus the matched general population

After 1:10 nearest-neighbour matching based on nationality, sex and age, data of 170 people from the Dutch general population and data of 80 people from the British general population were selected to compare scores between DTF patients and the general population. Four British patients were excluded from this analysis due to missing data regarding their age at the time of questionnaire completion. Dutch patients had a score of 77 for global health and a summary score of 87.2, whereas scores for the matched Dutch population were 78.7 and 89.8 for global health and the summary score respectively. British patients (n = 8) had a score of 59.4 for global health and a summary score of 68.2, whereas scores for the matched British population were 60.2 and 76.7 for global health and the summary score respectively (Table 3) [16]. Dutch participating patients scored lower on all functioning scales compared to the general Dutch population, although only the physical functioning score ($p = 0.019$) and the role functioning score ($p = 0.021$) showed a statistically significant difference (Table 3). No statistically significant differences were found comparing EORTC QLQ-C30 scores between the British patients and British general population.

Discussion

The purpose of this study was to identify the most important HRQoL-issues for patients with sporadic DTF and rank them according to relevance. The most highly ranked HRQoL-issues by patients with DTF were related to the unpredictable disease trajectory of DTF. Additionally, issues regarding the rarity, aggressiveness and the benign classification of DTF received high scores. From the patient perspective, this benign classification was seen as misleading, as DTF can display aggressive growth. In terms of the health care system, the benign disease classification, not being cancer, can have both pros and cons as it can have consequences for insurances and covering of expenses, depending on the country of residence. As the aforementioned items are not included in the EORTC QLQ-C30 questionnaire, a tailored DTF HRQoL-tool could capture these issues. Physical symptoms

such as pain, fatigue and loss of muscle strength also received high priority scores of 2.4, 2.3 and 2.3 respectively. Although these items are covered by the EORTC QLQ-C30 questionnaire, the results of this study highlight the importance of physical symptoms, caused by the tumour or as a side effect of treatment, and their impact on HRQoL. Patients identified several important issues that were not covered by other questionnaires. These could be considered in the development of a future DTF-specific HRQoL-tool.

In a rare and heterogeneous disease, such as DTF, measuring the impact of the disease for patients can be challenging. This can be due to the variable disease presentation, course and response to treatment, and due to the knowledge gap of the natural history of the disease [20]. Moreover, the limited number of responses challenges research in this field. Our cohort may not be representative of the entire DTF population as the majority of patients in this cohort had an intra-abdominal tumour and many patients received one or multiple active forms of treatment.

In addition to physical, emotional and psychological problems, patients with DTF might also experience social isolation due to lack of peers with the same condition [20]. This was reflected in the current study by a relatively high score for the issue "not knowing peers with the same disease". Furthermore, lack of information was identified as a relevant topic as the following issues "DTF is unknown among most doctors", and "lack of information received about DTF", received M-scores of 2.6, and 1.8 respectively.

HCPs may treat a limited number of patients with this rare disease, therefore patients may receive an incorrect diagnosis or delay in diagnosis due to lack of experience in recognizing and treating the disease [20]. The comparison in relevance scores between patients and HCPs show that HCPs scored significantly higher on a large number of issues, suggesting that they recognize and acknowledge problems that faced by this patients group. The issue "reaching a definite diagnosis is time consuming" received an M-score of 2.3, showing that this is a relevant problem for this patient group. Whilst the future DTF-specific HRQoL tool will be available upon diagnosis, it is important for HCPs to consider that patients may have encountered difficulties reaching the correct diagnosis and so provision of clear information and support at this time is essential. Accessing specialists with knowledge of DTF can be challenging, as they may be located in regional specialist centres. This can result in patients receiving multiple treatment recommendations before seeing a specialist.

Financial consequences, due to insurance problems, the need to take time off work or increasing traveling costs can also affect HRQoL, although the issues regarding these subjects all received relatively low scores in the current study [21]. Social problems, such as the burden of having a rare disease on family and carers, as well as having this diagnosis at a young age, can also have a negative impact on HRQoL [20, 21].

This unique study identified important issues for DTF patients and compared the views of British and Dutch patients. Most issues were scored higher by British patients compared to Dutch patients (indicating a higher relevance for the specific issue). This phenomenon was also seen comparing EORTC QLQ-C30 scores, as British patients scored statistically significantly lower (indicating worse functioning) on four out of five function scales, and for the symptom scales insomnia, pain and fatigue. Although both participating centres are tertiary centres visited by patients with more complex or advanced disease, the catchment area of the Royal Marsden Hospital (London, UK) is larger than of the Erasmus MC (Rotterdam, NL) possibly creating selection bias during this study. Norm data obtained by the EORTC of the general Dutch and British population showed a comparable trend with higher scores on symptom scales and single items scales (indicating greater symptom burden) and lower scores on functioning scales (indicating worse functioning) comparing the data from the general Dutch and British population. Data from 2017 of The Organisation for Economic Co-operation and Development show similar results with lower scores (indicating a lower well-being) of British participants compared to Dutch participants on several measures of well-being (e.g., housing, income, education and health and life satisfaction) [22]. This suggest that, although our data might show differences between both countries of "impact of disease" on HRQoL, baseline scores in the normal population differ and that the experience of HRQoL-issues depends on where you live [16, 22].

Comparisons between patients and a matched cohort of the general population based on nationality, sex and age, did not yield significant results, except for "physical functioning" and "role functioning" comparing the Dutch patients with the Dutch general population. Additionally, we compared the scores of HCPs and participating patients. An important finding of this study was the clear overlap of issues that were important to patients and HCPs. The HCPs rated various issues higher than patients particularly with regard to pain, stress about the diagnosis, and concerns about the future.

We acknowledge that this study has several limitations. The small sample size is explained by the rarity of DTF. A larger cohort is needed to test the psychometric aspects of a DTF-

specific HRQoL-tool in future studies. The response rate was lower than we had hoped for, but similar response rates have been published in studies describing more common diseases such as cancer [23]. In the current study the relatively low response rate may have been due to the length of the questionnaire, the single centre setup (one centre in each country), the timing of sending out the questionnaire (mid-summer) and/or the overall reluctance to participate in a survey study. Furthermore, many patients also need to complete questionnaires as part of their regular health care; therefor patients might be less willing to complete questionnaires for research purposes. Sending out a reminder to patients would have been a valid option to increase the response rate. Selection bias may have led to an overestimation of HRQoL-problems in our cohort. As the primary aim was to identify the most relevant issues in this patient group, the effect of this overestimation is less relevant. A population-based cohort is required to determine true prevalence of issues and perhaps a more representative result. Lastly, interpretation of the questions is influenced by the current health situation of each patient. We tried to eliminate such influencing factors by excluding patients with a diagnosis of cancer and FAP-associated DTF. However, patients HRQoL might also be influenced by disease stage, tumour location and treatments, and by other comorbidities and personal circumstances. This impact on HRQoL-issues could be evaluated in a future population-based cohort study and stresses the need for validation of our findings in a large, international DTF cohort to evaluate the prevalence of HRQoL-issues.

Today, solely one DTF-specific questionnaire, the Gounder/DTRF Desmoid Symptom / Impact Scale, is available, and currently mainly used in the setting of clinical trials [24-26]. The findings of our study will be used for the development of a DTF-specific tool, according to the EORTC guidelines, which can be used accompanied by the EORTC QLQ-C30 HRQoL-instrument and will be useful for observational studies, clinical trials and clinical care. Implementation of this tool and action on abnormal findings, concerns or poor experiences of patients might improve satisfaction with health care, symptom management and HRQoL [27]. Health care providers may benefit from being able to anticipate and identify problems earlier, thereby improving work efficiency and promoting patient-centred care through shared decision-making [28-30]. In order for a tailored HRQoL-tool to work in clinical practice, this tool should add value to the clinical workflow without disrupting it [31]. Our results will be used in the development of an international, multicentre, population-based study in line with the EORTC guidelines for developing a questionnaire [14]. This study includes pre-testing and content validation of a DTF-specific questionnaire. This questionnaire will assess the prevalence of HRQoL-issues and will identify risk factors for the development of HRQoL-issues patients experience. Patients will receive an invitation to participate in an online survey and one reminder for completing

the questionnaire. When this tool has been developed and validated, it will reflect overall patient experience and its multidimensional contributing factors by including important non-symptom, disease specific areas regarding the unpredictable course of this rare disease. The tool could be used alongside the EORTC QLQ-C30, to gain more insight into HRQoL-issues of the patient at diagnosis. Additionally, longitudinal studies could evaluate HRQoL-issues of DTF patients during their disease trajectory and the questionnaire can potentially be used in both clinical and research setting.

Conclusions

This study identified relevant issues for DTF patients to be considered in the future development of a DTF-specific HRQoL-questionnaire. Issues regarding the unpredictable growth behaviour and rarity of DTF were the most highly ranked by patients and HCPs. Additionally, this study identified differences in priority scores between British and Dutch patients. Although this could be due to selection-bias, field-testing in a large, international cohort is needed to confirm any potential cultural findings.

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Supplemental Table 1. Sociodemographic characteristics of 29 participating patients

		Total group (%)[a]		Dutch patients (n = 17)		British patients (n = 12)	
Marital status	Single	5	(17%)	1	(6%)	4	(33%)
	Dating /in a relationship	7	(24%)	5	(29%)	2	(17%)
	Married	10	(35%)	5	(29%)	5	(42%)
	Living common	4	(14%)	3	(18%)	1	(8%)
	Widowed	1	(3%)	1	(6%)	0	(0%)
	Separated	1	(3%)	1	(6%)	0	(0%)
	Divorced	1	(3%)	1	(6%)	0	(0%)
Current living situation	Living with child(ren) and spouse /partner	14	(48%)	8	(47%)	6	(50%)
	Living with child(ren)	2	(7%)	2	(12%)	0	(0%)
	Living with spouse /partner	6	(21%)	4	(24%)	2	(17%)
	Living alone	5	(17%)	2	(12%)	3	(25%)
	Living with parent(s)	1	(3%)	0	(0%)	1	(8%)
	Missing value	1	(3%)	1	(6%)	0	(0%)
Caring responsibilities for children (<18 years)	Yes	14	(48%)	9	(53%)	5	(42%)
	No	15	(52%)	8	(47%)	7	(58%)
Highest completed education	High education	16	(55%)	10	(59%)	6	(50%)
	Intermediate education	11	(38%)	5	(29%)	6	(50%)
	Low education	2	(7%)	2	(12%)	0	(0%)
Current employment status	Employed full-time (including self-employed)	14	(48%)	7	(41%)	7	(58%)
	Employed part-time (including self-employed)	6	(21%)	4	(24%)	2	(17%)
	Unemployed	2	(7%)	2	(12%)	0	(0%)
	Permanently unable to work	6	(21%)	3	(18%)	3	(25%)
	Retired	1	(3%)	1	(6%)	0	(0%)
Physical demanding job	Not applicable	12	(41%)	8	(47%)	4	(33%)
	No physical demanding job	5	(17%)	2	(12%)	3	(25%)
	Physically light	7	(24%)	4	(24%)	3	(25%)
	Physically medium	5	(17%)	3	(18%)	2	(17%)
Mentally challenging job	Not applicable	9	(31%)	6	(35%)	3	(25%)
	Not at all challenging	1	(3%)	1	(6%)	0	(0%)
	Rarely challenging	1	(3%)	0	(0%)	1	(8%)
	Occasionally challenging	8	(28%)	4	(24%)	4	(33%)
	Frequently challenging	7	(24%)	4	(24%)	3	(25%)
	Constantly challenging	3	(10%)	2	(12%)	1	(8%)

[a] *Percentages may not add up to 100% due to rounding up of decimals*

Supplemental Table 2. Missing issues and quotes from patients

	Missing issues	Related quotes
Financial Issues	Questions about health care insurance and coverage for medical aids	• "It is not a 'cancer' and although benign, insurance companies won't cover as it falls under sarcoma which they assume is cancer. Yet, we want to try new medicines and treatments but it is not cancer. I feel we don't get the same medical urgency. Sometimes you feel you wish it was cancer to get the help we need. We should be entitled to be discriminated against, either way, is how many of us are left feeling." • "Feeling that you have to fight this disease, and the injustice surrounding it: e.g. life insurance" • "My health care insurance caused problems when I wanted a bigger breast prosthetic in my unaffected breast to make my breasts symmetrical" • "Restricted access to the National Health Service when professionals did not know whether to treat as a cancer or not. For example; I was entitled to free patient transport which is available to those with cancer (to my particular hospital) when I was trying to sort out transport for 25 straight days."
Information	Adequate online information	• "Lack of trusted information about desmoid-type fibromatosis online, or any research" • "I'm not a Facebook fan and I don't use it for anything else, but I can't tell you how much I have learned from reading about the experiences of others. And it is truly heart breaking when you see a newcomer to the site having plucked up the courage to post and say 'help' because they have a diagnosis and literally no information, support or route to other knowledge"
	Treatment options outside the region or country	• "The only additional worry I have is certain treatment options are only available depending on where in the country you live. For example, one patient was offered cryotherapy in Manchester, UK, but that is not offered in London, UK."
	Interaction with other pre-existing conditions. For example: fatigue from pain in one condition seemed to set off the pain in the other	

Supplemental Table 2. *(continued)*

	Missing issues	Related quotes
Hobbies/leisure	Problems with cycling	
	Lack of advice regarding eating or playing sports	
Health care facilities	Pain management and referral to a pain specialist	• "Certainly my greatest issues are with pain management and effect this has on sleep and concerns for the future and what changes could happen at any time, positive and negative"
Symptoms	Intestinal problems e.g. diarrhoea or constipation, needing a stoma	
	Specific pain during certain movements (e.g. turning)	
	Trouble tying shoelaces	
	Extra tumour pain during menstruation	
	Constant worries	"Constant trepidation about what it is going to do next, whether further growth" "Feeling like a hypochondriac" "The constant worry about 'what is it doing now' is always to the forefront, to the point that I started to keep a daily note during certain periods of what felt like the same feelings when it was very actively growing"

Supplemental Table 3. General remarks and quotes from patients

Remarks	Quotes
Difficult to interpret some of the questions since many issues change over time	
Due to multiple recurrences; 'fear for a new tumour rather than recurrent tumours'	
Psychological support at the beginning of the disease	• "I would have loved at the beginning is psychological support. Being a very active and confident person, adjusting to initial limitations was very hard and I really struggled both physically and mentally"
Some issues are caused by the operation rather than the tumour itself which is difficult to distinguish from each other	
Having a benign tumour which is aggressive, but no cancer	• "The biggest problem is having an aggressive recurrent tumour treated by oncologist with cancer treatment being less than cancer" • "It's benign so it's not going to kill you; the only thing it could do to kill you would be to suffocate you if it overwhelms your organs"
A good band with your treating physician	• "I learned thinking positive from my treating physician"
Fear of tumour growth during and after pregnancy	• "Especially post pregnancy with fear of further desmoid developing from scar from the caesarean section"

Supplemental Table 4. Mean (M) scores per issue ranked according to their relevance

Issues		Total Patients (n = 29)	Total HCPs (n = 31)	p-value	Dutch patients (n = 17)	British patients (n = 12)	p-value	Dutch HCPs (n = 21)	British HCPs (n = 10)	p-value
1	Worries about tumour growth	3.0	3.4	0.186	2.6	3.5	0.020*	3.2	3.8	0.172
2	Feeling that there is something in your body that does not belong there	2.9	2.8	0.889	2.5	3.3	0.066	2.7	3.1	0.287
3	Fear of tumour growth/fear of the tumour growing into adjacent tissues or organs	2.8	2.9	0.572	2.4	3.3	0.066	2.7	3.4	0.039*
4	Desmoid-type fibromatosis is unknown among most doctors	2.6	2.8	0.453	2.1	3.5	0.001*	2.7	3.2	0.209
5	Stress around check-ups during the follow-up	2.6	3.0	0.071	2.6	2.5	0.926	3.1	2.8	0.237
6	Contemplation about the disease	2.6	2.8	0.404	2.4	2.8	0.227	2.8	2.8	0.846
7	Experience of uncertainty during the course of disease	2.6	3.2	0.039*	2.1	3.3	0.011*	3.1	3.3	0.533
8	Feeling that the disease is aggressive	2.5	2.8	0.237	2.0	3.2	0.008*	2.6	3.4	0.005*
9	Change in life perspective (e.g. feeling thankful not having cancer)	2.5	2.2	0.314	2.4	2.6	0.711	2.1	2.4	0.287
10	Fear of recurrence after treatment	2.5	3.0	0.063	2.1	3.1	0.014*	3.0	3.0	1.000
11	Family members and/or friends worrying about your disease	2.5	2.4	0.776	1.8	3.3	0.000*	2.0	3.0	0.010*
12	Feeling frustrated about the “benign” diagnosis with malignant features	2.4	2.9	0.203	2.0	3.1	0.018*	2.7	3.2	0.205
13	Changed sensitivity of the of the tumour area	2.4	2.7	0.375	1.9	3.3	0.011*	2.4	3.3	0.011*
14	Pain	2.4	3.2	0.016*	1.8	3.3	0.002*	2.9	3.9	0.007*
15	Stress about the diagnosis	2.4	3.2	0.007*	2.5	2.3	0.677	3.2	3.3	0.530
16	Fear of second tumours	2.4	2.3	0.948	1.9	3.1	0.003*	2.2	2.6	0.266
17	Concerns about the future	2.4	3.0	0.012*	2.1	2.8	0.053	2.8	3.4	0.142
18	Not being able to lean on the tumour site	2.4	2.3	0.994	1.8	3.1	0.013*	2.0	3.0	0.011*

Supplemental Table 4. *(continued)*

19	Fatigue	2.3	2.4	0.687	2.0	2.8	0.122	2.1	3.0	0.022*
20	Downplaying symptoms in order not to upset family/ friends	2.3	2.1	0.721	1.7	3.1	0.002*	2.0	2.3	0.287
21	Reaching a definite diagnosis is time consuming	2.3	2.7	0.115	2.0	2.7	0.152	2.6	2.9	0.448
22	Not having cancer but having the same treatments and side effects	2.3	2.8	0.083	1.5	3.4	0.000*	2.6	3.1	0.183
23	Loss of muscle strength	2.3	2.3	0.718	1.8	3.0	0.009*	1.9	3.3	0.000*
24	Reassurance because of the benign tumour	2.2	2.5	0.214	2.4	2.0	0.318	2.5	2.6	0.929
25	Feeling exhausted	2.2	2.2	0.923	1.8	2.8	0.042*	2.0	2.8	0.050
26	Problems with playing sports	2.2	2.9	0.019*	2.0	2.5	0.264	2.7	3.3	0.055
27	Feeling like you don't have a clear prognosis	2.2	2.9	0.015*	1.7	2.9	0.003*	2.7	3.2	0.266
28	Fear of treatment	2.2	2.5	0.235	2.1	2.4	0.444	2.2	3.2	0.013*
29	Lack of optimal treatment options and/or uncertainty about preferred treatment	2.2	2.8	0.039*	1.8	2.7	0.026*	2.5	3.3	0.055
30	Life changing disease in a negative manner	2.2	2.7	0.021*	1.8	2.7	0.059	2.5	3.2	0.059
31	Problems with sleeping	2.2	2.3	0.528	1.6	3.0	0.003*	2.0	2.9	0.006*
32	Worries about decreased muscle strength after treatment	2.2	2.3	0.502	1.6	3.0	0.004*	2.0	2.8	0.019*
33	Not knowing peers with the same disease	2.1	2.6	0.068	1.9	2.5	0.329	2.3	3.3	0.013*
34	Deterioration in physical fitness	2.1	2.2	0.812	1.7	2.7	0.030*	1.7	3.3	0.000*
35	Feeling asymmetrical/misshaped	2.1	2.3	0.284	1.8	2.5	0.059	2.0	3.1	0.001*
36	Limited movement of a limb	2.1	2.9	0.004*	1.6	2.8	0.007*	2.6	3.5	0.013*
37	Continuous worries about the disease	2.1	2.7	0.005*	1.8	2.5	0.053	2.5	3.1	0.031*
38	Trying to relieve the burden on family members	2.1	2.2	0.423	1.8	2.4	0.227	2.1	2.6	0.169
39	Feeling disappointed about the course of disease	2.1	2.8	0.012*	1.6	2.8	0.018*	2.5	3.6	0.003*
40	Doubting the efficacy of treatments	2.0	2.8	0.011*	1.5	2.8	0.008*	2.5	3.4	0.019*

Supplemental Table 4. *(continued)*

41	Not being able to sleep because of pain	2.0	2.7	0.021*	1.4	3.0	0.001*	2.2	3.7	0.000*
42	Frustrated having to explain the meaning of this disease to others	2.0	2.5	0.047*	1.6	2.6	0.048*	2.4	2.8	0.349
43	Feeling that you have to fight this disease	2.0	2.3	0.169	1.5	2.8	0.004*	2.1	2.8	0.039*
44	Stiffness of a limb	2.0	2.5	0.056	1.5	2.8	0.004*	2.3	3.1	0.048*
45	Lack of information about recurrences rates	2.0	2.5	0.003*	1.5	2.6	0.013*	2.2	3.2	0.019*
46	Covering up tumour(s) and/or scar(s)	2.0	2.4	0.031*	1.8	2.2	0.505	2.2	2.6	0.186
47	Poor communication between GP's and specialist, and also between professionals	1.9	2.1	0.297	1.6	2.4	0.080	2.0	2.5	0.281
48	Feeling that you have to explain your circumstances	1.9	2.3	0.088	1.6	2.5	0.033*	2.0	2.9	0.025*
49	Feeling that you have a chronic disease	1.9	2.8	0.003*	1.5	2.6	0.035*	2.7	3.1	0.209
50	Negative responses to appearance	1.9	2.5	0.005*	1.8	2.0	0.499	2.1	3.2	0.002*
51	Missing psychological support	1.9	2.3	0.019*	1.5	2.4	0.053	2.1	2.7	0.096
52	Lack of libido	1.8	2.0	0.208	1.3	2.6	0.005*	1.8	2.5	0.025*
53	Having to take sick leave	1.8	2.4	0.008*	1.6	2.1	0.286	2.1	3.0	0.004*
54	Change of career ambitions after the diagnosis	1.8	2.0	0.144	1.4	2.4	0.016*	1.9	2.4	0.214
55	Lack of continuity of doctors during follow-up	1.8	2.2	0.058	1.4	2.4	0.089	1.9	2.8	0.035*
56	Lack of information received about desmoid-type fibromatosis	1.8	2.6	0.003*	1.3	2.4	0.016*	2.4	2.9	0.234
57	Long distance to travel to the hospital	1.8	2.4	0.002*	1.5	2.2	0.208	2.2	3.0	0.063
58	Short temper	1.8	1.9	0.329	1.5	2.2	0.122	1.6	2.7	0.002*
59	Lack of confidence	1.7	2.4	0.006*	1.5	2.0	0.329	2.0	3.1	0.009*
60	Problems with concentration	1.7	1.9	0.247	1.6	1.9	0.378	1.6	2.6	0.005*
61	Feeling that you are a burden on your family and/or partner	1.7	2.4	0.007*	1.3	2.4	0.025*	2.1	2.9	0.019*
62	Negative body image	1.7	2.7	0.000*	1.6	1.9	0.404	2.3	3.7	0.000*

Supplemental Table 4. *(continued)*

63	Forgetfulness	1.7	1.5	0.602	1.5	2.0	0.147	1.2	2.2	0.004*
64	Worries about possible inheritance/passing condition to children	1.7	1.9	0.079	1.4	2.1	0.161	1.6	2.5	0.019*
65	Difficulty explaining the disease to others	1.7	2.6	0.001*	1.3	2.3	0.082	2.5	2.8	0.504
66	Problems with activities of daily living (e.g. washing/ dressing)	1.7	2.4	0.001*	1.4	2.1	0.048*	2.0	3.2	0.001*
67	Change in lifestyle	1.7	2.3	0.034*	1.4	2.3	0.022*	2.1	2.7	0.022*
68	Not being able to enjoy hobbies	1.6	2.5	0.001*	1.3	2.2	0.007*	2.1	3.2	0.147
69	Problems with receiving treatment(s) in a cancer hospital	1.6	2.2	0.014*	1.6	1.7	1.000	2.0	2.7	0.150
70	Not being able to work at all	1.6	2.4	0.000*	1.4	1.9	0.403	2.2	2.9	0.039*
71	Change of hair colour	1.6	1.4	0.245	1.8	1.4	0.941	1.2	1.8	0.031*
72	Feeling that you are standing still in life	1.6	2.4	0.003*	1.4	2.0	0.329	2.1	2.9	0.065
73	Feeling less feminine/feeling less masculine	1.6	2.0	0.027*	1.4	1.9	0.147	1.7	2.7	0.002*
74	Feeling depressed	1.6	2.7	0.001*	1.4	2.0	0.161	2.3	3.5	0.001*
75	Having different recommendations about treatment options given to you	1.6	2.6	0.001*	1.2	2.3	0.047*	2.5	2.7	0.681
76	Fear of dying	1.6	2.1	0.030*	1.6	1.5	0.863	1.7	2.9	0.002*
77	Lack of understanding from family members and/or friends	1.6	2.3	0.002*	1.4	1.9	0.140	2.0	3.0	0.007*
78	Being addicted to pain medication	1.6	2.2	0.003*	1.2	2.2	0.117	1.9	2.8	0.006*
79	Changing jobs	1.5	2.3	0.001*	1.4	1.8	0.285	2.0	3.0	0.017*
80	Feeling depressed	1.5	2.4	0.000*	1.4	1.8	0.134	2.0	3.2	0.001*
81	Problems with getting dressed	1.5	1.8	0.048*	1.1	2.2	0.003*	1.6	2.4	0.007*
82	Breathless on minimal exertion	1.5	1.7	0.286	1.4	1.7	0.505	1.3	2.5	0.000*
83	Being less independent	1.5	2.2	0.001*	1.1	2.1	0.037*	1.9	3.0	0.004*

Supplemental Table 4. *(continued)*

84	Fear of amputation of a limb	1.5	2.1	0.005*	1.4	1.6	0.817	1.9	2.6	0.077
85	Weight loss	1.5	1.5	0.277	1.5	1.5	0.890	1.3	2.0	0.017*
86	Problems walking	1.5	2.2	0.005*	1.4	1.7	0.353	1.8	3.0	0.002*
87	Feeling that you are wasting the time of cancer professionals	1.5	1.5	0.284	1.1	2.1	0.088	1.3	2.0	0.039*
88	Negative influence of the disease on wishes to become a parent	1.5	2.4	0.000*	1.2	1.9	0.264	2.3	2.7	0.348
89	Feelings of isolation	1.5	2.0	0.007*	1.1	2.0	0.042*	1.6	2.9	0.000*
90	Altered nutritional intake	1.5	1.4	0.615	1.2	1.8	0.100	1.3	1.7	0.082
91	Lack of information about treatment side effects	1.5	2.3	0.000*	1.1	2.0	0.015*	2.1	2.6	0.131
92	Feeling disabled	1.4	2.4	0.000*	1.2	1.8	0.286	2.0	3.2	0.003*
93	Negative impact of the disease on family (life)	1.4	2.5	0.000*	1.2	1.8	0.187	2.2	3.3	0.002*
94	Being upset because of referral to a cancer hospital	1.4	2.4	0.000*	1.7	1.0	0.023*	2.3	2.6	0.429
95	Problems sitting down	1.4	2.1	0.000*	1.2	1.7	0.458	2.0	2.4	0.105
96	Missing online support (forum/chat group)	1.4	2.2	0.000*	1.3	1.6	0.611	2.1	2.6	0.164
97	Financial problems because you have had to change your job	1.4	2.1	0.002*	1.2	1.8	0.074	1.9	2.8	0.040*
98	Hair loss	1.4	1.4	0.245	1.5	1.3	0.781	1.3	1.8	0.045*
99	Missing out on social occasions	1.4	2.2	0.000*	1.1	1.9	0.002*	1.9	2.9	0.005*
100	Financial problems due to travel expenses	1.4	2.1	0.001*	1.2	1.7	0.074	1.7	3.0	0.002*
101	Marital/relational problems	1.4	2.1	0.001*	1.2	1.6	0.187	1.8	2.9	0.003*
102	Financial problems due to the disease or treatment(s)	1.4	2.3	0.000*	1.1	1.8	0.155	2.1	2.9	0.035*
103	Worries about tumour growth during pregnancy	1.4	2.5	0.000*	1.4	1.3	0.505	2.4	2.8	0.390
104	Nausea	1.4	1.5	0.296	1.1	1.7	0.111	1.2	2.2	0.001*
105	Feeling lonely	1.4	2.0	0.008*	1.1	1.8	0.012*	1.6	2.9	0.001*

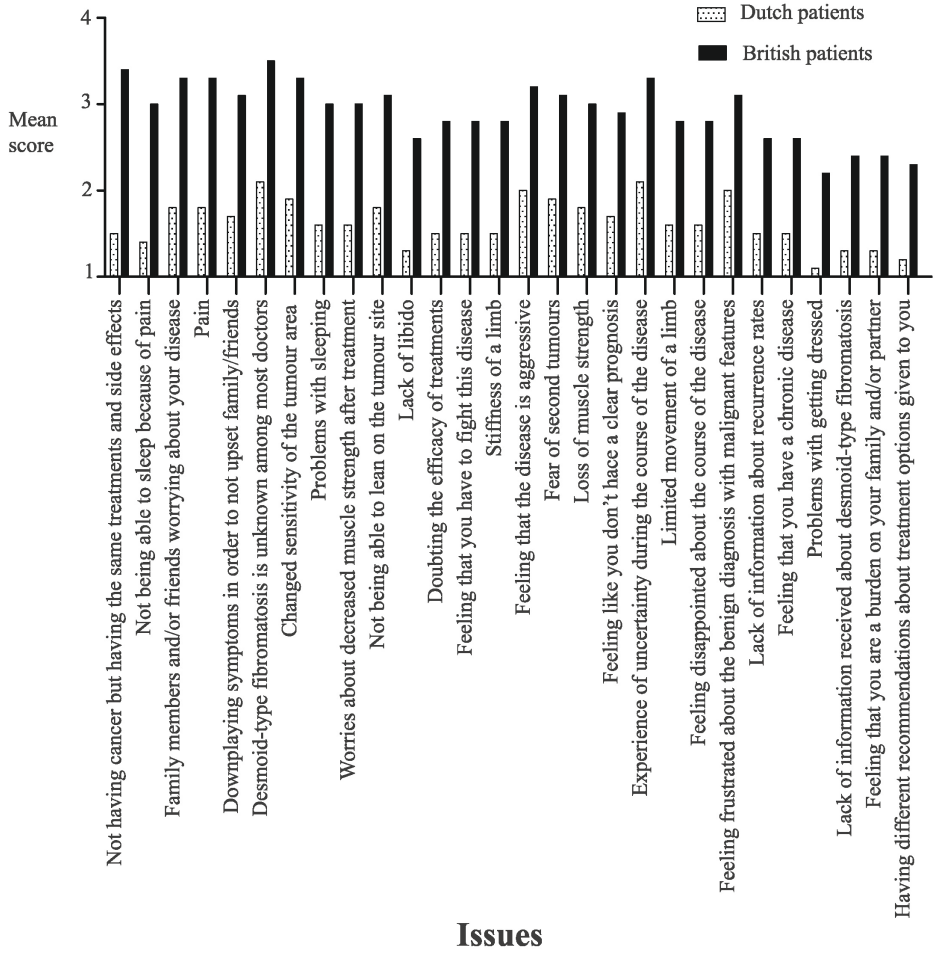
Supplemental Table 4. *(continued)*

106	Change of dominant side during writing	1.4	1.7	0.005*	1.0	1.9	0.111	1.5	2.1	0.049*
107	Change in appetite	1.3	1.8	0.019*	1.1	1.6	0.042*	1.5	2.4	0.002*
108	Oedema (abnormal accumulation of fluid)	1.3	1.8	0.002*	1.3	1.4	0.890	1.7	2.0	0.231
109	Problems with fertility	1.3	2.0	0.001*	1.1	1.6	0.342	1.8	2.8	0.016*
110	Parental role affected	1.3	2.2	0.000*	1.3	1.3	0.522	1.9	2.8	0.048*
111	Vomiting	1.3	1.5	0.106	1.1	1.6	0.141	1.2	1.9	0.008*
112	Eczema	1.3	1.3	0.643	1.2	1.3	0.963	1.2	1.6	0.449
113	Less attention from family and friends because of the "benign" disease	1.2	2.1	0.000*	1.1	1.5	0.370	1.8	2.7	0.013*
114	Lack of trust in your treating physician	1.2	2.0	0.000*	1.2	1.3	0.711	1.9	2.3	0.307
115	Feeling guilty towards siblings because you receive more attention because of your disease	1.2	1.6	0.004*	1.1	1.4	0.611	1.4	1.9	0.114
116	Shipped from hospital to hospital to hospital	1.2	2.2	0.000*	1.3	1.1	0.711	2.3	2.1	0.769
117	Missing school	1.2	2.1	0.000*	1.3	1.1	0.897	1.8	2.7	0.014
118	Feeling ashamed to use support tools (e.g. wheelchair, scooter, electric bike etc.)	1.2	1.9	0.000*	1.1	1.4	0.537	1.7	2.4	0.074
119	Lack of support from family/friends	1.2	1.9	0.000*	1.2	1.2	0.941	1.7	2.3	0.048*
120	Suicidal thoughts	1.2	1.6	0.009*	1.2	1.2	0.643	1.2	2.6	0.000*
121	Problems with driving a car	1.2	2.0	0.000*	1.1	1.3	0.537	1.7	2.6	0.003*
122	Loss of friendships	1.1	1.9	0.000*	1.1	1.2	0.890	1.6	2.6	0.010*
123	Hives	1.1	1.3	0.211	1.1	1.2	0.890	1.1	1.6	0.173
124	Change of living accommodation	1.1	1.7	0.001*	1.0	1.2	0.414	1.3	2.4	0.001*
Total mean		**1.8**	**2.3**		**1.5**	**2.2**		**2.0**	**2.8**	

** statistically significant difference; HCP, health care provider*

Grey marked areas are issues with an M-score of >1.5 given by either patients or HCPs, the cut of value for inclusion in the DTF-specific HRQoL-tool. The p-value represents the comparison of the scores of the participating patients and HCPs, the comparison of the scores of the Dutch and British participating patients, and the comparison of the scores of Dutch and British HCPs.

Supplemental Figure 1. Differences in score of more than 1 point between Dutch and British patients



IV

General discussion and Future Perspectives

11

General Discussion

General Discussion

The knowledge about desmoid-type fibromatosis (DTF) has grown significantly over the past decades. Various, often patient-initiated, projects have led to more awareness for this rare disease. Today the research community still faces many challenges in DTF research; gathering knowledge about tumour genetics, biology, growth behaviour, and risk prediction. The efficacy of existing treatments are evaluated and the search for novel therapeutic targets continues. Lastly, the impact of the disease on patients' health-related quality of life (HRQoL) is increasingly acknowledged and incorporated in both research and clinical decision-making. The aim of this thesis was to describe the many faces of desmoid-type fibromatosis, to contribute to the current knowledge of this peculiar disease.

Genetics and Molecular Biology

DTF is rare with an incidence of 5 patients per million people per year in the Netherlands [1]. Several treatments options are currently available including active surveillance, surgery, and several systemic treatments [2]. In **Chapter 2**, we evaluate existing biological evidence for the activity of common cell-signalling pathways in DTF including Wnt/β-catenin, JAK/STAT, Notch, PI3 kinase/AKT, mTOR, Hedgehog, the oestrogen pathway, and the growth regulatory pathway in order to identify potential therapeutic opportunities. Furthermore, we summarized the results of completed clinical trials studying targeted drug regimens in the DTF setting. The evidence for most of these treatment regimens are based on small sized, retrospective cohort studies with heterogeneous study populations, and the biological rationale for the use of these targeted therapies is often limited. The variable efficacy of systemic treatments in DTF may also be caused by the absence of recommendations on the sequence of systemic treatments in international treatment guidelines [3]. This leaves room for individual physicians to follow their preferred indication for starting systemic treatment and their drugs of choice [4]. Thus many current treatments, administered to DTF patients lack the solid evidence base for efficacy that is preferred. Nevertheless, initiation of systemic treatments in a stepwise fashion, starting with the least toxic agent, is recommended [3].

The current systemic regimens include traditional chemotherapy such as methotrexate alone [5] or combined with vinblastine/vinorelbine [6], anthracycline-containing regimens (i.e., dacarbazin and/or (liposomal pegylated) doxorubicin) [5, 7], but also targeted therapies. The later encompasses non-steroidal anti-inflammatory drugs, such as celecoxib or sulindac, alone [8] or in combination with selective oestrogen receptor modulators (i.e., tamoxifen, toremifene, or raloxifene [9-13]), and tyrosin kinase inhibitors (i.e., imatinib, nilotinib, and

pazopanib) [14-18]. The evidence for most of these treatment regimens are based on small sized retrospective cohort studies with heterogeneous study populations.

Drugs that form an attractive therapeutic option include gamma-secretase inhibitors. These drugs interfere with the Notch signalling pathway by inhibiting the final Notch processing step by which intra-cellular domain is released to act in the nucleus, consequently blocking Notch signalling [19]. The gamma-secretase inhibitor PF-03084014, later named Nirogacestat, was developed based on the positive results of decreased Notch target protein expression in *in vitro* studies. This led to the development of several clinical trials [21-23]. The results of the phase 3 trial (Nirogacestat vs. placebo) are currently awaited and will determine if Nirogacestat can be seen as a valid treatment option for DTF [24].

Other randomized data regarding systemic treatments for DTF that is currently available include two tyrosin kinase inhibitors: sorafenib and pazopanib. The first trial reported an advantage for sorafenib in the 2-year progression-free survival (PFS) over placebo (81% (95% confidence interval [CI], 69-96) versus 36% (95% CI, 22-57). The second trial reported an advantage for pazopanib over methotrexate-vinblastin in progression-free proportion of patients measured at 6 months (83.7% (95% CI 69.3–93.2) versus 45% (95% CI 23.1–68.5) [25, 26]. The result of these trials suggest that there seems to be a beneficial effect of tyrosin kinase inhibitors on DTF tumours. These drugs could therefore be seen as a valid treatment option, taking potential side effects and clinical benefits into account [27].

The increased level of β-catenin and consequent aberrant Wnt/β-catenin signalling are characteristics of DTF, but so far they have not yielded any therapeutic opportunities [28]. The cause of nuclear β-catenin accumulation are specific, non-synonymous mutations in the *CTNNB1* gene. About 85% of DTF tumours contain a mutation located in exon 3 causing one of the following substitutions: a replacement of threonine to alanine at codon 41 (T41A), a replacement of serine for phenylalanine (S45F), or a replacement of serine for proline (S45P) at codon 45. The relevance for these mutations and their impact on prognosis and clinical growth behaviour remains uncertain. Clinical studies demonstrate clear differences in tumour behaviour between the most common mutation types. S45F-mutated DTF tumours exhibit a higher recurrence rate after primary resection than wild-type (WT) and T41A mutant tumours [29-32]. Several studies demonstrate that S45F tumours have an increased resistance to systemic treatments such as meloxicam [33], sorafenib [34], or doxorubicin [35] and that they are more resistant to apoptosis and exhibit deregulation of apoptotic-related genes [35].

The biological explanation for the aforementioned differences remains unclear. In pre-clinical research studying liver cancer, different *CTNNB1* mutations are linked to different levels of β-catenin activation: an S45F mutations led to a weaker activation compared to T41A, but the duplication of weak S45F alleles resulted in a final higher β-catenin activity [36]. In desmoid cells, Hamada et al. reported that S45F desmoid cells have a stronger nuclear β-catenin staining and observed an upregulation of Wnt target genes *AXIN2* and *CCND1* compared to WT and T41A cells [37]. In this thesis we sought to explain these clinical differences based on mRNA expression data and DNA methylation patterns. In **Chapter 3**, we compared Wnt signalling activity between the mutation types based on the expression of Wnt target genes. We were not able to identify differences comparing the different mutations types. In **Chapter 4**, we used a novel technique to uncover whole genome DNA-methylation patterns. No distinct DNA-methylation patterns were found between DTF with a S45F or T41A mutation. From this research we tentatively conclude that S45F and T41A are biologically similar and that observed clinical differences may be caused by other factors such as tumour location or size [38]. A possible confounder in our studies is that the WT group, now defined by the absence of mutations in exon 3 of *CTNNB1*, needs to be screened more thoroughly for *CTNNB1* mutations. This group of DTF tumours remain peculiar, as previous studies show that a large part of these "wild-type" contain other mutations in *APC* or *CTNNB1* which can be detected using more sensitive methods than Sanger Sequencing (e.g., Next Generation Sequencing) [39, 40]. In this group, other genomic alterations can occur like *APC* loss, chromosome 6 loss and alterations such as *BMI1* mutations are seen. All of these alterations are linked to Wnt/β-catenin activation [40] and this pathway seems to play a pivotal role in the development of DTF tumours. More insight into the genes that drive this subgroup of tumours and their biological and clinical differences with the more common mutation is encouraged.

Diagnosis and Treatment

About one third of DTF patients is misdiagnosed at the first presentation [41]. Early recognition and referral to a sarcoma centre with a medical specialist with affinity for DTF, is vital for a patient to establish a correct diagnosis and treatment plan [3]. Magnetic resonance imaging (MRI) is the preferred imaging modality for extra-abdominal or abdominal wall soft tissue sarcomas (STS) and DTF. However, these two entities can display similar imaging characteristics such as fascial crossing and invasive growth [42]. As DTF is rare, there are no established imaging protocols and diagnosing can be challenging. Radiomics is a promising technique that links large amounts of quantitative imaging features with

clinical outcome [43-47]. In **Chapter 5**, we used radiomics to create a prediction model for the differential diagnosis of DTF (DTF versus STS) and the different genetic mutations (S45F, T41A, and WT) observed in DTF. We showed that our radiomics model was capable of distinguishing DTF from STS based on T1-weighted and T2-weighted MR images, and the model outperformed the two experienced radiologists. Unfortunately, the model was not able to predict the *CTNNB1* mutation type of the included DTF tumours. The results of our study encourages the use of computer-aided models in the diagnostic trajectories of rare diseases.

After establishing the correct diagnosis, treatment decisions are based on the patients' preferences, comorbidities, symptoms, tumor site and tumour size. Treatment decisions are preferably made in a multidisciplinary team meeting in the presence of a radiologist, a pathologist, a surgeon, an oncologist, and a radiotherapist, all with relevant expertise regarding soft tissue sarcomas and DTF. The increasing knowledge about the biology of DTF has taught us that active surveillance can be a safe approach [3, 48]. Consequently, the rate of surgery as an initial treatment therefore decreased from 55% to 42% between 2010 and 2015 in a French nationwide cohort study [49]. Three prospective clinical trials (NTR 4714, the Netherlands, NCT02547831, Italy, and NCT01801176, France) are conducted in Europe to evaluate the percentage of patients that do not need to receive an active form of treatment [50-52]. The results of those trials are still awaited. In an attempt to systematically evaluate the results of the active surveillance using retrospective data, we performed a systematic literature review in **Chapter 6**. This study showed that a majority of patients undergoing active surveillance have either stable disease or partial response. The overall median percentage of patients with partial response in the included studies was 20% (95% CI 10-24) which demonstrates the capacity of DTF tumours of undergoing spontaneous regression. About one-third of patients shift to an active form of treatment. Although the active surveillance approach avoids the complications seen in active treatments, patients should be made aware of the benefits and risks to increase the chance of a successful active surveillance. Moreover, there is a need for identification of patients that will benefit from this approach.

A way of stratifying the patients that will benefit from a certain treatment approach is to determine the *CTNNB1* mutation type. Several studies implicate a prognostic role for these mutations specifically after surgical resection [29-32]. These studies imply that the S45F mutated desmoid tumour has an increased risk of recurrence compared to other *CTNNB1* mutations and WT desmoid tumours. However, these studies were all small-sized

heterogeneous retrospective studies and other report contradictory results [53, 54]. To assess the true prognostic value of the S45F mutation for recurrence in a homogeneous group of patients, we performed a meta-analysis with individual patient data of patients with primary DTF treated with surgery only (reported in **Chapter 7**). We found that S45F mutated tumours do have a higher chance of recurrence compared to WT and T41A mutations. However, this risk of recurrence appeared to be mediated by tumour size and a higher chance of recurrence for S45F tumours was only valid for small tumours (<5 cm). This study suggests that a prognostic nomogram should not only focus on tumour site, size and age, but should also include *CTNNB1* mutation status when predicting the risk of recurrence [38].

Health-related Quality of Life

Whilst decreasing the risk of recurrence or achieving tumour stabilization or regression are the goal of many treatments, the effect of lowering symptoms must not be underestimated [55]. DTF remains a tumor with many faces; varying from a small, asymptomatic lump to a large infiltrative debilitating tumour and multiple recurrences. Especially in an unknown disease with a wide variety of clinical outcomes, evaluating HRQoL can be relevant to meet a patients' need. To achieve this, more knowledge about the impact of the disease on a patients' life is a necessity. Furthermore, a tool, designed specifically for DTF required. In **Chapter 8**, **9** and **10** we describe the development of a DTF-specific questionnaire, the DTF-QOL, according to the guidelines of the European Organization for Research and Treatment for Cancer (EORTC) [56]. In **Chapter 8**, we conducted a systematic literature review and organized patient focus groups to identify relevant HRQoL-issues. In **Chapter 9**, the issues are ranked and prioritized by patients and health-care professionals. These two studies showed the impact of the disease on various domains of DTF patients and led to the initiation of the QUALIFIED study (The evaluation of health-related quality of life issues experienced by patients with desmoid-type fibromatosis), of which the protocol is described in **Chapter 10**. This study includes the DTF-specific questionnaire, the 'DTF-QOL' which covers all HRQoL-domains. The results of this study will hopefully provide insight into the prevalence of HRQoL-issues and can be used to identify patients at risk for impaired HRQoL to improve our DTF care.

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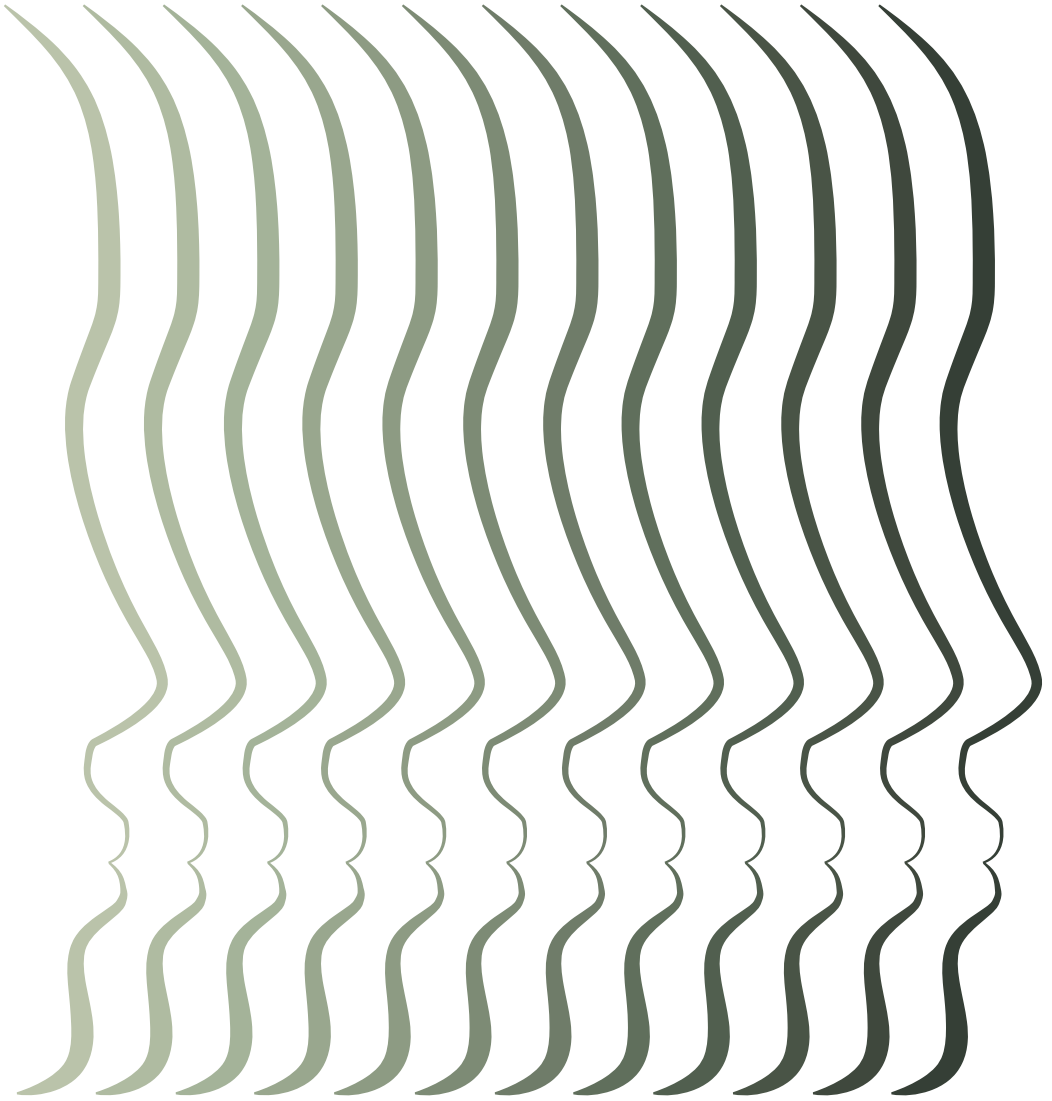

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Future Perspectives

Investing in *in Vivo* and *in Vitro* Models to Understand DTF Biology

The clinical course of DTF tumours varies widely. Although several factors have been mentioned to influence risk of recurrence or to reflect tumour aggressiveness such as tumour site, size and type of *CTNNB1* mutation, up until now, for a large part, DTF biology is still unknown. The current thesis focuses mainly on the hypothesized biological differences between the most common mutation types of DTF. Our translational research was mainly challenged by the limited number of available biomaterials and corresponding clinical data. Therefore, inevitably given the rarity of DTF, the used study sample sizes were small, potentially contributing to inconclusive or negative results. Moreover, types of analysis were restricted by the use of FFPE material and lack of fresh frozen tissues and micro-array data. Commercially available desmoid cell lines are scarce and culturing of DTF tumour tissue remains challenging. This is mainly due to overgrowth of fibroblastic cells without a *CTNNB1* mutation.

Currently, clonal expansion techniques to separate tumour cells (containing an *CTNNB1* mutation) from stromal cells are being exploited [1]. Although cell lines are a corner stone of many translational studies, one should also consider the role of the tumour microenvironment in DTF pathogenesis and growth. DTF tumours are known to be heterogeneous, and unpublished work from M. Al-Jazrawe and B.A. Alman showed that there is a large variation in the proportion of stromal cells and mutated cells per tumour [1]. The clinical consequences of these differences are still not clear but could explain the observed differences in tumour behaviour in culture, and possibly in patients. The role of the tumour microenvironment is well recognized in cancer, and the cross talk between stromal and neoplastic cells are thought to play an important role in tumour initiation, progression, and invasion [2, 3]. Future studies should therefore acknowledge the contribution of the tumour microenvironment for example by using representative animal models rather than cell lines. A murine model; *APC+/APC1638N*^{I1638N} and a Xenopus tropicalis model have been developed. Unfortunately, both models are *APC*-based and may not be representative for the sporadic DTF [4,5]. Currently, mouse models with a mutation in *CTNNB1* (T41A and S45F) are being developed for research purposes [6]. These animal models could be of use to study the DTF pathogenesis, microenvironment, and tumour growth. Furthermore, they could be of value when testing the efficacy of existing treatments *in vivo*.

(Inter)national Case Registration

In order to increase the availability of biomaterials accurate identification and registration of patients is a necessity. As the disease is not classified as a cancer [7], cases are not registered in the Dutch national cancer registry. Currently, our research is depending on the Dutch national pathology database: Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief (PALGA) [8]. This registry provides pathology reports, but contains little clinical information, and results can be distorted due to the use of general terms like "fibrosis". Furthermore, obtaining clinical data is challenging as patients are often referred to their local hospitals for initial diagnosis before referral to one of the seven Dutch sarcoma-expertise clinics. This could lead to fragmentation of patient data, as the first diagnostic specimen remains in the local hospital, whilst data of additional molecular analysis and the clinical data are localized in the expert clinic. Currently, both pathological analysis (first diagnosis and additional molecular analyses) are registered separately in PALGA, and patient linkage is not always possible. This type of registration leads to missing data or incorrect data interpretation. Especially due to the rarity of the disease, a complete registration system to identify patients and their diagnostic pathways on a national level must be possible. This national registry could be complemented by an imaging database, such as the radiomics platform, in order to recognize patters on imaging, to predict clinical behaviour.

Several worldwide initiatives have been made to improve the registration process of DTF cases. For example, the American patient association, the Desmoid Tumor Research Foundation (DTRF), developed the DTRF registry [9]. This patient registry is an online system completed by patients, and designed to understand patients' experiences and the natural history of DTF. The French sarcoma network has also made tremendous efforts to improve registration by including DTF patients in the national sarcoma registry [10]. This effort decreased the number of misdiagnosis, shortened the diagnostic delay, and increased the number of patients treated in an expert centre [11]. Accurate case registration would aid translational research projects by identification of biomaterial available for research purposes. Clinical research would benefit by quick identification of eligible patients for both retrospective and prospective studies. Additionally, such a registration would be helpful for quick and clear communication about important research updates and it will assist international collaborations. Therefore, a national DTF registration system is advised.

The Usefulness of RECIST in DTF

Tumour size in DTF remains an ambiguous variable since DTF can display variable shapes with irregular margins and infiltrative growth [12, 13]. Currently, Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) [14, 15] is used for determining treatment success using the categories; complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) [15]. However, these criteria assumes spherical-shaped tumours and a uniform decrease in size [16]. To obtain a "partial response" status, a 30% tumour size reduction has to be achieved. This may not always be feasible in DTF; hence, the relatively high number of reported SD in retrospective trials evaluating systemic treatments and active surveillance. Furthermore, distinction of natural DTF tumour behaviour and the true efficacy of treatment can be challenging [17, 18]. This is illustrated in the phase 3 sorafenib versus placebo trial, in which 20% of patients on placebo display a partial response [19]. Therefore, we encourage to use a volumetric parameter, alongside RECIST, to evaluate treatment response and/or to observe the natural growth of DTF [16]. Our radiomics model might provide a platform to obtain such volume measurements as it uses semi-automatic segmentation of the entire tumour in multiple dimensions. Moreover, it can be used in various imaging modalities such as ultrasound, computed tomography, and magnetic resonance imaging (MRI). It can also provide insights into changes in histological activity, reflected by hyperintense signals on T2 weighted MRI, which can be diminished without changes in tumour size [20]. We encourage efforts to improve the radiomics platform, exploring automatic segmentation options to make the process less time consuming, and to include volumetric parameters when evaluating treatment response or natural growth of DTF. This radiomics platform could also be used in a longitudinal manner to map tumour growth, and to predict tumour behaviour.

Health-Related Quality Of Life

HRQoL is increasingly used as an endpoint in clinical trials for DTF (e.g., NCT04195399 [21], NCT01876082 [22], and NCT03966742 [23]). Measuring HRQoL in DTF is relevant as the mortality rate is low and traditional oncologic endpoints are less relevant. However, there are some factors to consider. First, appropriate time points for assessing HRQoL have to be identified so that treatment arms are evaluated in a fair and similar manner. The accurate choosing of time points to assess HRQoL can be challenging by the unpredictable response of DTF to certain treatments, for example delayed response or late recurrences [24-26]. Additionally, measurable response does not necessarily mean a decrease in symptoms [26-28]. Choosing accurate time points depends on the treatment type, the type and timing of

expected effects of the treatment and this should be individually established for each clinical trial. Second, trial information should be presented as objective as possible to minimize influence on treatment decisions and HRQoL-issues. This could be established by using standardized information videos to inform patients about the study design, potential adverse effects and trial endpoints. Third, the balance between accurate assessment of HRQoL and patient response burden must be evaluated carefully; as too many questionnaires and/or to many questions can lead to a decrease in response rates and missing data [29]. Fourth, the research team should establish a baseline HRQoL-value for their cohort and have consensus on the definition of the desirable 'effect' on HRQoL of the investigated treatment strategy. Insight into common HRQoL-problems can serve as a source of information for new patients and their loved ones. Despite the aforementioned challenges, incorporation of HRQoL as a clinical endpoint will provide evidence for the efficacy of a certain treatment on symptom relieve and improvement of HRQoL. The use of a DTF-specific measurement is encouraged as such as tool captures disease-specific problems.

Final Remark

The knowledge about desmoid-type fibromatosis (DTF) has grown significantly over the past decades and it continues to grow. Strengthened collaborations, accurate case registration, and the use of new technologies and representative tumour models will lay the foundation towards of a global consensus for the optimal treatment of this rare disease. Such a network will accumulate valuable insights, experiences and knowledge from all over the world and it will act as a powerful platform for health care providers committed to this rare disease.

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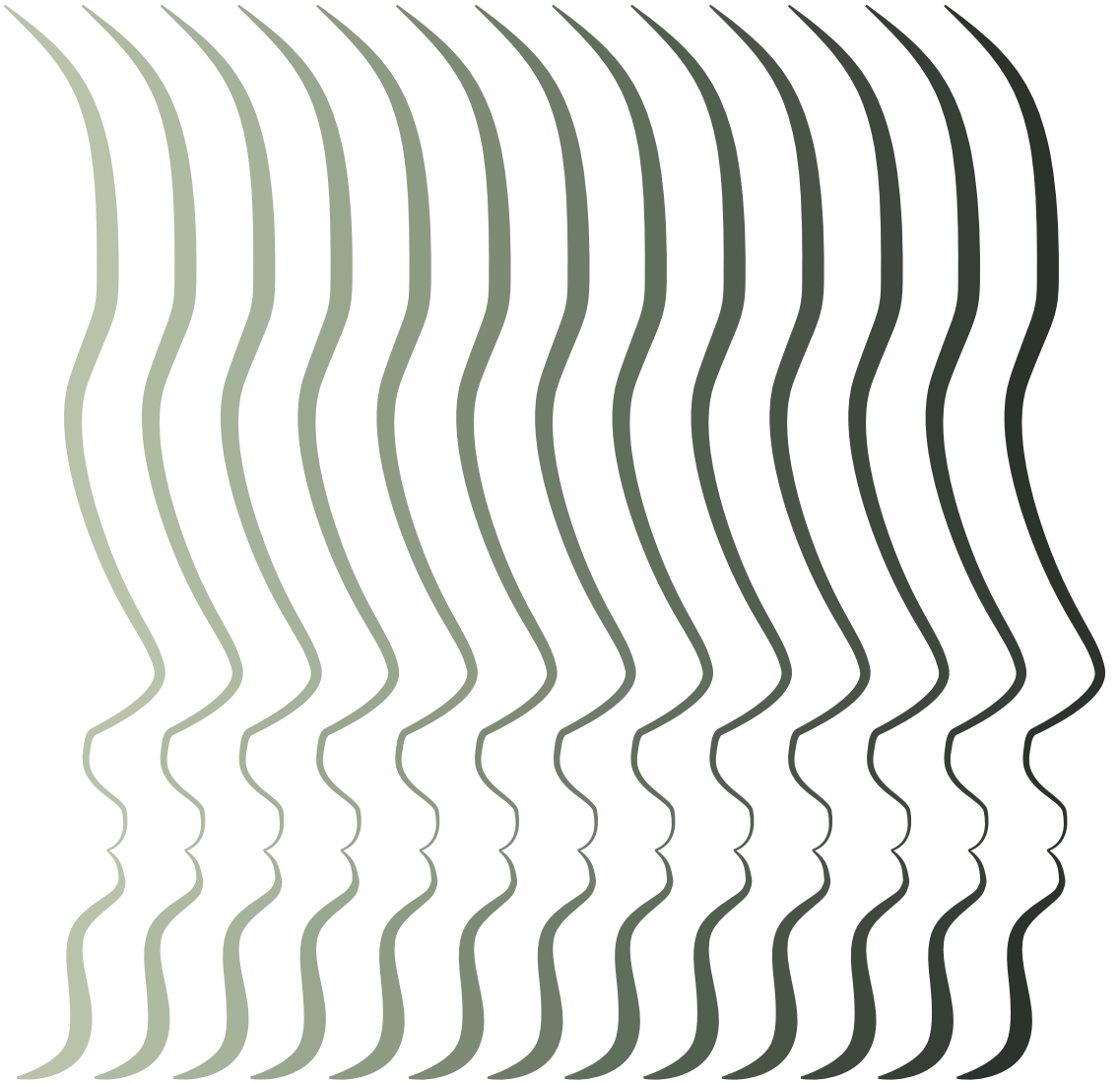

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Summary

Summary

This thesis consists of four parts. **Part I** of this thesis aims to gain insight into the genetics and biology of desmoid-type fibromatosis (DTF). The first chapter describes the role of the various cell signalling pathways. The other chapters aim to explain potential differences in clinical behaviour by comparing Wingless (Wnt)/ β-catenin signalling and DNA methylation patterns of the most common *CTNNB1* mutation types. **Part II** describes a novel technique named radiomics, evaluates the success of the active surveillance approach and investigates whether primary S45F-mutated DTF tumours have an increased risk on recurrence after surgery. **Part III** describes the health-related quality of life (HRQoL) in the setting of DTF.

Part I - Genetics and Molecular Biology

Chapter 2 describes the current evidence for the use of targeted therapies in the treatment of DTF. Specific attention was paid to *in vivo*/*in vitro* studies. This literature review aimed to review the common cell signalling pathways Wnt/β-catenin, JAK/STAT, Notch, PI3 kinase/ AKT, mTOR, Hedgehog, the oestrogen pathway, and the growth regulatory pathway, which might play a role in the pathogenesis of DTF. Additionally, an overview on the currently available targeted therapies targeting the aforementioned pathways is provided. This review underlined the existing evidence for activation of the Wnt/β-catenin cell signalling pathway, but currently there are no clinically available treatments targeting this pathway. The evidence for activation of the other signalling pathways is weak and mainly based on the expression of certain genes or reports of observed clinical response after treatment with available targeted therapies. Currently, few randomized clinical trials investigate the effect of targeted therapies. This study raises awareness to the need to better understand the biology of DTF and identify novel therapeutic drug targets.

Chapter 3 describes the role of the Wnt/β-catenin cell signalling pathway. β-catenin is a major player in this pathway and this chapter investigated the differences in expression of Wnt-related genes between the different mutation types of DTF (S45F, T41A, S45P and wild-type (WT)). This study uses mRNA data of 128 desmoid patients from an Affymetrix dataset (gene Expression Omnibus series matrix file, GSE58697). Additionally, the expression of *AXIN2*, *CCND1*, and *DKK1* was calculated in 64 desmoid patients by real-time polymerase chain reactions (RT-PCR). There were no statistically significant differences in relative expression levels of the Wnt-related genes *AXIN2*, *CCND1*, and *DKK1* between the β-catenin mutants. Hierarchical cluster analyses using selected Wnt targets did not discriminate between different *CTNNB1* mutation types.

Chapter 4 examines the genome-wide DNA methylation profiles of S45F and T41A mutated DTF. MeD-seq analysis was performed on 29 primary DTF tumours including 15 T41A samples and 14 S45F samples. In total, 365 regions were identified as differentially methylated regions (DMRs). Clustering analysis yielded no clear separation was observed between the two mutation groups. Only few DMRs had a fold change of ≥ 1.5 implicating no difference in methylation between mutation types. No differences in expression of the corresponding genes of those DMRs between the two mutation types were confirmed in an independent mRNA Affymetrix dataset. Immunoprecipitation did not reveal an association of wild-type β-catenin or mutant variants with DNMT1.

Part II - Diagnosis and Treatment

Chapter 5 describes the use of a new technology: radiomics that related large amounts of quantitative imaging features to clinical outcome. In this chapter we used radiomics in an effort to simplify the diagnosis of DTF by creating a model that could distinguish extremity DTF from other extremity soft tissue sarcoma (STS) on a pre-treatment T1-weighted (T1w) magnetic resonance imaging (MRI) scan. Furthermore a prediction model was created for determining the *CTNNB1* mutation type. The MRI data encompassed tumours from 203 patients and included 72 patients with DTF and 131 patients with STS. Tumours were semi-automatically annotated on the T1w images, from which 424 features were extracted. The T1w radiomics model showed a mean area under the curve (AUC) of 0.79 on the full dataset. Addition of T2w and post-contrast sequences did not improve the performance. On the location matched cohort, the T1w model had a mean AUC of 0.88 while the radiologists had an mean AUC of 0.80 and 0.88, respectively. For the prediction of the mutations (S45F, T41A and wild-type), the T1w model showed an mean AUC of 0.61, 0.56, and 0.74. From this research we have to conclude that the radiomics model was able to distinguish DTF from STS with high accuracy similar to two radiologists, but was not able to predict the *CTNNB1* mutation status.

In **Chapter 6** we performed a systematic literature search to systematically evaluate the results of retrospective studies describing the active surveillance approach. A total of 24 articles were included describing a total of 3541 patients of which 40.7% (n = 1404) received active surveillance. The majority were females and the majority had a primary tumour. About 20% of patients had progressive disease, 58% had stable disease and 20% of patients had a partial response. About 30% of patients needed shift to an active form of

treatment most commonly surgery and systemic treatment. The reported median follow-up time ranged between 8 and 73 months, and the reported median time to progression and/or initiation of the subgroup shifting from active surveillance to 'active' therapy ranged from 6.5 months to 19.7 months. Selecting patients who will benefit from this active surveillance approach upfront should be the priority of future studies.

Chapter 7 describes a meta-analysis of seven retrospective studies with individual patient data of 329 patients to analyse differences in risk of recurrent according to the *CTNNB1* mutation status. From the total group of patients, 154 had a tumour with a T41A mutation, 66 patients presented tumours with a S45F mutation, 24 tumours displayed a S45P mutation and 85 contained WT *CTNNB1* tumours. Eighty-three patients (25.2%) experienced a tumour recurrence after surgery. Multivariable analysis, adjusting for sex, age and tumour site yielded a p-value of 0.011 for *CTNNB1* mutation and risk of recurrence after surgery. Additional adjustment for tumour size yielded a p-value of 0.082 with hazard ratio's (HR) of 0.83 (95% confidence interval [CI] 0.48-1.42), 0.37 (95% CI 0.12-1.14) and 0.44 (95% CI 0.21-0.92) for T41A, S45P and WT DTF tumours compared to S45F DTF tumours. From this study we concluded that primary, sporadic DTFs harbouring a *CTNNB1* S45F mutation have a higher risk of recurrence after surgery compared to T41A, S45P and WT DTF, but this association appears to be mediated by tumour size.

Part III - Health-Related Quality of Life

Chapter 8 investigated the impact of DTF on HRQoL. A mixed methods methodology was used consisting of a systematic literature review to provide an overview of measures previously used to evaluate HRQoL among DTF patients and focus groups, to gain insight into HRQOL-issues experienced by DTF patients. Thirteen articles reporting HRQoL-measures using a wide variety of cancer-specific HRQoL tools, functional scores, symptom scales, and single-item outcomes (e.g., pain and functional impairment) were identified but no DTF specific HRQoL-tool was found. Qualitative analysis of three focus groups (6 males, 9 females) showed that participants emphasised the negative impact of DTF and/or its treatment on several HRQoL-domains. Six themes were identified including diagnosis, treatment, follow-up and recurrence, the physical domain, the psychological and emotional domain and the social domain. This study indicates that HRQoL of DTF patients was negatively affected in several domains. A DTF-specific HRQoL-measure could improve our understanding of short- and long-term effects and, ideally, can be used in both clinic and for research purposes.

In **Chapter 9**, we used the results of chapter 8 and the corresponding study from the United Kingdom (UK) of Husson et al. (2018, Support Care Cancer) to create a list of issues regarding diagnosis, treatment, follow-up, recurrence, living with DTF, healthcare and supportive care experiences. DTF patients and health care providers (HCPs) from the Netherlands and the UK individually ranked 124 issues according to their relevance. The most highly ranked issues by 29 patients were issues concerning "tumour growth", "feeling that there is something in the body that does not belong there", and "fear of tumour growth into adjacent tissues or organs". British patients scored higher on most issues compared to Dutch patients and 31 HCPs gave higher scores on most issues compared to patients. Field-testing in a large, international cohort is needed to confirm these findings and to devise a comprehensive and specific HRQoL-questionnaire for DTF patients.

Chapter 10 describes the protocol of the QUALIFIED study (The evaluation of health-related quality of life issues experienced by patients with desmoid-type fibromatosis), an international, multicentre, cross-sectional, observational cohort study. The DTF-specific questionnaire, the DTF-QOL supplements existing questionnaires such as the European Organization for Research and Treatment for Cancer Quality of Life Questionnaire (EORTC QLQ-C30), and the EuroQol 5D (EQ-5D) and questionnaires compiled specifically for this study to gather sociodemographic and clinical characteristics. All adult (≥18 years) patients diagnosed between January 1990 and October 2019, with pathologically proven, sporadic DTF and a recent (between October 2014 and October 2019) visit to the hospital for their DTF are eligible for inclusion. Questionnaires will be completed using PROFILES (Patient Reported Outcomes Following Initial treatment and Long term Evaluation of Survivorship), a system to electronically capture questionnaire responses. It aims to gain insight into DTF-specific HRQoL-problems, to compare HRQoL-scores of DTF patients in the general Dutch and British population; and to identify subgroups at risk for impaired HRQoL.

Samenvatting

Dit proefschrift bestaat uit vier delen. In **Deel I** van dit proefschrift wordt gepoogd inzicht te geven in de genetica en de biologie van desmoïd-type fibromatose (DTF). Het eerste hoofdstuk beschrijft de rol van verschillende signaaltransductie cascades. De andere hoofdstukken proberen het verschil in klinisch gedrag van desmoïd tumoren te verklaren door de Wingless (Wnt)/β-catenine signaaltransductie cascade en DNA methylatie patronen tussen de verschillende veel voorkomende *CTNNB1* mutatie types te vergelijken. **Deel II** beschrijft de toepassing van een nieuwe techniek, genaamd radiomics, om het diagnostische proces van DTF te optimaliseren. Verder wordt het succes van de actieve observatie (in het Engels: active surveillance) geëvalueerd en wordt er onderzocht of primaire tumoren die een S45F mutatie bezitten een hoger risico op een recidief hebben na chirurgische resectie. **Deel III** beschrijft de gezondheid-gerelateerde kwaliteit van leven van patiënten met DTF.

Deel I – Genetica en Moleculaire Biologie

In **hoofdstuk 2** is de huidige bewijslast voor het geven van doelgerichte, oftewel "targeted" therapieën voor DTF. Samengevat werd hierbij gelet op de bewijslast van *in vivo* en *in vitro* experimenten. Voor deze studie werd er middels een literatuuronderzoek een overzicht gegeven van bekende signaaltransductie cascades welke mogelijk een rol spelen in de pathogenese van DTF. De volgende signaaltransductie cascades werden bekeken: Wnt/β-catenine, JAK/STAT, Notch, PI3 kinase/AKT, mTOR, Hedgehog, de oestrogeen signaaltransductie cascade en de groeifactor signaaltransductie cascade. Ook werd er een overzicht gegeven van de beschikbare therapieën door aan te geven op welke manier zij mogelijk op deze signaaltransductie cascades inwerken. Er zijn veel aanwijzingen dat activatie van de Wnt/β-catenine signaaltransductie cascade een belangrijke rol speelt in DTF, maar helaas is er tot op heden nog geen klinisch beschikbaar middel op de markt dat hierop ingrijpt. Voor de andere genoemde cascades is het bewijs dat zij geactiveerd zijn in DTF schaars en met name gebaseerd op expressie van bepaalde genen of gebaseerd op werkzaamheid van reeds bestaande middelen die in de setting van DTF werden uitgeprobeerd. Tevens zijn er momenteel weinig medicamenteuze studies die het effect van bepaalde medicamenten in gerandomiseerde studies hebben onderzocht. Op basis van de resultaten van deze studie kan er mogelijk meer onderzoek gedaan worden naar de biologische rationale voor de werkzaamheid van reeds beschikbare medicamenten in DTF.

Hoofdstuk 3 beschrijft de rol van de Wnt/β-catenine signaaltransductie cascade. β-catenine is een belangrijk eiwit in deze signaaltransductie cascade en in dit hoofdstuk werd er onderzocht of er verschillen zijn tussen de expressie van Wnt-gerelateerde genen tussen de

verschillende *CTNNB1* (β-catenine) mutaties die voorkomen in desmoïd tumoren. Voor deze studie werd er gebruik gemaakt van mRNA expressie data van 128 desmoïd patiënten uit een openbare Affymetrix dataset. Aanvullend werd er gekeken naar de expressie van *AXIN2*, *CCND1* en *DKK1* in 64 andere DTF-patiënten met real-time polymerase chain reaction (RT-PCR). Uit deze studie blijkt dat er geen verschil is in de expressie van de Wnt-gerelateerde genen *AXIN2, CCND1* en *DKK1* tussen β-catenine mutanten. Tevens bleek uit clusteranalyse, op basis van expressieprofielen van genen die het product zijn van de Wnt/β-catenine signaaltransductie cascade, dat de verschillende mutanten niet van elkaar konden worden onderscheiden.

Hoofdstuk 4 onderzoekt DNA methylatie profielen van S45F en T41A mutaties in DTF. MeD-seq analyse werd uitgevoerd op 29 primaire DTF tumoren waarvan 14 een T41A mutatie en 15 een S45F mutatie hadden. In totaal werden 365 regio's geïdentificeerd als "anders gemethyleerde regio's" (in het Engels: differentially methylated regions (DMR's)) echter hadden slechts zes DMR's hadden een fold-change van ≥1.5. Een cluster analyse liet zien dat er geen duidelijk te onderscheiden methylatiepatronen per mutatie groep zijn. Ook in een onafhankelijke mRNA expressie dataset konden er geen duidelijke expressie verschillen gevonden worden tussen mutanten waarbij er specifiek gekeken is naar de genen die aan de DMR's gerelateerd zijn. Immunoprecipitatie liet geen associatie zien tussen wild-type β-catenine of gemuteerde β-catenin varianten en DNMT1.

Deel II – Diagnose en Behandeling

Hoofdstuk 5 beschrijft het gebruik van een nieuwe technologie; radiomics welke grote hoeveelheden beeldeigenschappen relateert aan klinische uitkomsten. In dit hoofdstuk werd radiomics ingezet om te kijken of het diagnosticeren van DTF versimpeld kon worden door het creëren van een radiomics model. Met dit model wordt er gekeken of DTF in de extremiteiten onderscheiden kan worden van andere wekedelen sarcomen in de extremiteiten op T1-gewogen magnetic resonance imaging (MRI) beelden welke vóór de start van de behandeling vervaardigd werden. Verder werd er een voorspel model gemaakt om te voorspellen welke *CTNNB1* mutatie een desmoïd tumor bezit. In totaal werden 203 patiënten geïncludeerd; 72 patiënten hadden een desmoïd tumor, 131 patiënten hadden een wekedelen sarcoom. Tumoren werden semiautomatisch geannoteerd op T1-gewogen MRI-beelden. In totaal werden hier 424 beeld karakteristieken uit onttrokken. Het T1-gewogen radiomics model had een gemiddeld gebied onder de curve (in het Engels Area Under the

Curve (AUC)) van 0.79 op de volledige dataset. Toevoeging van T2-gewogen en post-contrast beelden verbeterde de prestaties van het model niet. In het locatie gematchte-cohort, waarin alleen patiënten met extremiteit tumoren werden geïncludeerd, had het T1-gewogen model een gemiddelde AUC van 0.88 terwijl de radiologen een gemiddelde AUC van 0.80 en 0.88 hadden. Voor het voorspellen van de mutaties (S45F, T41A en WT, liet het T1-gewogen model een gemiddelde AUC van 0.61, 0.56 en 0.75 respectievelijk zien. Uit deze studie concluderen we dat het radiomics model in staat is om DTF van wekedelen sarcomen van de extremiteiten te onderscheiden met een hoge accuratesse, welke gelijk staat aan twee radiologen. Het model was niet in staat om het type *CTNNB1* mutatie te voorspellen.

In **Hoofdstuk 6** werd een systematisch literatuuronderzoek uitgevoerd om de resultaten van actieve observatie (in het Engels: active surveillance) beschrijven, die werd beschreven in verschillende retrospectieve studies, samen te vatten. In totaal werden 24 artikelen geïncludeerd welke een totaal van 3541 patiënten beschreven. 40.7% van deze patiënten (n = 1404) ondergingen actieve observatie. De meerderheid was vrouw en de meerderheid had een primaire tumor. Ongeveer 20% van de patiënten had progressieve ziekte, 58% had stabiele ziekte en 20% van de patiënten had een gedeeltelijke regressie van de tumor. Circa 30% van de patiënten moest veranderen van behandelingsvorm, dit betrof meestal chirurgische resectie of systemische behandeling. De gerapporteerde mediane follow-up tijd varieerde tussen de 8 en 73 maanden en de gerapporteerde mediane tijd tot progressie en/of initiatie van een actieve behandeling varieerde tussen 6.5 maanden tot 19.7 maanden. Selectie van patiënten die van deze actieve observatie profiteren moet de prioriteit worden bij het ontwerpen van toekomstige studies.

Hoofdstuk 7 beschrijft een meta-analyse van zeven retrospectieve studies met individuele patiënten data van 329 patiënten om de verschillen in het risico op het krijgen van een recidief na chirurgie te analyseren tussen de verschillende *CTNNB1* mutaties. Van een totale groep hadden 154 patiënten een T41A mutatie, 66 patiënten een S45F mutatie, 24 patiënten een S45P mutatie en 85 patiënten een WT DTF tumor. Van de totale groep had uiteindelijk 83 patiënten (25.2%) een recidief. Een multivariabele analyse, waarbij er gecorrigeerd werd voor geslacht, leeftijd en tumor locatie toonde een p-waarde van 0.011 voor de *CTNNB1* mutatie. Een additionele correctie voor tumor grootte toonde echter een p-waarde van 0.082 voor *CTNNB1* mutatie met hazard ratio's van 0.83 (95% betrouwbaarheidsinterval [BI] 0.48-1.42), 0.37 (95% BI 0.12-1.14) en 0.44 (95% BI 0.21-0.92) voor T41A, S45P en WT DTF tumoren in vergelijking met S45F DTF-tumoren. Uit deze studie concluderen we dat primaire, sporadische DTF-tumoren die een *CTNNB1* S45F mutatie hebben een hoger

risico hebben op recidief na operatie in vergelijking met T41A, S45P en WT, maar dat deze associatie gemedieerd wordt met door tumor grootte.

Deel III – Gezondheids-Gerelateerde Kwaliteit Van Leven

In **Hoofdstuk 8** wordt de impact van deze zeldzame ziekte op gezondheid gerelateerde kwaliteit van leven beschreven. Een systematisch literatuuronderzoek geeft een overzicht van de kwaliteit-van-leven-instrumenten die op dit moment ingezet worden om de kwaliteit van leven van patiënten met DTF te beoordelen. Daarnaast werden er focus groepen georganiseerd om te evalueren welke problemen er bij deze patiënten spelen. Dertien artikelen rapporteren kwaliteit-van-leven-instrumenten met een variëteit aan kanker-specifieke instrumenten, functie scores, symptoom schalen en lijsten die een enkele uitkomst (vb. pijn of functionele beperking) meten. Er werd geen DTF-specifiek kwaliteit-van-leven instrument gevonden. Kwalitatieve analyse van 3 focusgroepen waar 6 vrouwen en 9 mannen aan deelnamen toonden dat de ziekte een negatieve impact had op de volgende domeinen: 1) diagnose, 2) behandeling, 3) follow-up en recidief, 4) het fysieke domein, 5) het psychologische en emotionele domein 6) en het sociale domein. Deze studie impliceert dat de gezondheid gerelateerde kwaliteit van leven van DTF-patiënten negatief wordt beïnvloed in verschillende domeinen. Een DTF-specifiek meetinstrument kan het begrip van korte- en lange termijneffecten van de ziekte verbeteren en kan zowel in de kliniek als in het onderzoek gebruikt worden.

In **Hoofdstuk 9** gebruikten we de resultaten van hoofdstuk 8 en de soortgelijke studie uit het Verenigd Koningrijk van Husson et al. (2018, Support Care Cancer) om een lijst met problemen te ontwikkelen. DTF-patiënten en zorgprofessionals uit Nederland en het Verenigd Koningrijk scoorde de relevantie van elk probleem. In totaal deden 29 patiënten mee en 31 zorg professionals. De meest relevante problemen gingen over: “tumorgroei”, “het gevoel dat er iets in het lichaam zit wat daar niet thuishoort”, en “angst van tumorgroei in omliggend weefsel en organen”. Britse patiënten scoorde hoger op de meeste problemen in vergelijking met Nederlandse patiënten. Zorgprofessionals gaven hogere scores aan de meeste problemen in vergelijking met patiënten. Op basis van de meest relevante problemen werd een vragenlijst samengesteld. Deze vragenlijst, de DTF-QOL, zal in een groot internationaal cohort getest worden om de bevindingen van deze studie te bevestigen en om de specifieke kwaliteit van leven vragenlijst voor desmoïd patiënten verder te ontwikkelen.

In **Hoofdstuk 10** wordt het protocol van de QUALIFIED studie (The evaluation of health-related quality of life issues experienced by patients with desmoid-type fibromatosis), beschreven. Dit is een internationale, multicentrische, cross-sectionele, observationele cohortstudie. De DTF-specifieke vragenlijst, de DTF-QOL, die ontwikkeld is op basis van de data uit **Hoofdstuk 8** en **9**, wordt samen met bestaande vragenlijsten zoals de European Organization for Research and Treatment for Cancer Quality of Life Questionnaire (EORTC QLQ-C30) en de EuroQol 5D (EQ-5D) en vragenlijsten special samengesteld voor deze studie voorgelegd aan patiënten om socio-demografische en klinische karakteristieken te verzamen. Alle volwassen (≥18 jaar) patiënten, gediagnosticeerd tussen Januari 1990 en Oktober 2019 met een pathologisch bewezen, sporadische DTF-tumor en een recent bezoek (tussen oktober 2014 en oktober 2019) aan het ziekenhuis voor hun desmoïd zijn geschikt voor inclusie. Vragenlijsten worden ingevuld via PROFIEL (PROFILES: (Patient Reported Outcomes Following Initial treatment and Long term Evaluation of Survivorship) een systeem dat elektronisch vragenlijsten kan verzamelen. De QUALIFIED studie beoogt inzicht te krijgen in DTF-specifieke gezondheid gerelateerde kwaliteit van leven problemen door scores van DTF patiënten met scores van de populatie te bekijken en door subgroepen die risico lopen op kwaliteit van leven problemen te identificeren.

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Appendices

List of contributing authors

J. Boers
Department of Developmental Biology, Erasmus University Medical Center, Rotterdam, The Netherlands

R. Boers
Department of Developmental Biology, Erasmus University Medical Center, Rotterdam, The Netherlands

J.J. Bonenkamp
Department of Surgical Oncology, Radboud University Medical Center, Nijmegen, The Netherlands

F. Chibon
Department of Pathology, Institut Claudius Regaud, IUCT-Oncopole, Toulouse, France.

C. Colombo
Sarcoma Service, Department of Surgery, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

P. Colombo
Department of Pathology, Humanitas Clinical and Research Center, Milan, Italy

L. Dean
Sarcoma Unit, Royal Marsden NHS Foundation Trust, London, United Kingdom

A. Dunlop
Sarcoma Unit, Royal Marsden NHS Foundation Trust, London, United Kingdom

S. Gennatas
Department of Medical Oncology, Royal Marsden Hospital, London, United Kingdom

W.T. van der Graaf,
Department of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
Department of Medical Oncology, Radboud University Medical Center, Nijmegen, The Netherlands

J. Gribnau
Department of Developmental Biology, Erasmus University Medical Center, Rotterdam, The Netherlands

A. Gronchi
Sarcoma Service, Department of Surgery, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

D.J. Grünhagen
Department of Surgical Oncology, Erasmus University Medical Center, The Netherlands

D. Hanff
Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands

W. van Houdt
Department of Surgical Oncology, The Netherlands Cancer Institute, Antoni van Leeuwenhoek, Amsterdam, The Netherlands

O. Husson
Clinical Trials Unit, Royal Marsden NHS Foundation Trust, London, United Kingdom
Division of Psychosocial Research and Epidemiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
Department of Research, Netherlands Comprehensive Cancer Organisation (IKNL), Eindhoven, The Netherlands

W.F.J. van IJcken
Center for Biomics, Erasmus University Medical Center, Rotterdam, The Netherlands

M.L Janssen
Department of Surgical Oncology, Erasmus University Medical Center, Rotterdam, The Netherlands

R. L. Jones
Sarcoma Unit, Royal Marsden NHS Foundation Trust, London, United Kingdom

H.S. Kim
Department of Pathology, College of Medicine, Chung-Ang University, Seoul 06973

S. Klein
Department of Medical Informatics, Erasmus University Medical Center, Rotterdam, The Netherlands
Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands

M. Lavrijnsen
Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands

G.J.LH. van Leenders,
Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands

E. Lidington
Sarcoma Unit, Royal Marsden NHS Foundation Trust, London, United Kingdom

J.T. Mullen,
Department of Surgery, Harvard Medical School and the Massachusetts General Hospital, Boston Massachusetts, United States of America.

W.J. Niessen
Department of Medical Informatics, Erasmus University Medical Center, Rotterdam, The Netherlands
Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands
Faculty of Applied Sciences, Delft University of Technology, Delft, The Netherlands

Y. Nishida
Department of Rehabilitation and Orthopedic Surgery, Nagoya University Graduate School of Medicine, Nagoya, Aichi 466-8550, Japan

G.A. Padmos
Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands

L.V. van de Poll-Franse
Division of Psychosocial Research and Epidemiology, The Netherlands Cancer Institute, Amsterdam, the Netherlands
Department of Medical and Clinical Psychology, Tilburg University, The Netherlands
Department of Research, Netherlands Comprehensive Cancer Organisation (IKNL), Eindhoven, The Netherlands

M. Renckens
Department of Radiology and Nuclear Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

J. van Rosmalen
Department of Biostatistics, Erasmus Medical Center, Rotterdam, The Netherlands

S, Salas
Aix-Marseille Université, Hôpital de la Timone, Department of Medical Oncology and Palliative Care, Marseille, France

A.W. Schut
Department of Surgical Oncology, Erasmus MC Cancer Institute, Erasmus Medical Center Rotterdam, the Netherlands
Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus Medical Center Rotterdam, The Netherlands

S. Sleijfer
Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus Medical Center Rotterdam, The Netherlands

M. Smid
Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus Medical Center Rotterdam, The Netherlands

R. Smits
Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands

M.P.A. Starmans,
Department of Medical Informatics, Erasmus University Medical Center, Rotterdam, The Netherlands
Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands

C. Verhoef
Department of Surgical Oncology, Erasmus University Medical Center, Rotterdam, The Netherlands

J.J. Visser
Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands

A.L.M. Vriends
Department of Medical Oncology, Erasmus University Medical Center, Rotterdam, The Netherlands

E.A.C. Wiemer
Department of Medical Oncology, Erasmus University Medical Center, the Netherlands

E. Younger
Department of Surgical Oncology, The Netherlands Cancer Institute, Antoni van Leeuwenhoek, Amsterdam, The Netherlands
Sarcoma Unit, Royal Marsden NHS Foundation Trust, London, United Kingdom

About the author

Milea J.M. Timbergen was born on the 6th of September, 1990, in Rotterdam. In 2009 she graduated secondary school, Thorbecke College Rotterdam. In 2009 she moved to Maastricht to start her medical training. During this time she enjoyed student life in Maastricht, conducted research in the department of surgery (Maastricht University Medical Center) and was involved in organizing the Rosalind Franklin Contest, a national medicine competition. During her medical training she developed an interest for research. For her master thesis she moved back to Rotterdam and conducted research (Erasmus University Medical Center (MC)) on pancreatic cancer under the supervision of prof. dr. C.M.J. van Eijck. After obtaining her medical degree she started working as a surgical resident at the department Surgery of the Erasmus MC in Rotterdam. In December 2016 she seized the opportunity to start a fulltime PhD program conducting the research described in this thesis, under the supervision of prof. dr. C. Verhoef (Surgical oncology, Erasmus University MC), prof. dr. S. Sleijfer (Internal Oncology, Erasmus University MC), dr. D.J. Grünhagen, (Surgical oncology, Erasmus University MC), and dr. E.A.C. Wiemer (Internal Oncology, Erasmus University MC). From 2020 onwards, Milea combined research with clinical work at the department of Intensive Care at Franciscus Gasthuis & Vlietland in Rotterdam.

Milea met Arco van der Vlist in 2013 as he was a participant of the Rosalind Franklin Contest. They got married in October of 2019 and currently they live together in Rotterdam. Milea will start her residency training at the department of Anesthesiology of the Sint Antonius Hospital, Nieuwegein, from April 2021.

PhD Portfolio

Year		Workload ECTS
	Research Skills	
2017	Workshops Systematic Literature Retrieval and EndNote	1.0
2017	Basic Introduction Course on SPSS	1.0
2017	Open Clinica Course	1.0
2017	Basic and Translational Oncology	1.8
2017	Biomedical English Writing Course	2.0
2017	BROK 'Basiscursus Regelgeving Klinisch Onderzoek'	1.5
2018	Research Integrity	0.3
2018	Coach training	0.3
2018	NIHES Biostatistical Methods I: Basic Principles Part A	2.0
2019	Organiseer jezelf en je werk	0.6
	Attendance to Seminars and workshops	
2017	Focus group Royal Marsden, London, United Kingdom	0.5
2017	Wetenschapsdag chirurgie 2017, Rotterdam, the Netherlands	0.3
2018	Wetenschapsdag chirurgie 2018, Rotterdam, the Netherlands	0.3
2017	Annual Molmed day 2017, Rotterdam, the Netherlands	0.3
2018	Annual Molmed day 2018, Rotterdam, the Netherlands	0.3
2019	Annual Molmed day 2019, Rotterdam, the Netherlands	0.3
2017	Chirurgen dagen, 2017, NVVH, Veldhoven, the Netherlands	1.0
2017	Wondcongres 2017, Rotterdam, the Netherlands	0.5
2018	SEOHS 2018, Rotterdam, the Netherlands	0.5
	Oral Presentations	
2017	DTRF 2017, Philadelphia, United States of America	1.0
2018	DTRF 2018, Philadelphia , United States of America	1.0
2018	Chirurgen dagen, 2018, NVVH, Veldhoven, the Netherlands	1.0
2018	ESSO 2018, Budapest, Hungry (2 orals)	1.0
2018	Medical Oncology Research meeting, Rotterdam,the Netherlands	1.0
2018	Medical Oncology Science day, Rotterdam, the Netherlands	1.0
2019	SPAEN, Athens, Greece	1.0
2019	Patiënten dag Sarcomen patiëntenplatform	0.5
2019	DTRF 2019, Philadelphia , United States of America	1.0
	Poster presentations	
2018	CTOS, Rome, Italy (2 poster presentations)	1.0
2019	ESSO, Rotterdam, the Netherlands (2 poster presentations)	1.0
2019	ESMO, Barcelona, Spain	0.5
	Teaching	
2017	Supervising master thesis Medicine Erasmus University G. Padmos	2.0
2018	Supervising master thesis Medicine Erasmus University M. Renckens	2.0
2018	Coaching bachelor medicine students	2.0

List of publications:

This thesis:

Identification and Assessment of Health-Related Quality of Life Issues in Patients with Sporadic Desmoid-Type Fibromatosis: A Literature Review and Focus Group Study.
Timbergen, M.J.M., van de Poll-Franse, L.V., Grünhagen, D.J., van der Graaf, W.T., Sleijfer, S., Verhoef, C., Husson, O., Quality of Life Research. 2018 Dec;27(12):3097-3111.
doi: 10.1007/s11136-018-1931-3.

Wnt Targets Genes are not Differentially Expressed in Desmoid Tumors Bearing Different Activating β-catenin Mutations.
Timbergen, M.J.M., Janssen, M.L., Verhoef, C., Grünhagen, D.J., Chibon, F., Smid, M., Sleijfer, S., Wiemer E.A.C., European Journal of Surgical Oncology. 2019 Apr;45(4):691-698.
doi: 10.1016/j.ejso.2018.09.019.

Activated Signaling Pathways and Targeted Therapies in Desmoid-Type Fibromatosis: A Literature Review.
Timbergen, M.J.M., Smits, R., Grünhagen, D.J., Verhoef, C., Sleijfer S., Wiemer E.A.C., Frontiers in Oncology. 2019 May 17;9:397.
doi: 10.3389/fonc.2019.00397.

The Prognostic Role of β-Catenin Mutations in Desmoid-type Fibromatosis Undergoing Resection Only: A Meta-analysis of Individual Patient Data.
Timbergen, M.J.M., Colombo, C., Renckens, M., Kim, H.S., Rosmalen, J.V., Salas, S., Mullen, J.T., Colombo, P., Nishida, Y., Wiemer, E.A.C., Verhoef, C., Sleijfer, S., Gronchi, A., Grünhagen D.J., Annals of Surgery. 2019 Dec 2. Online ahead of print.
doi: 10.1097/SLA.0000000000003698

Active Surveillance in Desmoid-Type Fibromatosis: A Systematic Literature Review.
Timbergen, M.J.M., Schut, A.W., Grünhagen D.J., Sleijfer, S., Verhoef, C., European Journal of Cancer. 2020 Jul 29;137:18-29.
doi: 10.1016/j.ejca.2020.06.022.

Assessing the Desmoid-Type Fibromatosis Patients' Voice: Comparison of Health-Related Quality of Life Experiences from Patients of Two Countries.
Timbergen, M.J.M., van der Graaf, W.T.A., Grünhagen, D.J., Younger, E., Sleijfer, S., Dunlop, A., Dean L., Verhoef, C., van de Poll-Franse, L.V., Husson O. Sarcoma. E-Collection, 2020 Jul 26;2020:2141939.
doi: 10.1155/2020/2141939.

Differential Diagnosis and Mutation Stratification of Desmoid-Type Fibromatosis on MRI using Radiomics.
Timbergen, M.J.M.*, Starmans M.P.A.*, Padmos G.A., Grünhagen D.J., van Leenders G.J.LH., Hanff D.F., Verhoef C., Niessen W.J., Sleijfer S., Klein S., Visser J.J., European Journal of Radiology, 2020 Oct;131:109266.
doi: 10.1016/j.ejrad.2020.109266.
** Shared first authorship*

Differentially Methylated Regions in Desmoid-Type Fibromatosis: A Comparison Between CTNNB1 S45F and T41A Tumors
Timbergen, M.J.M., Boers, R., Vriends, A.L.M., Boers, J., van IJcken W.F.J., Lavrijsen M., Grünhagen, D.J., Verhoef C., Sleijfer S., Smits R., Gribnau J., Wiemer E.A.C., Frontiers in Oncology., 29 October 2020
doi: 10.3389/fonc.2020.565031

Other publications:

Radiomics approach to distinguish between well differentiated liposarcomas and lipomas on MRI.
Vos, M.*, Starmans, M.P.A.*, Timbergen, M.J.M., van der Voort, S.R., Padmos, G.A., Kessels, W., Niessen WJ, van Leenders GJLH, Grünhagen DJ, Sleijfer S, Verhoef C, Klein S, Visser JJ.British Journal of Surgery. 2019 Dec;106(13):1800-1809.
doi: 10.1002/bjs.11410.
** Shared first authorship*

[An uncommon tumour following breast enlargement]
Burlage, L.C., Timbergen M.J.M., Verhoef C., Corten E.M.L. Nederlands Tijdschrift voor Geneeskunde. 2018

Venous in-stent thrombosis treated by ultrasound accelerated catheter directed thrombolysis.
Strijkers, R.H., de Wolf, M.A., Arnoldussen, C.W., Timbergen, M.J.M., de Graaf, R., ten Cate-Hoek, A.J., Wittens, C.H., European Journal of Vascular Endovascular Surgery 2015 Apr;49(4):440-7.
doi: 10.1016/j.ejvs.2015.01.006.

[A woman with increasing pain after cholecystectomy].
Timbergen M.J.M., Fransen SA, Bell AV., Nederlands Tijdschrift voor Geneeskunde. 2015;159:A8720.

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