Ontario
Cancer Care Ontario
Action Cancer Ontario

Guideline 11-12 ARCHIVED

A Quality Initiative of the Program in Evidence-Based Care (PEBC), Cancer Care Ontario (CCO)

Molecular Analyses in the Diagnosis, Prognosis, and Selection of Therapy in non-GIST Soft Tissue Sarcomas

R.A. Kandel, X. Yao, B.C. Dickson, M. Ghert, S Popovic, B.M. Purgina, S. Verma, J. Werier, and the Sarcoma Disease Site Group

Report Date: April 25, 2018

Guideline 11-12 was ARCHIVED in 2023. This means that the document will no longer be maintained but may still be useful for academic or other information purposes. The PEBC has a formal and standardized process to ensure the currency of each document (PEBC Assessment & Review Protocol)

Guideline 11-12 is comprised of 5 sections. You can access the summary and full report here:
https://www.cancercareontario.ca/en/guidelines-advice/types-of-cancer/53401

Section 1:	**Recommendations Summary**
Section 2:	**Guideline**
Section 3:	**Guideline Methods Overview**
Section 4:	**Evidence Review**
Section 5:	**Internal and External Review**

For information about this document, please contact R.A. Kandel and M. Ghert through the PEBC via:
E-mail: ccopgi@mcmaster.ca Fax: 905 526-6775 Phone: 905-527-4322 ext. 42822

For information about the PEBC and the most current version of all reports, please visit the CCO website at http://www.cancercare.on.ca/ or contact the PEBC office at:
E-mail: ccopgi@mcmaster.ca Fax: 905 526-6775 Phone: 905-527-4322 ext. 42822

PEBC Report Citation (Vancouver Style): R.A. Kandel, X. Yao, B.C. Dickson, M. Ghert, S Popovic, B.M. Purgina, S. Verma, J. Werier, and the Sarcoma Disease Site Group. Molecular analysis in the diagnosis, prognosis, and selection of therapy in non-GIST soft tissue sarcomas. Toronto (ON): Cancer Care Ontario; *2018 April 25* [Archived]. Program in Evidence-Based Care Guideline No.: 11-12 ARCHIVED.

PUBLICATIONS FROM THIS REPORT

1. R.A. Kandel, X. Yao, B.C. Dickson, M. Ghert, S. Popovic, B.M. Purgina, S. Verma, J. Werier. Molecular analyses in the diagnosis and prediction of prognosis in non-GIST soft tissue sarcomas: a systematic review and meta-analysis. Cancer Treat Rev. 2018;66:74-81.
2. X. Yao, M. Ghert, B.C. Dickson, S. Popovic, B.M. Purgina, S. Verma, J. Werier, R.A. Kandel. An evidence-based guideline on the application of molecular testing in the diagnosis, prediction of prognosis, and selection of therapy in non-GIST soft tissue sarcomas. Cancer Treat Rev. Volume 85, April 2020, 101987. https://doi.org/10.1016/j.ctrv.2020.101987

Table of Contents

Molecular Analyses in the Diagnosis, Prognosis, and Selection of Therapy in non-GIST Soft Tissue Sarcomas

Section 1: Recommendations

This section is a quick reference guide and provides the guideline recommendations only. For key evidence associated with each recommendation, see Section 2.

Strength of Recommendations for This Guideline

Strength	Definition
Strong Recommendation for/against the molecular test	The guideline Working Group* believes the benefits of molecular testing in the diagnosis and prognosis of soft tissue sarcomas clearly outweigh the harms (or vice versa) for nearly all patients and the group strongly supports the recommended action.
Recommendation for/against the molecular test	The guideline Working Group* believes the benefits and harms of molecular testing in the diagnosis and prognosis of soft tissue sarcomas are closely balanced or are more uncertain but still adequate to support the recommended action.
No Recommendation for/against the molecular test	The guideline Working Group* is uncertain whether the benefits and harms of molecular testing in the diagnosis and prognosis of soft tissue sarcomas are balanced and does not recommend a specific action.
	The factors considered in the above judgments include test accuracy, desirable and undesirable effects, the quality of evidence, the guideline Working Group* members' clinical experience, and patient preference.

*The guideline Working Group includes multidisciplinary clinical experts.

GUIDELINE OBJECTIVES

1. To identify molecular tests that improve the classification of non-gastrointestinal stromal tumour (non-GIST) soft tissue sarcomas (STS)
2. Identify genetic tests that are of prognostic significance in non-GIST STS
3. Identify genetic tests that inform treatment decisions based on the type of non-GIST STS

TARGET POPULATION

For Objective 1: Adult patients (≥18 years) with suspected non-GIST STS
For Objective 2: Adult patients with non-GIST STS
For Objective 3: Adult patients with non-GIST STS

INTENDED USERS

Pathologists, general surgeons, orthopedic oncology surgeons, medical oncologists, and other clinicians who are involved in the treatment of the target patients in the province of Ontario.

RECOMMENDATIONS

Each recommendation is labelled as to whether it pertains to diagnosis, prognosis, or treatment.

Diagnosis:

Thresholds for making recommendations regarding diagnostic tests: in order to identify accurate molecular tests to assist clinicians to make a diagnostic decision, we recommended for a molecular test if it met our pre-planned threshold of both sensitivity and specificity of ≥90% with the lower limit of the 95% confidence interval (CI) of ≥50% (details in **Section 4**), and we recommended against a molecular test with both sensitivity and specificity of <80%. These thresholds were based on the clinical opinion of the guideline Working Group members. For molecular tests with diagnostic accuracy between these two pre-planned thresholds, no recommendation was made (certain exceptions are outlined in the ***Qualifying Statements of Recommendation Part II*** below).

Prognosis:

For prognostic tests, the following threshold criteria were applied: we recommended for the particular molecular alteration as a predictive factor for better prognosis if the hazard ratio (HR) was ≤1.0 with a p-value of ≤0.05 and against it as a predictive factor if the p-value was >0.05; we recommended for the particular molecular alteration as a predictive factor for worse prognosis when the HR was >1.0 with a p-value of ≤0.05 and against it as a predictive factor if the p-value was >0.05.

Treatment:

No recommendations can be made for tests to inform treatment selection because no eligible studies were found.

If a type of STS is not mentioned below, such as sclerosing epithelioid fibrosarcoma, it is because no evidence was found in the current medical literature by the search date (October 2016) that met our pre-planned study selection criteria. Full names of all molecular tests are listed in Appendix 1.

Part I — Strong Recommendations

It is strongly recommended to USE these gene tests

1. Liposarcoma

—— Diagnosis

- *MDM2* amplification by fluorescence in situ hybridization (FISH) is recommended as a sensitive and specific test to differentiate patients with atypical lipomatous tumour (ALT), well-differentiated liposarcoma (WDL), or dedifferentiated liposarcoma (DDL) from benign tumours (mainly lipoma) and other STS in the differential diagnosis.

2. Synovial sarcoma

—— Diagnosis

- *SS18 (SYT)* break-apart by FISH or *SS18-SSX (SYT-SSX)* fusion by reverse transcription-polymerase chain reaction (RT-PCR) is recommended as a sensitive and specific test to differentiate patients with synovial sarcoma from other sarcomas.

3. Desmoid tumour

—— Prognosis

- *CTNNB1* S45F mutation by PCR is recommended as a prognostic factor for decreased recurrence-free survival (RFS) in patients with desmoid tumours.

Part II — Recommendations

Evidence May Support to USE These Gene Tests

1. Desmoid tumour

—— Diagnosis

- *CTNNB1* mutation by next-generation sequencing may be a sensitive and specific test to differentiate patients with desmoid tumour from histological mimickers.

2. Epithelioid sarcoma

—— Diagnosis

- *SMARCB1* deletion by FISH may be a sensitive and specific test to differentiate patients with epithelioid sarcoma from mimickers.

3. Liposarcoma

—— Diagnosis

- *DDIT3* rearrangement by FISH may be a sensitive and specific test to differentiate patients with myxoid liposarcoma (ML) from other STS or lipoma.
- 12q13-15 amplification/rearrangement by chromosomal microarray (CMA)/FISH may be a sensitive and specific test to differentiate patients with WDL/DDL from benign tumours (mainly lipoma).

—— Prognosis

- 19q13 loss by CMA may be a prognostic factor for decreased local RFS in patients with DDL.

4. Malignant peripheral nerve sheath tumour (MPNST)

—— Diagnosis

- DNA copy number changes by CMA may be a sensitive and specific test to differentiate MPNST from cutaneous neurofibromas;

—— Prognosis

- Loss from Xq or 10q, or gain at 16p by CMA may be a prognostic factor for decreased overall survival (OS).
- *CDK4* gain or amplification by CMA/FISH may be a prognostic factor for decreased OS.

5. Angiosarcoma

—— Prognosis

- *MYC* amplification by FISH may be a prognostic factor for decreased OS, but not for decreased disease-free survival (DFS) in patients with breast radiation-induced secondary angiosarcoma (SAS).
- *CIC* alteration by FISH may be a prognostic factor for decreased DFS in patients with angiosarcoma.

Evidence May NOT Support to USE These Gene Tests

1. Endometrial stromal sarcoma

—— Diagnosis

- *JAZF1* rearrangement by FISH may not be a sensitive and specific test to differentiate patients with endometrial stromal sarcoma from endometrial stromal nodule or undifferentiated uterine sarcoma.

2. Liposarcoma

—— Prognosis

- *MDM2* amplification by FISH or real-time PCR may not be a prognostic factor for local recurrence, disease-specific survival (DSS), progression-free survival (PFS), or OS in patients with WDL/DDL or ML.
- *HMGA2* amplification by FISH may not be a prognostic factor for increased RFS in patients with ALT/WDL/DDL.

3. MPNST

—— Diagnosis

- *MDM2* amplification by FISH may not be a sensitive test to differentiate patients with MPNST from neurofibroma and schwannoma.

—— Prognosis

- *FOXM1* gain, *NOL1* gain, *SOX5* gain, or *MYC* gain may be not a prognostic factor for OS.

Qualifying Statements under Recommendation Part II

1. Angiosarcoma

—— Diagnosis

- *MYC* amplification analyzed by FISH may be a specific test to rule in patients with breast radiation-induced SAS from patients with primary angiosarcoma and/or atypical vascular lesions when the test is positive.

2. Clear cell sarcoma

—— Diagnosis

- *EWSR1* rearrangement by FISH or EWSR1 break-apart analyzed by FISH may be a specific test to rule in patients with clear cell sarcoma from malignant melanoma when the test is positive.

3. Dermatofibrosarcoma protuberans

—— Diagnosis

- *COL1A1*/*PDGFB* fusion by RT-PCR or PDGFB break-apart by FISH may be a specific test to rule in patients with dermatofibrosarcoma protuberans from dermatofibromas when the test is positive.

4. Desmoid tumour

—— Diagnosis

- *CTNNB1* mutation by direct Sanger sequencing or PCR may be a specific test to rule in patients with desmoid tumour from histological mimickers when the test is positive.

5. Hemangiopericytoma

—— Diagnosis

- *NAB2*ex6-*STAT6*ex16/17 fusion by RT-PCR may be a specific test to rule in patients with hemangiopericytoma from solitary fibrous tumour when the test is positive.

6. Liposarcoma

—— Diagnosis

- *MDM2* amplification by real-time PCR may be a sensitive and specific test to differentiate patients with ALT/WDL/DDL with benign tumours (mainly lipoma); and it may be a sensitive test to rule out patients with ALT/WDL/DDL from patients with other STS if the test is negative.
- *CDK4* amplification by FISH/real-time PCR/CMA may be a sensitive and specific test to differentiate patients with ALT/WDL/DDL from benign tumours (mainly lipoma) or other STS.

- *FUS* rearrangement by FISH may be a sensitive test to rule out patients with ML from other myxoid soft tissue tumours when the test is negative.

7. Low-grade fibromyxoid sarcoma
—— Diagnosis
- *FUS-CREB3L1* or *FUS-CREB3L2* fusion by RT-PCR may be a specific test to rule in patients with low-grade fibromyxoid sarcoma from mimickers when the test is positive.

8. Synovial sarcoma
—— Prognosis
- Compared with tumours with *SS18-SSX2*, tumours with *SS18-SSX1 by RT-PCR* may be a prognostic factor for decreased metastasis-free survival.

Part III — No Recommendations (Uncertainty)

1. Epithelioid hemangioendothelioma
—— Diagnosis
- There is no recommendation as to whether either the *CAMTA1* rearrangement test or *WWTR1* rearrangement test by FISH can differentiate patients with epithelioid hemangioendothelioma from epithelioid angiosarcoma.

2. Hybrid hemosiderotic fibrolipomatous tumour with myxoinflammatory fibroblastic sarcoma
—— Diagnosis
- There is no recommendation as to whether either the *TGFBR3* or *MGEAS* rearrangement test by FISH can differentiate patients with hybrid hemosiderotic fibrolipomatous tumour and myxoinflammatory fibroblastic sarcoma from myxoinflammatory fibroblastic sarcoma.

3. Liposarcoma
—— Diagnosis
- There is no recommendation as to whether the *HMGA2* rearrangement test determined by FISH or real-time PCR can differentiate patients with WDL/DDL from lipoma.

—— Prognosis
- There is no recommendation as to whether the *CDK4* amplification test determined by either by FISH, real-time PCR, or *CMA* is predictive for OS, RFS, DSS, and PFS in patients with ALT/WDL/DDL.
- There is no recommendation for the *PIK3CA* amplification test determined by FISH as a prognostic factor for DFS or OS in patients with primary liposarcoma.

4. Synovial sarcoma
—— Prognosis
- There is no recommendation for the *SS18-SSX* test by RT-PCR as a prognostic factor for OS or DSS in patients with synovial sarcoma.

5. Any STS types
—— Treatment Selection

- No recommendations are made for genetic tests for any STS type to inform treatment selection.

Molecular Analyses in the Diagnosis, Prognosis, and Selection of Therapy in non-GIST Soft Tissue Sarcomas

Section 2: Guideline – Recommendations and Key Evidence

GUIDELINE OBJECTIVES

1. To identify molecular tests that improve the classification of non-gastrointestinal stromal tumour (non-GIST) soft tissue sarcomas (STS)
2. Identify genetic tests that are of prognostic significance in non-GIST STS
3. Identify genetic tests that inform treatment decisions based on the type of non-GIST STS

TARGET POPULATION

For Objective 1: Adult patients (≥18 years) with suspected non-GIST STS
For Objective 2: Adult patients with non-GIST STS
For Objective 3: Adult patients with non-GIST STS

INTENDED USERS

Pathologists, general surgeons, orthopedic oncology surgeons, medical oncologists, and other clinicians who are involved in the treatment of the target patients in the province of Ontario.

RECOMMENDATIONS

Each recommendation is labelled as to whether it pertains to diagnosis, prognosis, or treatment.

Diagnosis:

Thresholds for making recommendations regarding diagnostic tests: in order to identify accurate molecular tests to assist clinicians to make a diagnostic decision, we recommended for a molecular test if it met our pre-planned threshold of both sensitivity and specificity of ≥90% with the lower limit of the 95% confidence interval (CI) of ≥50% (details in **Section 4**), and we recommended against a molecular test with both sensitivity and specificity of <80%. These thresholds were based on the clinical opinion of the guideline Working Group members. For molecular tests with diagnostic accuracy between these two pre-planned thresholds, no recommendation was made (some exceptions are outlined in the ***Qualifying Statements of Recommendation Part II*** below).

Prognosis:

For prognostic tests, the following threshold criteria were applied: we recommended for the particular molecular alteration as a predictive factor for better prognosis if the hazard ratio (HR) was ≤1.0 with a p-value of ≤0.05 and against it as a predictive factor if the p-value was >0.05; we recommended for the particular molecular alteration as a predictive factor for worse prognosis when the HR was >1.0 with a p-value of ≤0.05 and against it as a predictive factor if the p-value was >0.05.

Treatment:

No recommendations can be made for tests to inform treatment selection because no eligible studies were found.

If a type of STS is not mentioned below, such as sclerosing epithelioid fibrosarcoma, it is because no evidence was found in the current medical literature by the search date (October

2016) that met our pre-planned study selection criteria. Full names of all molecular tests are listed in Appendix 1.

Part I — Strong Recommendations

It is strongly recommended to USE these gene tests

1. Liposarcoma

—— Diagnosis

- *MDM2* amplification by fluorescence in situ hybridization (FISH) is recommended as a sensitive and specific test to differentiate patients with atypical lipomatous tumour (ALT), well-differentiated liposarcoma (WDL), or dedifferentiated liposarcoma (DDL) from benign tumours (mainly lipoma) or other STS.

2. Synovial sarcoma

—— Diagnosis

- *SS18 (SYT)* break-apart by FISH or *SS18-SSX (SYT-SSX)* fusion by reverse transcription-polymerase chain reaction (RT-PCR) is recommended as a sensitive and specific test to differentiate patients with synovial sarcoma from other sarcomas.

3. Desmoid tumour

—— Prognosis

- *CTNNB1* S45F mutation by PCR is recommended as a prognostic factor for decreased recurrence-free survival (RFS) in patients with desmoid tumours.

Key Evidence for Part I Recommendations

- **Key evidence from diagnostic studies.**

Differentiating purpose	Molecular test (positive threshold)	Sample size, (number of studies), [reference]	Diagnostic outcomes	
			Sensitivity % (95% CI)	Specificity % (95% CI)
Liposarcoma				
ALT/WDL/DDL from benign tumours (mainly lipoma)	*MDM2* amplification by FISH (A ratio of *MDM2* to CEP12 ≥3 in 3 studies, ≥2 in 9 studies; fluorescent signa¤s/cell >5 in 1 study)	971 (10) [1-10]	95 (89 to 98)	100 (89 to 100)
ALT/DDL/DDL from other STS		347 (4) [4,7,8,11]	99 (71 to 100)	90 (78 to 95)
ALT/WDL/DDL from lipoma or other STS		96 (2) [12,13]	92 (75 to 99) to 94 (81 to 99)	96 (79 to 100) to 100 (69 to 100)
Synovial sarcoma (SS)				
SS from other sarcomas	*SS18* break-apart by FISH (various[a])	258[b] (4) [14-17]	94 (89 to 97)	97 (60 to 100)
SS from other sarcomas	*SS18-SSX* fusion by RT-PCR (NR)	532[b] (4) [14,16,18,20]	93 (85 to 96)	100 (97 to 100)

Abbreviations: ALT = atypical lipomatous tumour, CEP = centromere-specific probe for chromosome, CI = confidence interval, DDL = dedifferentiated liposarcoma, FISH = fluorescence in situ hybridization, NR = not reported, RT-PCR = reverse transcription-polymerase chain reaction, STS = soft tissue sarcoma, WDL = well differentiated liposarcoma.

[a]Four studies reported FISH results with four different thresholds: (1) sample score ratio (defined as the ratio of paired signals to unpaired signals) is equal to 3.46, (2) ≥16.39% cell nuclei had orange and green signals, (3) ≥15% of cell nuclei had split orange and green signals, and (4) ≥10% of cells showed gene rearrangement.

[b]STATA 11 software (TX: StataCorp LP) did not produce an output initially and showed "initial values not feasible", which may be because there were several "0" values in the four studies. Arbitrary values were input as rarely as possible in only one study in order to generate an output. These would have underestimated the sensitivity or specificity of the test. However, both of the calculated sensitivity and specificity reached the pre-planned threshold of 90%. Therefore, we believe this arbitrary change did not impact the conclusions.

- **Key evidence from prognostic studies.**

Sarcoma type	Molecular test (positive threshold)	Sample size, (number of studies), [reference]	Prognostic outcomes
Desmoid tumour	*CTNNB1* mutation by PCR (NR)	418 (3) [21-23]	At median 41 to 60 months, *CTNNB1* S45F mutation vs. wild-type/other *CTNNB1* mutation for RFS: HR, 2.59 (95% CI, 1.19 to 5.65) to HR, 6.20 (95% CI, 2.24 to 17.15); p≤0.05

Abbreviations: CI = confidence interval, HR = hazard ratio, NR = not reported, PCR = polymerase chain reaction, RFS = recurrence-free survival, vs. = versus.

Interpretation of Evidence for Part I Recommendations

- Based on the GRADE assessment criteria, the quality of the aggregate evidence for each molecular test of every diagnostic or prognostic question was low (see the details in **Section 4. Evidence Review**) [24].
- From a diagnostic perspective:
    - The Working Group made a strong recommendation for the *MDM2* amplification test determined by FISH to be an accurate test for differentiating patients with ALT/WDL/DDL from benign tumours (mainly lipoma) or other STS because data from more than 1300 patients from 13 studies consistently supported this result with both a calculated sensitivity and specificity ≥90%. Also, the Working Group made a strong recommendation for the *SS18* break-apart test by FISH or *SS18-SSX (SYT-SSX)* fusion test by RT-PCR to differentiate synovial sarcoma from other sarcomas because data from more than 800 patients from six studies consistently supported this result with both calculated sensitivity and specificity ≥91% (a sample size of >400 is an appropriate size for a rare disease [25]).
    - A limitation of the existing evidence is that the eligible papers used different definitions for the threshold of the positive molecular tests, which made it impossible to add this information into the recommendation. However, the diagnostic accuracy outcomes were strongly consistent across all studies for each type of STS listed above supporting the confidence of the Working Group members to make these recommendations.
    - The Working Group set a high threshold of both sensitivity and specificity of ≥90% to make a recommendation, which means ≥90% of patients can be diagnosed accurately and get appropriate treatment afterwards. Although no eligible studies reported the change of management and patient outcomes after diagnosis based on molecular tests, the management of different benign and malignant tumours should be different in most cases. It is reasonable to believe that accurate classification of non-GIST STS should lead to more appropriate management and better patient outcomes (e.g., RFS). There is no potential harm to the patients from the genetic test itself. Thus, the desirable effects are greater than undesirable effects in general. However, all the studies were retrospective studies and the investigators chose the study populations in each study, so the diagnostic accuracy of the molecular tests may be overestimated, especially for specificity. The potential for false-positive and false-negative tumours should be considered. Also, patients' values, preferences, and levels of acceptance should be considered.
- From a prognostic perspective:
    - The Working Group made a strong recommendation for desmoid tumours with *CTNNB1* S45F mutation analyzed by PCR to predict a decreased RFS because data from more than 400 patients (which is an appropriate size for a rare disease) [24] from three studies consistently supported this result at a median 41 to 60 months follow-up time.

- The Working Group believed that the results from the prognostic studies provided patients with useful information to understand and predict the possible course of their diseases, and further helped them make some necessary decisions with their family members and healthcare providers. For example, patients with the *CTNNB1* S45F mutation may need to be considered more optimal treatment strategies to prevent recurrence. The potential undesirable effects may include the mental burden associated with receiving the information that they have a poor prognosis and the potential overtreatment effects on the patient's health if the predicted prognosis from the molecular test is inaccurate. However, the estimate of effect in prognosis was relatively large and precise between patients with and without the molecular marker and the Working Group expected few patients would receive an inaccurate prognosis.
- Different patients may value and accept these outcomes differently, thus, patient preference should be considered.

- When making these recommendations, the Working Group predicted that these recommendations could be generalizable to the entire target population in Ontario.

Part II — Recommendations

Evidence May Support to USE These Gene Tests

1. Desmoid tumour

—— Diagnosis

- *CTNNB1* mutation by next-generation sequencing may be a sensitive and specific test to differentiate patients with desmoid tumour from histological mimickers.

2. Epithelioid sarcoma

—— Diagnosis

- *SMARCB1* deletion by FISH may be a sensitive and specific test to differentiate patients with epithelioid sarcoma from mimickers.

3. Liposarcoma

—— Diagnosis

- *DDIT3* rearrangement by FISH may be a sensitive and specific test to differentiate patients with myxoid liposarcoma (ML) from other STS or lipoma.
- 12q13-15 amplification/rearrangement by chromosomal microarray (CMA)/FISH may be a sensitive and specific test to differentiate patients with WDL/DDL from benign tumours (mainly lipoma).

—— Prognosis

- 19q13 loss by CMA may be a prognostic factor for decreased local RFS in patients with DDL.

4. Malignant peripheral nerve sheath tumour (MPNST)

—— Diagnosis

- DNA copy number changes by CMA may be a sensitive and specific test to differentiate MPNST from cutaneous neurofibromas;

—— Prognosis

- Loss from Xq or 10q, or gain at 16p by CMA may be a prognostic factor for decreased overall survival (OS).
- *CDK4* gain or amplification by CMA/FISH may be a prognostic factor for decreased OS.

5. Angiosarcoma
—— **Prognosis**
- *MYC* amplification by FISH may be a prognostic factor for decreased OS, but not for decreased disease-free survival (DFS) in patients with breast radiation-induced secondary angiosarcoma (SAS).
- *CIC* alteration by FISH may be a prognostic factor for decreased DFS in patients with angiosarcoma.

Evidence May NOT Support to USE These Gene Tests
1. Endometrial stromal sarcoma
—— Diagnosis
- *JAZF1* rearrangement by FISH may not be a sensitive and specific test to differentiate patients with endometrial stromal sarcoma from endometrial stromal nodule or undifferentiated uterine sarcoma.

2. Liposarcoma
—— Prognosis
- *MDM2* amplification by FISH or real-time PCR may not be a prognostic factor for local recurrence, disease-specific survival (DSS), progression-free survival (PFS), or OS in patients with WDL/DDL or ML.
- *HMGA2* amplification by FISH may not be a prognostic factor for increased RFS in patients with ALT/WDL/DDL.

3. MPNST
—— Diagnosis
- *MDM2* amplification by FISH may not be a sensitive test to differentiate patients with MPNST from neurofibroma and schwannoma.

—— Prognosis
- *FOXM1* gain, *NOL1* gain, *SOX5* gain, or *MYC* gain may be not a prognostic factor for OS.

Qualifying Statements under Recommendation Part II

1. Angiosarcoma
—— Diagnosis
- *MYC* amplification analyzed by FISH may be a specific test to rule in patients with breast radiation-induced SAS from patients with primary angiosarcoma and/or atypical vascular lesions when the test is positive.

2. Clear cell sarcoma
—— Diagnosis
- *EWSR1* rearrangement by FISH or EWSR1 break-apart analyzed by FISH may be a specific test to rule in patients with clear cell sarcoma from malignant melanoma when the test is positive.

3. Dermatofibrosarcoma protuberans
—— Diagnosis

- *COL1A1/PDGFB* fusion by RT-PCR or PDGFB break-apart by FISH may be a specific test to rule in patients with dermatofibrosarcoma protuberans from dermatofibromas when the test is positive.

4. Desmoid tumour

—— Diagnosis

- *CTNNB1* mutation by direct Sanger sequencing or PCR may be a specific test to rule in patients with desmoid tumour from histological mimickers when the test is positive.

5. Hemangiopericytoma

—— Diagnosis

- *NAB2*ex6-*STAT6*ex16/17 fusion by RT-PCR may be a specific test to rule in patients with hemangiopericytoma from solitary fibrous tumour when the test is positive.

6. Liposarcoma

—— Diagnosis

- *MDM2* amplification by real-time PCR may be a sensitive and specific test to differentiate patients with ALT/WDL/DDL with benign tumours (mainly lipoma); and it may be a sensitive test to rule out patients with ALT/WDL/DDL from patients with other STS if the test is negative.
- *CDK4* amplification by FISH/real-time PCR/CMA may be a sensitive and specific test to differentiate patients with ALT/WDL/DDL from benign tumours (mainly lipoma) or other STS.
- *FUS* rearrangement by FISH may be a sensitive test to rule out patients with ML from other myxoid soft tissue tumours when the test is negative.

7. Low-grade fibromyxoid sarcoma

—— Diagnosis

- *FUS-CREB3L1* or *FUS-CREB3L2* fusion by RT-PCR may be a specific test to rule in patients with low-grade fibromyxoid sarcoma from mimickers when the test is positive.

8. Synovial sarcoma

—— Prognosis

- Compared with tumours with *SS18-SSX2*, tumours with *SS18-SSX1 by RT-PCR* may be a prognostic factor for decreased metastasis-free survival.

Key Evidence for Part II Recommendations

- **Key evidence from diagnostic studies.**

Differentiating purpose	Molecular test (positive threshold)	Sample size, (number of studies), [reference]	Diagnostic outcomes	
			Sensitivity % (95% CI)	Specificity % (95% CI)
Desmoid tumour (DT)				
DT from mimics	*CTNNB1* mutation by NGS (≥5% variant allele fraction in a region with ≥500 reads/base)	159 (1) [26]	92 (87 to 96)	100 (78 to 100)
Endometrial stromal sarcoma				
Endometrial stromal sarcoma from endometrial stromal	*JAZF1* rearrangement by	36 (1) [27]	63 (38 to 84)	77 (50 to 93)

nodule and undifferentiated uterine sarcoma	FISH (≥1 copy arranged)			
Epithelioid sarcoma				
Epithelioid sarcoma from its mimickers	*SMARCB1* deletion by FISH (hemi or homozygous loss of FISH signals for SMARCB1)	81 (1) [28]	90 (76 to 97)	93 (80 to 99)
Liposarcoma				
ML from other STS and lipoma	*DDIT3* rearrangement by FISH (>10% rearrangement; ≥20% rearrangement)	189[a] (4) [29-32]	96 (69 to 100)	100 (80 to 100)
WDL/DDL from benign tumours (mainly lipoma)	12q13-15 amplification by CMA (DNA copy number gains with $\log_2$ ratio >1) / rearrangement by FISH (NR)	81 (2) [33,34]	100 (90 to 100)	100 (63 to 100
MPNST				
MPNST from cutaneous neurofibroma	DNA copy number changes by CMA (≥2)	58 (1) [35]	92 (80 to 98)	90 (56 to 100)
MPNST from neurofibroma and schwannoma	*MDM2* amplification by FISH (A ratio of *MDM2* to CEP12 >2)	44 (1) [36]	20 (4 to 48)	100 (88 to 1▫0)

Abbreviations: CMA = chromosomal microarray, CEP = centromere-specific probe for chromosome, CI = confidence interval, DDL = dedifferentiated liposarcoma, FISH = fluorescence in situ hybridization, ML = myxoid liposarcoma, MPNST = Malignant peripheral nerve sheath tumour, NR = not reported, NGS = next-generation sequencing, STS = soft tissue sarcoma, WDL = well-differentiated liposarcoma.

[a]STATA 11 software (TX: StataCorp LP) did not produce an output initially and showed "initial values not feasible", which may be because there were several "0" values in the four studies. Arbitrary values were input as rarely as possible in only one study in order to generate an output. These would have underestimated the sensitivity or specificity of the test. However, both of the calculated sensitivity and specificity reached the pre-planned threshold of 90%. Therefore, we believe this arbitrary change did not impact the conclusions.

- Key evidence from prognostic studies.

Sarcoma type	Molecular test (positive threshold)	Sample size, (number of studies), [reference]	Prognostic Outcomes
Angiosarcoma			
SAS	*MYC* amplification by FISH (*MYC*/CEP8≥2.0)	37 (1) [37]	At median 25 months, OS: HR, 3.47 (95% CI, 1.09 to 11.1) DFS: HR, 1.89 (95% CI, 0.78 to 4.55)
PAS/SAS	*CIC* alteration by FISH (≥20% of nuclei had a break-apart signal)	82 (1) [38]	At median 27 months, DFS: HR, 3.46; 95% CI, 1.42 to 8.44; p=0.006
Liposarcoma			
DDL	*MDM2* amplification by FISH (high level vs. low level; high	50 (1) [39]	At median 28 months, LR: HR, 1.92; 95% CI, 0.81 to 4.58; p=0.13

	level: ≥20) fluorescent signals/cell, low level: <20 but >5)		
WDL/DDL	*MDM2* amplification by real-time PCR (high level vs. low level; high level: copy number of *MDM2* to ALB ≥10, low level <10 but >2.2)	56 (1) [40]	At median 43 months, DSS: HR, 1.35; 95% CI, 0.144 to 12.5; p=0.794 PFS: HR, 1.22; 95% CI, 0.45 to 2.39;▫p=0.655
ML/round cell liposarcoma	*MDM2* amplification by PCR (>2-fold amplification)	120 (1) [41]	At 5 years, OS: p=0.179
ALT/WDL/ DDL	*HMGA2* amplification by FISH (≥10 fluorescent signals/cell in ≥1% of cells)	91 (1) [42]	At 2 years, RFS: p=NS
DDL	19q13 loss by CMA (NR)	40 (1) [43]	At median 28 months, Local RFS: HR, 2.99; 95% CI, 1.0 to 6.3; p=0.01
MPNST			
MPNST	DNA copy number changes by CMA (≥2 aberrations)	48 (1) [35]	At 10 years, OS: Loss from Xq or 10q or gain at 16p: HR, 11.0; 95% CI, 3.5 to 35; p<0.001
	CDK4 gain, *FOXM1* gain, *NOL1* gain, *SOX5* gain, *MYC* gain by *CMA* (NR)	38 (1) [44]	OS (follow-up: NR): CDK4 gain: HR, 4.22; 95% CI, 1.43 to 12.44; p=0.009 FOXM1 gain: p=not significant (NS) NOL1 gain: p=NS SOX5 gain: p=NS MYC gain: p=NS
	CDK4 amplification by FISH (NR)	87 (1) [44]	OS (follow-up: NR): HR, 2.04; 95% CI, 1.03 to 4.04; p=0.041

Abbreviations: ALT = atypical lipomatous tumour, CEP = centromere-specific probe for chromosome, CMA = chromosomal microarray, CI = confidence interval, DDL = dedifferentiated liposarcoma, DFS = disease-free survival, DNA = deoxyribonucleic acid, DSS = disease-specific survival, FISH = fluorescence in situ hybridization, HR = hazard ratio, LR = local recurrence, LRFS = local recurrence-free survival, MFS = metastasis-free survival, ML = myxoid liposarcoma, NR = not reported, NS= not significant, OS = overall survival, PAS = primary angiosarcoma, PCR = polymerase chain reaction, PFS = progression-free survival, RCL = round cell liposarcoma, RFS = recurrence-free survival, RR = relative risk, SAS = secondary angiosarcoma, vs. = versus, WDL = well differentiated liposarcoma.

Key Evidence for Qualifying Statements under Part II Recommendations

- **Key evidence from diagnostic studies.**

Differentiating purpose	Molecular test (positive threshold)	Sample size, (number of studies), [reference]	Diagnostic outcomes	
			Sensitivity % (95% CI)	Specificity % (95% CI)
Angiosarcoma				
SAS from PAS and/or AVL	*MYC* amplification FISH (clustered signals >9 in one study, clustered signals >8 in one study, *MYC*/CEP8 ratio of ≥2.0 in four studies)	329[a] (6) [37,45-49]	54 (37 to 71) to 100 (89 to▫100)	93 (68 to 100) to 100 (89 to 100)
Clear cell Sarcoma (CCS)				
CCS from malignant melanoma (MM)	*EWSR1* rearrangement by FISH (>10% tumour nuclei had *EWSR1* rearrangement)	42 (1) [50]	70 (35 to 93)	100 (89 to 100)

CCS from MM	*EWSR1* break-apart by FISH (a split red/green signal with a minimum diameter of one signal width)	45 (1) [51]	79 (49 to 95)	100 (89 to 100)
CCS from MM	*EWSR1/ATF1* fusion by RT-PCR (NR)	34 (1) [51]	70 (35 to 93)	100 (86 to 100)
Dermatofibrosarcoma protuberans (DFSP)				
DFSP from dermatofibroma (DF)	*PDGFB* break-apart by FISH (a split red/green signal with a minimum diameter of one signal width	151 (2) [52,53]	86 (68 to 96) to 91 (83 to 96)	100 (69 to 100) to 100 (79 to 100)
DFSP from DF	*COL1A1/PDGFB* fusion by RT-PCR (NR)	92 (1) [52]	72 (61 to 81)	100 (69 to 100)
Desmoid tumour (DT)				
DT from mimics	*CTNNB1* mutation by PCR (NR)	685 (3) [19,26,54]	82 (74 to 89) to 88 (83 to 92)	100 (78 to 100) to 100 (98 to 100)
DT from mimics	*CTNNB1* mutation by direct Sanger sequencing (NR)	95 (1) [55]	85 (75 to 92)	100 (72 to 100)
Hemangiopericytoma (HPC)				
HPC from solitary fibrous tumour	*NAB2-STAT6* fusion by RT-PCR (*NAB2*ex6-*STAT6*ex16/17)	52 (1) [56]	80 (44 to 98)	93 (81 to 99)
Liposarcoma				
ALT/WDL/DDL from benign tumours (mainly lipoma)	*MDM2* amplification by real-time PCR (copy number of *MDM2* to ALB ≥1.9, >6 gene copies with >3-fold, Peak value >2)	346 (4) [1,6,57,58]	88 (70 to 96)	98 (91 to 99)
ALT/WDL/DDL from other STS		187 (1) [57]	98 (93 to 100)	76 (66 to 84)
ALT/WDL/DDL from benign tumours (mainly lipoma)	*CDK4* amplification by FISH (a ratio of *CDK4* to CEP12 >2, Fluorescent signals/cell >5) or by real-time PCR (Peak value >2, a ratio of CDK4 to ALB ≥1.2 or by CMA (green-to-red fluorescence >1.4)	282 (3) [1,6,57]	76 (60 to 88) to 98 (93 to 100)	90 (78 to 97) to 100 (92 to 100)
ALT/WDL/DDL from other STS	*CDK4* amplification by real-time PCR (a ratio of *CDK4* to ALB ≥1.2) or by CMA (green-to-red fluorescence >1.4) or by FISH (a ratio of CDK4 to CEP12 >3)	235 (2) [11,57]	98 (93 to 100) to 100 (75 to 100)	75 (65 to 83) to 100 (90 to 100)
ML from other myxoid STT	*FUS* rearrangement by FISH (>10% rearrangement)	59 (1) [29]	94 (73 to 100)	83 (68 to 93)
Low-grade fibromyxoid sarcoma (LGFM)				
LGFM from mimickers	*FUS-CREB3L1* or *FUS-CREB3L2* fusion by RT-PCR (NR)	250 (2) [59,60]	81 (69 to 90) to 88 (62 to 86)	87 (74 to 94) to 100 (97 to 100)

Abbreviations: ALT = atypical lipomatous tumour, AVL = atypical vascular lesion, CEP = centromere-specific probe for chromosome, CMA = chromosomal microarray, CI = confidence interval, DDL = dedifferentiated liposarcoma, FISH = fluorescence in situ hybridization, ML = myxoid liposarcoma, NR = not reported, PAS = primary angiosarcoma, RT-PCR = reverse transcription-polymerase chain reaction, SAS = secondary angiosarcoma, STS = soft tissue sarcoma, STT = soft tissue tumour, WDL = well differentiated liposarcoma.
[a]One-half of patients overlapped in the Kacker et al. 2013 study [46] and the Manner et al. 2010 study [48].

- Key evidence from prognostic studies.

Sarcoma type	Molecular test (positive threshold)	Sample size, (number of studies), [reference]	Prognostic Outcomes
Synovial sarcoma			

Synovial sarcoma (SS18-SSX1 vs. SS18-SSX2)	*SS18-SSX* fusion by RT-PCR (NR)	340 (3) [61-63]	At 54 to 72 months, Metastasis-free survival: acceleration rate, -1.15; 95% CI, -2.12 to -0.19; p=0.019 in one study (n=132). RR, 1.86; 95% CI, 1.04 to 3.33; p=0.037 in one study (n=141). RR, 0.67; 95% CI, 0.25 to 1.60; p=0.38 in another study (n=67).

Abbreviations: NR = not reported, RR = relative risk, RT-PCR = reverse transcription-polymerase chain reaction, vs. = versus.

Interpretation of Evidence for Part II Recommendations and Qualifying Statements

- The overall aggregate quality of each molecular test for any outcome was low to very low (see the details in **Section 4. Evidence Review)**.
- From a diagnostic perspective:
  - According to GRADE criteria, low quality means that "our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect;" very low quality means that "We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect." [24]. Additionally, the sample size is <400 for each outcome. Thus, we are not confident enough to make strong recommendations for these molecular tests although they met our pre-planned threshold (both a sensitivity and specificity of ≥90%). For each recommended test, the Working Group members believed the desirable effects were moderate to large and undesirable effects might be small, (details described under ***Interpretation of Evidence for Part I — Strong Recommendation***). Thus, the benefits were greater than the harms. However, we still need to consider patients who have the potential of false-positive and false-negative tumours.
  - For the tests that were recommended not to be used, both the sensitivity and specificity were low (<80%). Given this evidence, the Working Group members believed it was reasonable to make these recommendations.
  - For the tests under Qualifying Statement, either the sensitivities or specificities were ≥90% with the lower limit of 95% CI ≥50%. Based on their clinical opinion, the Working Group members believed these tests were useful in clinical practice. When a test has a high specificity, a positive result "rules in" the diagnosis of the targeted type of non-GIST STS; when a test has a high sensitivity, a negative result "rules out" the diagnosis [64].
  - *CPM* amplification determined by FISH had both a sensitivity and specificity of 100% in one study. However, the Working Group members and one internal reviewer believed that as there was only one study of *CPM* amplification in the diagnosis of AWL/DDL liposarcoma and there were many studies demonstrating high sensitivity and specificity of *MDM2* amplification on the same chromosome, there was limited clinical rationale for testing of *CPM* amplification in Ontario. Thus, it was not recommended in this guideline.
  - A limitation of the existing evidence is that the eligible papers used different thresholds for the positivity of the molecular tests, making it impractical to add this information into the recommendation for so many different molecular tests in different types of STS. However, this information was listed in the Key Evidence table above for the readers' information.
- From a prognostic perspective:
  - The desirable effects of one test can be different in similar patient populations. The consideration of the undesirable effects by the Working Group is similar to that in ***Interpretation of Evidence for Recommendation Part I***. However, for certain tests,

the Working Group members could not decide significance after considering the potential harm and benefit, for example "*MDM2* amplification analyzed by FISH may not be a prognostic factor for local recurrence at a median 28-month follow-up in patients with DDL".

- The Working Group was also uncertain about the acceptable level of risk by patients or even clinicians. Furthermore, the Working Group was unclear whether the evidence was generalizable to the entire target population. Therefore, the recommendations for those tests were worded as "may". Patient preference should be strongly considered when determining if these tests should be used to predict prognosis.
- From the above evidence, two studies (n=273) showed that synovial sarcoma with *SS18-SSX1* was a prognostic factor for decreased metastasis-free survival, but one study did not support this outcome (n=67). Based on one external targeted reviewer's comment in Section 5 and given the inconsistent evidence for DSS and OS outcomes in ***Part III — No Recommendations (Uncertainty)*** below, the Working Group decided to move this Recommendation Statement to Qualifying Statement section.

Part III — No Recommendations (Uncertainty)

1. Epithelioid hemangioendothelioma

—— Diagnosis

- There is no recommendation as to whether either the *CAMTA1* rearrangement test or *WWTR1* rearrangement test by FISH can differentiate patients with epithelioid hemangioendothelioma from epithelioid angiosarcoma.

2. Hybrid hemosiderotic fibrolipomatous tumour with myxoinflammatory fibroblastic sarcoma

—— Diagnosis

- There is no recommendation as to whether either the *TGFBR3* or *MGEAS* rearrangement test by FISH can differentiate patients with hybrid hemosiderotic fibrolipomatous tumour and myxoinflammatory fibroblastic sarcoma from myxoinflammatory fibroblastic sarcoma.

3. Liposarcoma

—— Diagnosis

- There is no recommendation as to whether the *HMGA2* rearrangement test determined by FISH or real-time PCR can differentiate patients with WDL/DDL from lipoma.

—— Prognosis

- There is no recommendation as to whether the *CDK4* amplification test determined by either by FISH, real-time PCR, or *CMA* is predictive for OS, RFS, DSS, and PFS in patients with ALT/WDL/DDL.
- There is no recommendation for the *PIK3CA* amplification test determined by FISH as a prognostic factor for DFS or OS in patients with primary liposarcoma.

4. Synovial sarcoma

—— Prognosis

- There is no recommendation for the *SS18-SSX* test by RT-PCR as a prognostic factor for OS or DSS in patients with synovial sarcoma.

5. Any STS types

—— Treatment Selection

- No recommendations are made for genetic tests for any STS type to inform treatment selection.

Key Evidence for Part III Recommendations

- **Key evidence from diagnostic studies.**

Purpose	Molecular test (positive threshold)	Sample size, (number of studies), [reference]	Diagnostic outcomes	
			Sensitivity % (95% CI)	Specificity % (95% CI)
Epithelioid hemangioendothelioma (EHE)				
EHE from epithelioid angiosarcoma	*WWTR1* rearrangement by FISH (≥20% of nuclei showed a break-apart signal)	35 (1) [65]	87 (69 to 96)	100 (48 to 100)
	CAMTA1 rearrangement by FISH (≥20% of nuclei showed a break-apart signal)		87 (69 to 96)	80 (28 to 100)
Hybrid hemosiderotic fibrolipomatous tumour (HFLT)- myxoinflammatory fibroblastic sarcoma (MIFS)				
Hybrid HFLT-MIFS from MIFS	*TGFBR3* rearrangement by FISH (≥15% nuclei showed disruption of ≥1 fusion signal)	39 (1) [66]	75 (35 to 97)	100 (89 to 100)
	MGEA5 rearrangement by FISH (≥15% nuclei showed disruption of ≥1 fusion signal)		50 (16 to 84)	94 (79 to 99)
Liposarcoma				
WDL/DDL from lipoma	*HMGA2* rearrangement by FISH (NR)	50 (1) [67]	100 (75 to 100)	57 (40 to 73)
WDL/DDL from lipoma	*HMGA2* rearrangement by real-time PCR (a log10 value of >1 for exons 1-2, 3-4, or 4-5)	54 (1) [67]	100 (75 to 100)	17 (7 to 32)

Abbreviations: CI = confidence interval, DDL = dedifferentiated liposarcoma, FISH = fluorescence in situ hybridization, PCR = polymerase chain reaction, WDL = well differentiated liposarcoma.

- **Key evidence from prognostic studies.**

Sarcoma type	Molecular test (positive threshold)	Sample size, (number of studies), [reference]	Prognostic outcomes
Liposacoma			
Primary liposarcoma	*PIK3CA* amplification by FISH (>4 copies/cell or a ratio of PIK3CA/CEN3 ≥2)	101 (1) [68]	At median 51 months, DFS: HR, 2.40; 95% CI, 1.11 to 5.21; p=0.027 OS: HR, 1.63; 95% CI, 0.56 to 4.77; p=0.37
ALT/WDL/DDL	*CDK4* amplification by real-time PCR (high level vs. low level; high level: copy number of MDM2 to ALB ≥10, low level <10 but >2.2)	56 (1)[a] [40]	At median 43 months, DSS: HR, 2.19; 95% CI, 1.42 to 23.5; p=0.044 PFS: HR, 3.08; 95% CI, 1.03 to 10.81; p=0.048

	CDK4 amplification by real-time PCR (high level vs. low level; high level: copy number of *MDM2* to ALB ≥7.54, low level: <7.54 but >2)	48 (1)[a] [69]	At about 2 years, RFS: HR, 12.08; 95% CI, 1.48 to 98.83; p=0.20
	CDK4 amplification by FISH (≥10 fluorescent signals/cell in ≥1% of cells)	90 (1) [42]	At 2 years, OS: p=NS RFS: p=NS
Synovial sarcoma			
Synovial sarcoma (SS18-SSX1 vs. SS18-SSX2)	*SS18-SSX* fusion by RT-PCR (NR)	169 (2) [63,70]	At 43 to 54 months, OS: RR, 1.46; 95% CI, 0.64 to 3.28; p=0.36 in one study. RR, 2.34; 95% CI, 2.18 to 2.67; p=0.002 in another study.
		273 (2) [61,62]	At 54 to 72 months, DSS: AR, -0.22; 95% CI, -1.87 to 1.43; p=0.794 in one study. RR, 2.03, 95% CI, 1.26 to 3.28; p=0.004 in another study.

Abbreviations: ALT = atypical lipomatous tumour, AR = acceleration rate, CEN3 = centromere3, CI = confidence interval, DDL = dedifferentiated liposarcoma, DFS = disease-free survival, DSS = disease-specific survival, FISH = fluorescence in situ hybridization, HR = hazard ratio, NR = not reported, OS = overall survival, PFS = progression-free survival, RFS = recurrence-free survival, RR = relative risk, RT-PCR = reverse transcription-polymerase chain reaction, vs. = versus, WDL = well differentiated liposarcoma.
[a]Some patients in these two studies overlapped.

- **There is no evidence for Treatment Selection.**

Interpretation of Evidence for Part III Recommendations

- For diagnostic studies, there are no recommendations for the above tests for the corresponding types of STS based on the pre-planned criteria and literature available for review at the time.
- For prognostic studies, according to the pre-planned criteria on Page 3, in general, there should not be "No Recommendation" for a specific test as a prognostic indicator. However, there are several reasons for these tests. For example in the study evaluating, *PIK3CA* amplification determined by FISH, the investigators combined patients who had different liposarcoma subtypes, ALT/WDL, DDL, ML, and pleomorphic liposarcoma (PL), which are known to have different outcomes. No subtype analyses were conducted. Thus, no recommendation could be made regarding this molecular alteration. For some molecular alterations, if two studies with similar sample sizes have inconsistent results for similar outcomes, we expect to have extra data or studies using different technology to predict prognosis from the literature in the future.

IMPLEMENTATION CONSIDERATIONS

This comprehensive guideline may serve as a framework for the thoughtful implementation of molecular studies at cancer centres in Ontario and other jurisdictions. The Working Group members consider these recommendations to be feasible to implement and will not affect current health inequities. It is anticipated that some patients may view some recommended actions as unacceptable because the current evidence is insufficient to make strong recommendations for all different types of STS. The principal barrier would be cost but

one might argue that the avoidance of under-treatment and more importantly, over-treatment or over-investigation of patients should logically offset the cost of implementation.

Furthermore, the centralization of sarcoma services, including expert pathology assessment and review at three centres in Ontario, should permit the analyses of molecular markers in larger volumes (reducing 'per-test' costs), regular audits, and quality control and should contribute to further research in this critical area. Molecular and cytogenetic testing may be performed at ancillary testing centres in some cases to preserve the integrity of tissue depending on limitations of tissue quantity, quality, and time of collection. Testing sites are required to take part in ongoing external quality assurance surveys for any test offered. The cost-effectiveness is beyond the scope of the PEBC; the Working Group leaves resource considerations to other decision makers.

Please note that our knowledge of genetic alterations is growing rapidly and the technology used to identify these changes is improving. Thus, it is likely with time that there will be new evidence that may change some of the recommendations for diagnosis and predicting prognosis in this guideline. Additionally, it is anticipated that high-quality molecular-analysis studies will become available to guide treatment selection for different types of STS. All PEBC documents are maintained and updated through an annual assessment and subsequent review process (see the details in **Section 3: Guideline Methods Overview**).

Molecular Analyses in the Diagnosis, Prognosis, and Selection of Therapy in non-GIST Soft Tissue Sarcomas

Section 3: Guideline Methods Overview

This section summarizes the methods used to create the guideline. For the systematic review, see Section 4.

THE PROGRAM IN EVIDENCE-BASED CARE

The PEBC is an initiative of the Ontario provincial cancer system, CCO. The PEBC mandate is to improve the lives of Ontarians affected by cancer through the development, dissemination, and evaluation of evidence-based products designed to facilitate clinical, planning, and policy decisions about cancer control.

The PEBC supports the work of Disease Site Groups (DSGs) in the development of various PEBC products. The DSGs are composed of clinicians, other healthcare providers and decision makers, methodologists, and community representatives from across the province.

The PEBC is a provincial initiative of CCO supported by the Ontario Ministry of Health and Long-Term Care (OMHLTC). All work produced by the PEBC is editorially independent from the OMHLTC.

BACKGROUND FOR GUIDELINE

Diagnostic accuracy and prognostication is the backbone of cancer pathology and has a direct influence on the therapy that an individual patient might receive. Because of sarcoma's multiplicity of histology and challenging range of benign, borderline, and malignant tumours, molecular diagnostics may play a crucial role in sarcoma. The accurate differentiation of malignant tumours from benign tumours has a clear impact on all aspects of clinical care and is sometimes only discernible through molecular testing. Likewise, molecular markers that help define prognosis may influence whether a patient might receive adjuvant radiation or chemotherapy that differs from what might ordinarily be recommended, and may also lead to histology-specific therapies in a field. This guideline may serve as a framework for the thoughtful implementation of molecular studies at cancer centres in Ontario and other jurisdictions.

GUIDELINE DEVELOPERS

This guideline was developed by the Sarcoma DSG (see Appendix 2), led by a small Working Group, which was responsible for reviewing the evidence base, drafting the guideline recommendations, and responding to comments received during the document review process. The Working Group had expertise in pathology, surgical oncology, medical oncology, and health research methodology. Other members of the Sarcoma DSG served as the Expert Panel and were responsible for the review and approval of the draft document produced by the Working Group. Dr. Smith and Dr. Crocker were invited and agreed to join the Expert Panel due to their expertise. Conflict of interest declarations for all Expert Panel members are summarized in Appendix 2, and were managed in accordance with the *PEBC Conflict of Interest Policy*.

GUIDELINE DEVELOPMENT METHODS

The PEBC produces evidence-based and evidence-informed guidance documents using the methods of the Practice Guidelines Development Cycle [71, 72]. This process includes a systematic review, interpretation of the evidence by the Working Group and draft

recommendations, internal review by content and methodology experts and external review by Ontario clinicians and other stakeholders.

The PEBC uses the AGREE II framework [73] as a methodological strategy for guideline development. AGREE II is a 23-item validated tool that is designed to assess the methodological rigour and transparency of guideline development.

The up-to-date nature of each document is ensured through periodic review and evaluation of the scientific literature and, where appropriate, the addition of newer literature to the original evidence-base. This is described in the *PEBC Document Assessment and Review Protocol*. PEBC guideline recommendations are based on clinical evidence, and not on feasibility of implementation; however, a list of implementation considerations such as costs, human resources, and unique requirements for special or disadvantaged populations is provided along with the recommendations for information purposes. PEBC guideline development methods are described in more detail in the *PEBC Handbook* and the *PEBC Methods Handbook*.

Search for Existing Guidelines

As a first step in developing this guideline, a search for existing guidelines was undertaken to determine whether an existing guideline could be adapted or endorsed. To this end, the following sources were searched from 2013 to October 13 2016 for existing guidelines that addressed the research questions:

- Practice guideline databases: the Agency for Healthcare Research and Quality (AHRQ) National Guideline Clearinghouse, and the Canadian Medical Association Infobase.
- Guideline developer websites: National Institute for Health and Care Excellence (NICE), Scottish Intercollegiate Guidelines Network (SIGN), American Society of Clinical Oncology (ASCO), and National Health and Medical Research Council - Australia.

All the guidelines that were developed based on a systematic review would meet our guideline selection criterion. A search for existing guidelines for adaptation or endorsement did not yield an appropriate source document. Thus, a search of the primary literature was required (see **Section 4. Systematic Review**).

GUIDELINE REVIEW AND APPROVAL

Internal Review

For the guideline document to be approved, 75% of the content experts who comprise the Expert Panel must cast a vote indicating whether or not they approve the document, or abstain from voting for a specified reason, and of those that vote, 75% must approve the document. In addition, the PEBC Report Approval Panel (RAP), a three-person panel with methodology expertise, must unanimously approve the document. The Expert Panel and RAP members may specify that approval is conditional, and that changes to the document are required. If substantial changes are subsequently made to the recommendations during external review, then the revised draft must be resubmitted for approval by RAP and the Expert Panel.

External Review

Feedback on the approved draft guideline is obtained from content experts and the target users through two processes. Through the Targeted Peer Review, several individuals with content expertise are identified by the Working Group and asked to review and provide feedback on the guideline document. Through Professional Consultation, relevant care providers and other potential users of the guideline are contacted and asked to provide feedback on the guideline recommendations through a brief online survey. This consultation is intended to facilitate the dissemination of the final guidance report to Ontario practitioners.

ACKNOWLEDGEMENTS

The Sarcoma DSG would like to thank the following individuals for their assistance in developing this report:

- Melissa Brouwers, Sheila McNair, Emily Vella, Laurie Elit, Bill Evans, Hans Messersmith, Chris Fletcher, and Bret Wehrli for providing feedback on draft versions.
- Kristy Yiu, Max Chen, and Ruth Chau for conducting a data audit (everyone was in charge of one third of this task).
- Sara Miller for copy editing.

Molecular Analyses in the Diagnosis, Prognosis, and Selection of Therapy in non-GIST Soft Tissue Sarcomas

Section 4: Systematic Review

INTRODUCTION

STS are malignant tumours of cells of the connective and supporting tissues [74]. Although STS are not prevalent cancers, 1255 people were diagnosed with STS and 765 died from them in Canada in 2013 [74]. There are more than 50 types of STS and the most common STS are fat tissue tumour (e.g., liposarcoma), fibrous tissue tumours (e.g., dermatofibrosarcoma protuberans), muscle tissue tumours (e.g., leiomyosarcoma), blood and lymph vessel tumours (e.g., angiosarcoma), GISTs, nerve tissue tumours (e.g., malignant peripheral nerve sheath tumours), extraskeletal tumours of bone and cartilage (e.g., extraskeletal osteosarcoma), and tumours of uncertain tissue type (e.g., clear cell sarcoma) [74].

The treatments and outcomes for STS have remained relatively unchanged over the past 30 years. Furthermore, the histological features of these tumours are sufficiently variable that accurate classification by light microscopy can be challenging. In an attempt to more accurately diagnose tumours and to better predict outcome and treatment response, there has been a great interest in identifying molecular biomarkers. There is increasing evidence that STS commonly have disease-defining molecular alterations, including copy number changes, mutations, or translocations, etc. While relatively nascent, there have been numerous studies that report on the use of molecular markers as diagnostic methods, predictive markers, or treatment selection tools in STS. This guideline tries to objectively evaluate these papers in an attempt to guide physicians as to the utility of these markers for use in adult patients with non-GIST STS.

The Working Group (including four pathologists: RAK, BCD, SP, and BMP; two orthopedic oncologists: MG and JW; one medical oncologist: SV; and one methodologist: XY) of the Sarcoma DSG developed this evidentiary base to inform recommendations as part of a clinical practice guideline. Based on the objectives of this guideline (see **Section 2: Guideline - Recommendations and Key Evidence**), the Working Group derived the research questions as outlined below. The systematic review has been registered on the website of the International prospective register of systematic reviews (www.crd.york.ac.uk/prospero) as CRD42017061083.

RESEARCH QUESTIONS

1. Does genetic testing enable accurate typing of non-GIST STS? Specifically, can it be used to differentiate sarcomas from benign and/or locally aggressive tumours, and does it change management and further improve patient outcomes in adult patients with non-GIST STS?
2. Are there genetic tests that predict patient prognosis (including OS, PFS, local control rate, patient-reported outcomes, etc.) in adult patients with non-GIST STS?
3. Are there genetic tests that identify subgroups of adult patients who would derive greater benefit from specific treatments targeting non-GIST STS?

METHODS

This evidence-based review was conducted in two planned stages, including a search for systematic reviews followed by a search for primary literature. These stages are described in subsequent sections.

Search for Existing Systematic Reviews

A search was conducted for existing systematic reviews and meta-analyses. The MEDLINE, EMBASE, Cochrane Database of Systematic Reviews databases, and PROSPERO database were searched from January 2010 to October 13, 2016. The search terms for the molecular test names were collected by searching/checking the College of American Pathologists 2013 protocol for the examination of specimens from patients with tumours of soft tissue [75] and the 2016 NCCN guideline on soft tissue sarcoma [76], as well as the Working Group members' clinical expertise. The final search strategies are reported in Appendix 3. The eligible systematic reviews should describe study selection criteria and database search methods (including database names, search date, and search strategies), should state how many papers were included in, and should have at least one eligible article that met our following study selection criteria for the original studies. If no systematic reviews were found to cover all three research questions, then a search for primary literature was performed as described below.

Search for Primary Literature

Literature Search Strategy

MEDLINE, EMBASE, and the Cochrane Library were searched from January 2005 to October 13, 2016 to find full publications; and the American Society of Clinical Oncology Annual Meeting Abstracts and Connective Tissue Oncology Society Annual Meeting Abstracts were checked from 2013 to 2016 for abstracts that met the following study selection criteria. The search strategies are reported in Appendix 3.

Study selection criteria and process

Inclusion Criteria

An article was included if it met all of these pre-planned inclusion criteria:

1. A sample size of ≥30 adult patients (≥18 years old) analyzed who were suspected to have non-GIST STS.
2. For the diagnostic question (Q1), the study reported or provided sufficient data to create a 2 × 2 table for calculating diagnostic accuracy outcomes, and/or reported patient outcomes.
3. For the prognostic question (Q2), a multivariable analysis was performed or there was no statistically significant difference in patient characteristics between two groups (e.g., molecular alteration group versus without molecular alteration group).
4. For the therapy question (Q3), a randomized controlled trial **(**RCT**)** or a comparative study that showed no statistically significant difference in patient characteristics between two treatment groups, and reported the interaction results between patients with molecular alteration and patients without molecular alteration across the treatment groups.

Exclusion Criteria

An article or abstract was excluded if it met any of these pre-planned exclusion criteria:

1. The article was published in a language other than English, due to limited resources for translation.
2. The article was published in the form of a letter, editorial, commentary, or non-systematic review.
3. The study included >20% patients <18 years old and/or included patients with bone sarcoma with no subgroup analysis of **≥**30 adult patients or patients with STS.
4. For the diagnostic question (Q1), the study only included patients with the target sarcoma, or it treated the molecular test as the reference standard or a part of the reference standard.

Data Extraction and Study Risk of Bias Assessment

A review of the titles and abstracts that resulted from the search was performed by one reviewer (XY). For those that warranted full-text review, XY reviewed each article and discussed with the other Working Group members to confirm the final study selections. Data extraction was performed by XY. All extracted data and information were audited by independent auditors (KY, MC, and RC). For the diagnostic research question (Q1), the study quality for each eligible paper was assessed by the QUADAS-2 tool [77]. For the prognostic research question (Q2), the risk of bias for each eligible study was assessed by the QUIPs tool [78]. For the treatment selection research question (Q3), the risk of bias for each eligible study was assessed by the modified Cochrane Collaboration tools for randomized studies [79].

Synthesizing the Evidence

Statistical analyses were executed with the statistical software package STATA version 11 [80]. If no clinical heterogeneity was recognized, a meta-analysis was conducted. For diagnostic papers, a bivariate, random-effects meta-regression model was used to control for unexpected heterogeneity, to produce summary estimates of sensitivity and specificity, and to plot hierarchical summary receiver operating characteristic (HSROC) curves [81].

For some outcomes for which meta-analysis was inappropriate due to clinical heterogeneity, the results of each study were presented individually in a descriptive fashion.

The GRADE (Grading of Recommendations, Assessment, Development, and Evaluation) method for assessing the quality of aggregate evidence was used, and five factors were considered, including the risk of bias, inconsistency, indirectness, imprecision, and other considerations (e.g., publication bias) [24]. To identify accurate molecular tests that could be used to assist clinicians to make diagnostic decisions, the Working Group members' clinical experience was used to define the following thresholds and criteria for the diagnostic research question (Q1) before the literature search retrieval was screened:

1) We recommended the use of a molecular test if both its sensitivity and specificity were ≥90% with the lower limit of 95% CI ≥50%;
2) We recommended against the use of a molecular test if both its sensitivity and specificity were <80%;
3) We were uncertain of the suitability of a molecular test if its diagnostic accuracy was between these two pre-planned thresholds. However, if either a test's sensitivity or specificity was ≥90% with the lower limit of 95% CI ≥50%, based on the Working Group members' clinical opinion, the test might be useful in clinical practice.
4) The test diagnostic accuracy calculation focused on patients whose test results were interpretable [82].

For the prognostic research question (Q2), we recommended the particular molecular alteration as a predictive factor for better prognosis if the HR was ≤1.0 with a p-value of ≤0.05 or recommended against it as a predictive factor if the p-value was >0.05; we recommended the particular molecular alteration as a predictive factor for worse prognosis if the HR was >1.0 with a p-value of ≤0.05 or recommended against it as a predictive factor if the p-value was >0.05.

For the treatment selection question (Q3), an HR of ≤1.0 indicated improved efficacy for the experimental arm and an HR of >1.0 indicated improved efficacy for the control arm. A two-sided significance level of α=0.05 was assumed.

RESULTS

Literature Search Results

The PRISMA flow diagram (http://www.prisma-statement.org/statement.htm) of studies considered in the systematic review is shown in Appendix 4. Of 6674 review and original study articles identified from the MEDLINE and EMBASE searches and the Cochrane Clinical trial Register, 6107 articles were excluded after reviewing the titles and abstracts, and 77 [1-23,26-63,65-70,83-92] met our pre-planned study selection criteria after reviewing 567 full texts. Of these, 70 underwent data extraction and were analyzed in this systematic review, and their reference lists were manually searched but no further eligible papers were found. Two papers met inclusion criteria for both diagnostic and prognostic research questions [35,37]. Data were not extracted from seven articles on the prognostic research question for the following reasons: Although one existing systematic review [87] was relevant, it did not use the same pre-defined selection criteria as ours, missed one relevant paper, and also included several ineligible papers. However, its included studies were reviewed as potentially eligible studies for this systematic review. Five articles conducted a multivariable analysis but the molecular variable was not in the model because the molecular variable was not statistically significant in the univariable analysis [88-92]. One study recruited patients with different types of STS without subgroup analysis for an individual STS type [86], thus, we did not know whether the targeted molecular test was a prognostic test for one STS type or several different types. Therefore, these seven papers were not discussed further in this systematic review.

A screen of conference abstracts yielded three abstracts that met the study selection criteria [93-95]. However, all three abstracts had been published as full texts that were included in our literature retrieval.

The full names and abbreviations of the molecular tests are listed in Appendix 1.

Study Design and Quality Assessment

For the diagnostic research question (Q1), 51 articles on 13 types of sarcomas were eligible [1-20,26-37,45-60,65-67]. Their study designs were either retrospective cohort or retrospective case-control. The quality assessment result for each study is reported in Appendix 5. Forty-three studies were low quality, and eight were unclear quality. Thus, the aggregate diagnostic study evidence for each molecular test in every disease was low to very low after considering other four factors (inconsistency, indirectness, imprecision, and other factors) together from the GRADE approach. The traditional GRADE summary tables for each outcome were not presented because of the large number of molecular tests and different types of STS involved in this guideline. Additionally, there were fewer than four eligible studies for most molecular tests, thus meta-analyses were not performed for these tests.

For the prognostic research question Q2, 21 articles on five types of sarcomas were eligible [21-23,35,37-44,61-63,68-70,83-85]. Their study designs were either retrospective inception cohorts (IC) (IC was defined as patients at a similar point in the course of a sarcoma type) or retrospective non-IC. The assessment result of risk of bias for each study is reported in Appendix 6. Sixteen studies had high risk of bias, and five had moderate risk of bias. The quality of the aggregate evidence for each molecular test was low to very low when considering risk of bias, inconsistency, indirectness, imprecision, and other factors together. Again, the traditional GRADE summary tables were not presented for the same reasons described for Q1 above.

For the treatment selection question Q3, no articles met our pre-planned study selection criteria.

Outcomes by types of non-GIST STS

The following types of non-GIST STS had evidence to answer both diagnostic and prognostic research questions. The eligible diagnostic studies only reported diagnostic outcomes without patient outcomes.

1. Angiosarcoma

1). Diagnostic research question Q1

Six articles met the study selection criteria [37,45-49]. The key study characteristics and diagnostic outcomes for individual study are reported in Table 4-1. A meta-analysis of these studies results was not performed because patient populations at baseline and the definitions of the positive *MYC* amplification were different among the eligible studies (Table 4-1). In addition, 50% of patients overlapped with the Kacker et al. 2013 study [46] and the Manner et al. 2010 study [48]. To differentiate breast radiation-induced SAS from PAS or/and AVL, these six papers showed the sensitivity of *MYC* (*C-MYC*) amplification by FISH method ranged from 54% (95% CI, 37% to 71%) to 100% (95% CI, 89% to 100%) and the specificity ranged from 93% (95% CI, 68% to 100%) to 100% (95% CI, 89% to 100%). The sample size for these studies ranged from 33 to 69.

2). Prognostic research question Q2

Two studies met the study selection criteria for the prognostic question [37,38]. The key study characteristics are reported in Table 4-2. The Fraga-Guedes et al. 2015 paper found that among 37 patients by 25 months after treatment [37], those with SAS who had *MYC* amplification had lower OS than patients without *MYC* amplification (HR, 3.47; 95% CI, 1.09 to 11.1; p=0.035), but there was no statistically significant difference for the DFS rate (HR, 1.89; 95% CI, 0.78 to 4.55; p=0.155) between the two groups. The Huang et al. 2016 paper with a sample size of 82 reported that those with PAS or SAS who had *CIC* alteration had a lower DFS rate than patients without *CIC* alteration (HR, 3.46; 95% CI 1.42 to 8.44; p=0.006) [38].

2. Desmoid Tumour

1). Diagnostic research question Q1

Four studies met the study selection criteria for the diagnostic question [19,26,54,55]. The key study characteristics and diagnostic outcomes for individual studies are reported in Table 4-1. A meta-analysis of the study results was not performed because different detection methods were used among studies. Four studies with a total of 780 patients reported that the *CTNNB1* mutation test by PCR or direct Sanger sequencing method had sensitivities ranging from 82% to 88% and specificities of 100% to differentiate desmoid tumour from histological mimics. However, the Aitken et al. 2015 study [26] found that the *CTNNB1* mutation by next-generation sequencing method had good sensitivity (92%; 95% CI, 87% to 96%) and specificity (100%; 95% CI, 78% to 100%).

2). Prognostic research question Q2

Six studies met the study selection criteria [21-23,83-85]. The key study characteristics and prognostic outcomes are reported in Table 4-2. Three main mutations in *CTNNB1* have been identified: T41A, S45F, and S45P [22]. Since the comparisons were different in each study, it was inappropriate to perform a meta-analysis. Three studies [21-23] found that patients with tumours harbouring a *CTNNB1* S45F mutation had a higher recurrence rate at 41 to 60 months after treatment than patients with a T41A or S45P mutation or without a *CTNNB1* mutation (HR from 3.50 [95% CI, 1.51 to 8.14] to 6.20 [95% CI, 2.24 to 17.15]). Although the Romero et al. 2012 study [85] and Kim et al. 2016 study [84] reported that patients with or without a *CTNNB1* gene mutation had similar recurrence rates, these two studies combined patients with mutation type T41A, S45F, or S45P into one group. Also, the Romero et al. 2012 study showed that all

seven patients with the S45F mutation experienced recurrence. The Domont et al. 2010 study [83] found that patients with any *CTNNB1* mutation had a higher recurrence rate, but it included patients who had recurrent desmoid tumours at baseline as well. Thus, there was significant bias in the study that led to the uncertain result for recurrence.

3. Liposarcoma

Liposarcoma is the most common STS in adults [4]. The World Health Organization classifies liposarcoma into four types: ALT/WDL, ML, DDL, and PL [96]. From the molecular alteration perspective, ALT/WDL and DDL are similar, but they are different from ML or PL. Thus, accurately differentiating each subtype from its mimic diseases is important for clinicians and patients in order to be able to choose optimal treatments to obtain the best patient outcomes and prognoses.

1). Diagnostic research question Q1

Twenty-two articles met the study selection criteria [1-13,29-34,57,58,67]. The key study characteristics and diagnostic outcomes for individual study are reported in Table 4-1.

(1). MDM2 amplification by FISH

A meta-analysis of 10 studies with the sample size of 971 to differentiate ALT/WDL/DDL from benign tumours (mainly lipoma) by *MDM2* amplification determined by FISH was conducted. The calculated sensitivity was 95% (95% CI, 89% to 98%) and specificity was 100% (95% CI, 89% to 100%) [1-10] (Table 4-3). The HSROC curve is shown in Figure 4-1.

To differentiate ALT/WDL/DDL from other STS, sensitivity of 99% (95% CI, 71% to 100%) and specificity of 90% (95% CI, 78% to 95%) were calculated from a meta-analysis of four studies with a combined sample size of 347 [4,7,8,11] (Table 4-3). The HSROC curve is shown in Figure 4-2.

Two other studies [12,13] with a combined sample size of 96 reported that the sensitivity was 92% (95% CI, 75% to 99%) and 94% (95% CI, 81% to 99%); and specificity was 96% (95% CI, 79% to 100%) and 100% (95% CI, 69% to 100%), respectively, to differentiate ALT/WDL/DDL from lipoma and other STS. The results from these two studies supported the above meta-analyses to indicate that the *MDM2* amplification test by FISH method was accurate to differentiate ALT/WDL/DDL from benign tumours (mainly lipoma) or other STS.

(2). MDM2 amplification by real-time PCR

Four studies were included in a meta-analysis with a combined sample size of 346 patients to differentiate ALT/WDL/DDL from benign tumours [1,6,57,58]. The calculated sensitivity was 88% (95% CI, 70% to 96%) and specificity was 98% (95% CI, 91% to 99%). The HSROC curve is shown in Figure 4-3. Additionally, one study with 187 patients reported that the sensitivity was 98% (95% CI, 93% to 100%) and specificity was 76% (95% CI, 66% to 84%) to differentiate ALT/WDL/DDL from other STS [57].

(3). CDK4 amplification

Three studies investigated the diagnostic accuracy of *CDK4* amplification as determined by FISH or real-time PCR to differentiate ALT/WDL/DDL from benign tumours (mainly lipoma). One study [6] reported that it was not sensitive (sensitivity of 82% or 76%, respectively) in 94 patients, but another study [57] reported a sensitivity of 98% (95% CI, 93% to 100%) and a specificity of 97% (95% CI, 84% to 100%) using FISH in 129 patients. The third study [1] reported that the sensitivity was 90% (95% CI, 76% to 97%) using the FISH method, but 80% (95% CI, 64% to 91%) using the real-time PCR method in 67 patients.

To differentiate ALT/WDL/DDL from other STS, one study [57] showed a sensitivity of 98% (95% CI, 93% to 100%) but a specificity of 76% (95% CI, 66% to 84%) in 187 patients; another study [11] showed that the sensitivity was 100% (95% CI, 75% to 100%) and specificity was 100% (95% CI, 90% to 100%) in 48 patients.

(4). DDIT3 rearrangement

Four studies [29-32] using *DDIT3* rearrangement (by FISH) to differentiate ML from benign tumours or other STS (Table 4-3) were included in a meta-analysis with a combined sample size of 189. The calculated sensitivity was 96% (95% CI, 69% to 100%) and specificity was 100% (95% CI, 80% to 100%). The HSROC curve is shown in Figure 4-4.

(5). 12q13-15 amplification

Two studies [33, 34] reported that both sensitivity and specificity were 100% for either 12q13-15 amplification determined by *CMA* or 12q13-15 rearrangement determined by FISH that could differentiate WDL/DDL from benign tumours (mainly lipoma) in a total of 81 patients.

(6). CPM amplification

One study [2] found that *CPM* amplification determined by FISH was accurate to differentiate ALT/WDL from lipoma with a sensitivity of 100% (95% CI, 89% to 100%) and specificity of 100% (95% CI, 95% to 100%) in 106 patients.

(7). HMGA2 rearrangement

One study [67] found that *HMGA2* rearrangement determined by either FISH or real-time PCR was sufficiently sensitive to differentiate WDL/DDL from lipoma (sensitivity of 100%) but not specific (specificity of 57% or 17%) in 50 or 54 patients, respectively.

(8). FUS rearrangement

One study [29] found that the *FUS* (16p11) rearrangement test by FISH was sensitive (sensitivity, 94%; 95% CI, 73% to 100%) but not specific (specificity, 83%; 95% CI, 68% to 93%) to differentiate ML from other myxoid soft tissue tumours in 59 patients.

2). Prognostic research question Q2

Seven studies met the study selection criteria [39-43,68,69]. The key study characteristics and prognostic outcomes are shown in Table 4-2.

(1). MDM2 amplification

Three studies [39-41] met the study selection criteria and did not find *MDM2* amplification to be associated with OS (p=0.179) in 120 patients with ML at five-year follow-up, with DSS (HR, 1.35; 95% CI, 0.144 to 12.5; p=0.794) or with PFS (HR, 1.22; 95% CI, 0.45 to 2.39; p=0.655) in 56 WDL/DDL patients at 43 months, or with local recurrent rate (HR, 1.92; 95% CI, 0.81 to 4.58; p=0.138) in 50 patients with DDL at 28 months.

(2). CDK4 amplification

One study [40] reported that tumours with *CDK4* amplification were associated with a shorter DSS (HR, 2.19; 95% CI, 1.42 to 23.5; p=0.044) and a shorter PFS (HR, 3.08; 95% CI, 1.03 to 10.81; p=0.048) in 56 patients with WDL/DDL at a median follow-up of 43 months. Another paper [69], which included some of the same patients from the first study, reported that *CDK4* amplification was associated with a shorter local RFS (HR, 12.08; 95% CI, 1.48 to 98.83; p=0.02) in 48 WDL/DDL patients at approximately two-year follow-up. However, the third study [42]

did not find the association between *CDK4* amplification and a two-year OS (p=not significant [NS]) and a two-year RFS (p=NS) in 90 patients with ALT/WDL/DDL.

(3). 19q13 loss

One study [43] reported that at a median follow-up time of 28 months, 19q13 loss led to a shorter local RFS (HR, 2.99; 95% CI, 1.30 to 6.83; p=0.01) in 40 patients with DDL.

(4). HMGA2 amplification

One study [42] demonstrated that there was no relationship between *HMGA2* amplification a two-year RFS (p=NS) in 91 patients with ALTWDL/DDL.

(5). PIK3CA amplification

Kim et al [68] reported that *PIK3CA* amplification was associated with a poor DFS (HR, 2.40; 95% CI, 1.11 to 5.21; p=0.027) but not OS (HR, 1.63; 95% CI, 0.56 to 4.77; p=0.375) in 101 patients with primary liposarcoma at a median follow-up time of 51 months. However, ALT/WDL/DDL is different than ML or PL from a prognostic perspective. Thus, we do not know whether the *PIK3CA* amplification is a prognostic test for the different liposarcoma subtypes so no recommendation is made for this molecular alternation.

4. MPNST

1). Diagnostic research question Q1

The Brekke et al. 2010 study [35] reported that DNA copy number changes as determined using *CMA* differentiated MPNST from cutaneous neurofibromas with a sensitivity of 92% (95% CI, 80% to 98%) and specificity of 90% (95% CI, 56% to 100%) in 58 patients.

The Wallander et al. 2012 study [36] showed a sensitivity of 20% (95% CI, 4% to 48%) and the specificity of 100% (95% CI, 88% to 100%) for *MDM2* amplification detected by FISH to differentiate MPNST from neurofibroma and schwannoma in 44 patients.

2). Prognostic research question

Two studies met the study selection criteria [35,44]. The key study characteristics and prognostic outcomes are reported in Table 4-2. The Brekke et al. 2010 study [35] with a sample size of 48 found that patients with loss from Xq or 10q, or gain at 16p had a shorter OS than patients without these DNA changes (HR, 11.0; 95% CI 3.5 to 35; p<0.001). The Yu 2010 et al. study [44] reported that patients with *CDK4* gain or amplification had poorer survival (HR, 4.22; 95% CI 1.43 to 12.44; p=0.009, and HR, 2.04; 95% CI 1.03 to 4.04; p=0.041, respectively), but there were no statistically significant differences for patients with *FOXM1* gain, *NOL1* gain, *SOX5* gain, or *MYC* gain.

5. Synovial sarcoma

1). Diagnostic research question Q1

Six studies met the study selection criteria [14-18,20]. The key study characteristics and diagnostic outcomes for individual studies are reported in Table 4-1. A meta-analysis of four eligible studies with a total of 258 patients was performed for the SS18 (SYT) break-apart by FISH to differentiate synovial sarcoma from other sarcomas [14-17]. The calculated sensitivity was 94% (95% CI, 89% to 97%) and specificity was 97% (95% CI, 60% to 100%) (Table 4-3). The HSROC curve is shown in Figure 4-5.

Another meta-analysis of four eligible studies with 532 patients was performed for the *SS18-SSX* fusion test by reverse transcription-PCR (RT-PCR) [14,16,18,20]. One study included normal tissues in the control group [18]. Since its specificity was 100%, which was the same as the other three studies, we believed it was reasonable to conduct a meta-analysis for these

four studies. The calculated sensitivity was 93% (95% CI, 85% to 96%) and specificity was 100% (95% CI, 97% to 100%) (Table 4-3). The HSROC curve is shown in Figure 4-6.

2). Prognostic research question

Four studies [61-63,70] met the study selection criteria, which reported clinical outcomes for synovial sarcoma with *SS18-SSX1* (formerly called *SYT-SSX1*) versus *SS18-SSX2* (formerly called *SYT-SSX2*) translocations [87]. The key study characteristics and prognostic outcomes are reported in Table 4-2.

For the OS outcome, Takenaka et al. [63] reported no statistical difference between the two groups in 81 patients with local or metastatic synovial sarcoma (RR, 1.46; CI, 0.64 to 3.28; p=0.36] or in 67 patients with only local synovial sarcoma (RR, 1.57; CI, 0.55 to 4.24; p=0.38). However, Ren et al. [70] found a significant result (RR, 2.34; 95% CI, 2.18 to 2.67; p=0.002) in 88 patients with local or metastatic synovial sarcoma.

For the DSS outcome, the Canter 2008 et al. study [61] reported that acceleration rate was -0.22 (95% CI, -1.87 to 1.43; p=0.794) in 132 patients, However, the Sun et al. 2009 study with a sample size of 141 reported that patients with *SS18-SSX1* had a shorter DSS than those with *SS18-SSX2* (RR, 2.03; CI, 1.26 to 3.28; p=0.004). Both studies included patients with synovial sarcoma who had local or metastatic disease.

For the metastasis-free survival outcome, Takenaka et al. [63] reported that there was no statistical difference between the two groups in 67 patients (RR, 0.67; 95% CI, 0.25 to 1.60; p=0.38). However, Sun et al. [62] found a significant result (RR, 1.86; 95% CI, 1.04 to 3.33; p=0.037) in 141 patients and Canter et al [61] reported that acceleration rate was -1.15 (95% CI, -2.12 to -0.19; p=0.02) in 132 patients.

For the following diseases, the eligible studies provided only evidence relevant to the diagnostic question, and they only reported diagnostic outcomes without patient outcomes.

6. Clear Cell Sarcoma

Two studies met the study selection criteria [50,51]. The key study characteristics and diagnostic outcomes for each study are shown in Table 4-1. Both of them found that *EWSR1* rearrangement or *EWSR1* dual break-apart (by FISH) was not sensitive (sensitivity, 70% or 79%, respectively) but was specific (specificity, 100%) to differentiate clear cell sarcoma from malignant melanoma.

7. Dermatofibrosarcoma protuberans

Two studies met the study selection criteria (Table 4-1) [52,53]. Some patients overlapped in these two studies. The Salgado study [52] found that *PDGFB* break-apart detected by FISH was sensitive (sensitivity, 91%; 95% CI, 83% to 96%) and specific (specificity, 100%; 95% CI, 69% to 100%) in 106 patients to differentiate dermatofibrosarcoma protuberans from dermatofibromas. In contrast, RT-PCR was not sensitive enough to detect this translocation (sensitivity of 72% and specificity of 100%). The Segura et al. 2011 paper reported sensitivity of 86% (95% CI, 68% to 96%) and specificity of 100% for *PDGFB* break-apart by FISH in 45 patients [53].

8. Epithelioid sarcoma

One study [28] with a sample size of 81 (in which 49% of cases were epithelioid sarcoma) found that *SMARCB1* deletion detected by FISH was sensitive (sensitivity, 63%; 95% CI, 76% to 97%) and specific (specificity, 71%; 95% CI, 80 to 99) to differentiate epithelioid sarcoma from its mimickers.

9. Endometrial stromal sarcoma

Only one study [27] reported that *JAZF1* rearrangement evaluated by FISH was neither sensitive (sensitivity, 63%) or specific (specificity, 77%) to differentiate endometrial stromal sarcoma from endometrial stromal nodule or undifferentiated uterine sarcoma in 36 patients (Table 4-1).

10. Epithelioid hemangioendothelioma

Anderson et al [65] reported that *WWTR1* rearrangement by FISH had sensitivity of 87% and specificity of 100%, and *CAMTA1* rearrangement by FISH had sensitivity of 87% and specificity of 80% (Table 4-1) in differentiating epithelioid hemangioendothelioma from epithelioid angiosarcoma in 33 patients.

11. Hemangiopericytoma

One study of 52 patients [56] reported a sensitivity of 80% and specificity of 93% for *NAB2*ex6-*STAT6*ex16/17 fusion using RT-PCR to differentiate hemangiopericytoma from fibrous/cellular solitary fibrous tumour in 52 patients.

12. Hybrid hemosiderotic fibrolipomatous tumour (HFLT)-myxoinflammatory fibroblastic sarcoma (MIFS)

Zreik et al [66] reported that detection by FISH of both of *TGFBR3* rearrangement (detected by FISH) and *MGEA5* rearrangement (by FISH) were not sensitive (sensitivity was 75% and 50%, respectively) but were specific (specificity was 100% and 94%, respectively) in differentiating hybrid HFLT-MIFS from MIFS in 39 patients (Table 4-1).

13. Low-grade fibromyxoid sarcoma (LGFM)

Two studies [59, 60] with 268 patients reported a sensitivity of 81% and 88%, and a specificity of 87% and 100%, respectively for *FUS-CREB3L1/2* fusion detection by RT-PCR in differentiating LGFM from LGFM-like tumours (Table 4-1).

Ongoing, Unpublished, or Incomplete Studies

The National Cancer Institute Clinical Trials Database (http://www.clinicaltrials.gov/) was searched on April 27, 2017 for potential trials meeting the selection criteria for this systematic review. There are three ongoing, unpublished, or incomplete trials that should be checked for potential inclusion in the update of this guideline in the future (Appendix 7).

DISCUSSION

This systematic review targeted all STS except GIST. As there were many types and subtypes of sarcomas, the Working Group summarized the main findings in Table 4-4. Based on the GRADE criteria, the overall aggregate quality of each molecular test in all the diagnostic or prognostic studies was low to very low. Hence, the findings from this systematic review were not definitive except for three circumstances (bolded and italicized in Table 4-4): (1) *MDM2* amplification test determined by FISH is an accurate test to differentiate patients with ALT/WDL/DDL from patients with benign tumours (mainly lipoma) or other STS; (2) *SS18-SSX* (*SYT-SSX*) fusion analysis by FISH/RT-PCR is sensitive and specific to differentiate synovial sarcoma from other sarcomas; and (3) *CTNNB1* S45F (but not T41A and S45P) mutation determined by PCR is a risk factor for a poor RFS in patients with desmoid tumours at a median follow-up of 41 to 60 months. For these three outcomes, more than 400 patients from several studies were evaluated, the results were consistent across these studies, and the 95% CIs were narrow for the diagnostic outcomes.

The existing literature has several limitations regarding the three research questions. In terms of the diagnostic research question Q1: First, all the eligible studies were retrospective and 41 of 51 papers were case-controlled studies. This means that the investigators chose the patients with histological mimickers plus the patients with target disease to comprise the study population, which introduces a subjective component to the study. Thus, the diagnostic accuracy of molecular tests may be overestimated and might be one reason why some studies had a specificity of 100%. Second, to investigate the diagnostic accuracy of a biomarker for a rare disease, a retrospective cohort study may be a feasible and acceptable design. However, the interpretation of the biomarker test should at least be blinded to the results from the reference standard if the interpretation of the biomarker test is not objective; and at the same time, the final interpretation from the reference standard should be blinded to the biomarker results. But most eligible studies in this review did not state that blinding was implemented. Third, the eligible papers used different definitions for the positive threshold of the molecular tests for the diagnostic outcomes, which made it impossible to add this information into the recommendation. For example, 13 papers used *MDM2* amplification by FISH to differentiate patients with ALT, WDL, or DDL from patients with benign tumours (mainly lipoma) or other STSs (Table 4-1). The positive threshold was defined as a ratio of *MDM2* to CEP12 of ≥3 in three studies [2,11,12], ≥2 in 9 studies [1,3-5,7-10,13], and fluorescent signals/cell of >5 in one study [6]. The sensitivity and specificity will have the trade-off change with the different thresholds for the test positivity in the same population. However, the diagnostic accuracy outcomes were nearly consistent across all the eligible studies for every outcome. We listed this information in Table 4-1 for the readers' interest. Fourthly, there may be some limitations due to the technology used at the time of these eligible studies. For example, EWSR1 translocation can have different partners depending on the sarcoma type. If the test examines for only one component of a translocation then evaluation of the test specificity could be very misleading and introduce false negatives. This is all further complicated by the realization that molecular diagnostics is rapidly changing as next-generation sequencing and transcriptome analysis is being incorporated into the clinical laboratories. This can greatly influence the sensitivity and specificity of a test. Given the nature of this review, this could not be accounted for in the results and could potentially bias against a genetic test inappropriately. Fifthly, no eligible study reported patient management change outcomes after molecular testing, and further patient follow-up outcomes. However, the managements of different benign and malignant tumours should be different in most types of STS. Hence, accurate diagnosis of non-GIST STS by molecular tests should result in more appropriate management and better patient outcomes (e.g., RFS).

In terms of the prognostic research question Q2: First, most studies only provided significant variables from a univariable analysis into a multivariable model, which may not be the best statistical method to identify risk factors. In several studies, the molecular variable was not in the multivariable model because the molecular variable was not statistically significant in the univariable analysis. Also, different studies set different p-value cut-off points for a significant variable in the univariable model. Second, some eligible studies recruited mixed populations of patients with primary disease and recurrent disease. As these cohort patients were not at a similar point in their disease, they would be expected to have different outcomes independent of whether the tumour had molecular alterations or not, especially for the RFS outcome.

In terms of the treatment selection research question Q3, no evidence was found meeting our pre-determined study selection criteria. Therefore, high-quality observational studies are needed to address this question. Such a study should preferably be an RCT, or at least a comparative study that shows no statistically significant difference for patient characteristics between two treatment groups or that uses a multivariable model to control the

potential patient characteristic confounders. As well it should report the interaction results between patients with molecular alterations and patients without molecular alterations across the treatment groups.

Additionally, we required that a study could only be included if it had a sample size of ≥30 patients analyzed from a statistical perspective consideration (the Central Limit Theory [97]). That is why there is no evidence for many STS types (e.g., sclerosing epithelioid fibrosarcoma) to date. It is possible that the efficacy of some tests may have been underappreciated. Also, this systematic review focused on adult patients. Thus, some STS (e.g., rhabdomyosarcoma) whose target populations were mainly pediatric did not qualify for inclusion in this review.

CONCLUSION

Given all the limitations of this review, there are data that nevertheless show that molecular analysis is useful in differentiating selected sarcomas from benign tumours or other sarcomas and in predicting prognoses in some non-GIST sarcomas. As our understanding of molecular changes is growing rapidly, test technology is changing, and the cost of these tests is decreasing, we expect that new studies will be conducted that will provide more high-quality evidence in the near future to guide clinicians on diagnosis, prognosis prediction, and treatment selection for patients with STS.

Table 4-1. Characteristics and outcomes for diagnostic studies (different types of soft tissue sarcomas ordered alphabetically and eligible studies under each type ordered by publication year)

Study	Study design	Differentiation purpose	Sample size	Disease status	Mean or median age (range), years	Index test (positive threshold)	Reference standard	Sen (%) (95% CI)	Spec (%) (95% CI)
Angiosarcoma									
Manner 2010 [48]	Retro, case-control	SAS from PAS	61	Primary/ Secondary	71 (32 to 88)	*MYC* amplification by FISH (clustered signals >9)	Histological results	55 (36 to 72)	100 (88 to 100)

Mentzel 2012 [49]	Retro, case-control	SAS from AVL	37	Secondary	65 (29 to 95)	*MYC* amplification by FISH (clustered signals >8)	Histological results	100 (86 to 100)	100 (74 to 100)
Kacker 2013[a] [46]	Retro, case-control	SAS from PAS	69	Primary/ Secondary	NR	*MYC* amplification by FISH (*MYC*/centromere-signals ratio >2.0 in >25% tumour cells)	Histological results	58 (41 to 75)	100 (89 to 100)
Ginter 2014 [45]	Retro, cohort	SAS from AVL+PAS	33	Primary/ Secondary	63 (23 to 89)	*MYC* amplification by FISH (*MYC*/CEP8 ratio of ≥2.0)	Histological results	100 (66 to 100)	100 (86 to 100)
Fraga-Guedes 2015 [37]	Retro, cohort	SAS from AVL	66	Secondary	66 (37 to 88)	*MYC* amplification by FISH (*MYC*/CEP8 ratio of ≥2.0)	Histological results from core biopsy for SAS and PAS; punch biopsy and resection for AVL	54 (37 to 71)	100 (88 to 100)
		SAS from PAS	49	Primary/ Secondary	66 (30 to 88)			54 (37 to 71)	100 (74 to 100)
Lae 2015 [47]	Retro, cohort	SAS from PAS	47	Primary/ Secondary	56 (19 to 89)	*C-MYC* amplification by FISH (*MYC*/CEP8 ratio of ≥2.0)	Histological results	100 (89 to 100)	93 (68 to 100)
Clear cell sarcoma (CCS)									
Patel 2005 [50]	Retro, NR	CCS from MM	42	NR	NR	*EWSR1* rearrangement by FISH (>10% tumour nuclei had EWSR1 rearrangement)	Histological and IHC results	70 (35 to 93)	100 (89 to 100)
Yang 2012 [51]	Retro, case-control	CCS from MM	45	Primary/ Recurrent	66 (17 to 92)	*EWSR1* break-apart by FISH (a split red/green signal with a minimum diameter of one signal width)	Histological results	79 (49 to 95)	100 (89 to 100)
			34			*EWSR1/ATF1* fusion by RT-PCR (NR)		70 (35 to 93)	100 (86 to 100)
Dermatofibrosarcoma protuberans (DFSP)									
Salgado 2011 [52]	Retro, case-control	DFSP from DF	106	NR	45 (11 to 86)[b]	*PDGFB* break-apart by FISH (NR)	Histological results	91 (83 to 96)	100 (69 to 100)
			92	NR	45 (11 to 86)[b]	*COL1A1/PDGFB* fusion by RT-PCR (NR)	Histological results	72 (61 to 81)	100 (69 to 100)
Segura 2011[c] [53]	Retro, case-control	DFSP from DF	45	Primary/Recurrent	43 (15 to 87)[d]	*PDGFB* break-apart by FISH (a split red/green signal with a minimum diameter of one signal width)	Histological results	86 (68 to 96)	100 (79 to 100)
Desmoid tumour (DT)									
Amary and Pauwels 2007 [19]	Retro, case-control	DT from histological mimics	133	NR	37 (6 to 77)	*CTNNB1* mutation by MSRED (NR)	Histological results	87 (77 to 94)	100 (94 to 100)
Le Guellec 2012 [54]	Retro, case-control	DTF from histological mimics	429	NR	41 (1 to 88)[e]	*CTNNB1* mutation by PCR (NR)	Histological results	88 (83 to 92)	100 (98 to 100)
Huss 2012 [55]	Retro, case-control	DT from retroperitoneal fibrosis	95	NR	46 (8 to 87)	*CTNNB1* mutation by direct Sanger sequencing (NR)	Histological results	85 (75 to 92)	100 (72 to 100)
Aitken 2015 [26]	Retro, case-control	DT from histological mimics	159	Primary/ Recurrent	38 (8 to 81)[f]	*CTNNB1* mutation by NGS (≥5% VAF in a region with ≥500 reads/base)	Histological results	92 (87 to 96)	100 (78 to 100)
			123			*CTNNB1* mutation by MSRED (NR)	Histological results	82 (74 to 89)	100 (78 to 100)
Epithelioid sarcoma (ES)									
Le Loarer 2014 [28]	Retro, case-control	ES from ES mimickers	81	NR	36 (1 to 88)	*SMARCB1* deletion by FISH (hemi or homozygous loss of FISH signals for SMARCB1)	Histological results	90 (76 to 97)	93 (80 to 99)
Endometrial stromal sarcoma (ESS)									
Hodge 2016 [27]	Retro, case-control	ESS from ESN+UUS	36	Primary/Metastatic	NR	*JAZF1* rearrangement by FISH (≥1 copy arranged)	Histological results	63 (38 to 84)	77 (50 to 93)
Epithelioid hemangioendothelioma (EHE)									

Anderson 2015 [65]	Retro, case-control	EHE from EAS	35	NR	56 (22 to 84)[g]	*WWTR1* rearrangement by FISH (≥20% of nuclei showed a break-apart signal)	Histological results	87 (69 to 96)	100 (48 to 100)
						CAMTA1 rearrangement by FISH (≥20% of nuclei showed a break-apart signal)		87 (69 to 96)	80 (28 to 100)
Hemangiopericytoma (HPC)									
Barthelmeß 2014 [56]	Retro, case-control	HPC from SFT	52	Primary/Metastatic	61 (31 to 84)	*NAB2-STAT6* fusion by RT-PCR (*NAB2*ex6-*STAT6*ex16/17)	Histological results	80 (44 to 98)	93 (81 to 99)
Hybrid hemosiderotic fibrolipomatous tumour (HFLT)-myxoinflammatory fibroblastic sarcoma (MIFS)									
Zreik 2016 [66]	Retro, case-control	Hybrid HFLT-MIFS from MIFS	39	Primary/ Recurrent	50 (15 to 82)	*TGFBR3* rearrangement by FISH (≥15% nuclei showed disruption of ≥1 fusion signal)	Histological results	75 (35 to 97)	100 (89 to 100)
						MGEA5 rearrangement by FISH (≥15% nuclei showed disruption of ≥1 fusion signal)	Histological results	50 (16 to 84)	94 (79 to 99)
Liposarcoma									
Binh 2005 [57]	Retro, case-control	ALT/WDL/ DDL from benign tumours	129	NR	NR	*MDM2* amplification by CMA (green-to-red fluorescence >1.4) or by PCR (copy number of *MDM2* to ALB ≥1.9)	Histological results from resection	98 (93 to 100)	97 (84 to 100)
						CDK4 amplification by CMA (green-to-red fluorescence >1.4) or by PCR (copy number of *CDK4* to ALB ≥1.2)		98 (93 to 100)	97 (84 to 100)
		Identifying ALT/WDL/ DDL from other STS[h]	187			*MDM2* amplification by CMA (green-to-red fluorescence >1.4) or by PCR (copy number of *MDM2* to ALB ≥1.9)		98 (93 to 100)	76 (66 to 84)
						CDK4 amplification by CMA (green-to-red fluorescence >1.4) or by PCR (copy number of *CDK4* to ALB ≥1.2)		98 (93 to 100)	75 (65 to 83)
Shimada 2006 [11]	Retro, case-control	ALT/WDL/ DDL from other STS	48	NR	61 (17 to 84)	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 >3)	Histological results from resection	100 (75 to 100)	97 (85 to 100)
						CDK4 amplification by FISH (a ratio of *CDK4* to CEP12 ≥3)	Histological results from resection	100 (75 to 100)	100 (90 to 100)
Sirvent 2007 [6]	Retro, case-control	ALT/WDL/ DDL from benign tumours (mainly lipoma)	85	NR	NR	*MDM2* amplification by FISH (fluorescent signals/cell>5)	Histological results	95 (83 to 99)	100 (92 to 100)
			85			*MDM2* amplification by real-time PCR (a ratio of *MDM2* to ALB ≥1.9)		80 (64 to 91)	100 (92 to 100)
			85			*CDK4* amplification by FISH (fluorescent signals/cell>5)		90 (76 to 97)	100 (92 to 100)
			86			*CDK4* amplification by real-time PCR (a ratio of *CDK4* to ALB ≥1.2)		76 (60 to 88)	100 (92 to 100)
Downs-Kelly 2008 [29]	Retro, cohort	ML from other myxoid ST tumours	59	NR	NR	*DDIT3* rearrangement by FISH (>10% rearrangement)	Histological results	100 (82 to 100)	100 (91 to 100)

						FUS (16p11) rearrangement by FISH (>10% rearrangement)		94 (73 to 100)	83 (68 to 93)
Willmore-Payne 2008 [32]	Retro, case-control	ML from other liposarcomas+ lipoma	31	NR	NR	*DDIT3* rearrangement by FISH (>10% rearrangement)	Histological results	94 (73 to 100)	100 (75 to 100)
Weaver 2008[h] [8]	Retro, cohort	WDL/DDL from benign tumours (mainly lipoma)	57	NR	NR	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 ≥2)	Histological results from resection	100 (87 to 100)	100 (88 to 100)
		DDL from other STS	87					100 (77 to 100)	82 (72 to 90)
Erickson-Johnson 2009 [2]	Retro, cohort	ALT/WDL from lipoma	48	NR	NR	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 ≥3)	Histological results	100 (81 to 100)	100 (89 to 100)
			106			*CPM* amplification by FISH (a ratio of *CPM* to CEP12 ≥3)		100 (89 to 100)	100 (95 to 100)
Weaver 2009 [9]	Retro, case-control	WDL from SM and IRF	36	NR	NR	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 ≥2)	Histological results from resection	88 (64 to 99)	100 (82 to 100)
Weaver 2010 [10]	Retro, cohort	WDL from benign tumours (mainly lipoma)	51	NR	NR	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 ≥2)	Histological results from resection	94 (71 to 100)	100 (90 to 100)
			43				Histological results from core needle biopsy	100 (72 to 100)	100 (89 to 100)
Bianchini 2011[i] [56]	Retro, case-control	WDL/DDL from lipoma	50	NR	56 (0.5 to 81)	*HMGA2* rearrangement by FISH (NR)	Histological results from resection	100 (75 to 100)	57 (40 to 73)
			54		56 (0.5 to 81)	*HMGA2* mRNA overexpression by real-time PCR (a log10 value of >1 for exons 1-2, 3-4, or 4-5)		100 (75 to 100)	17 (7 to 32)
Ito 2011 [58]	Retro, case-control	WDL/DDL from benign tumours (mainly lipoma)	65	NR	NR	*MDM2* amplification by real-time PCR (>6 gene copies with >3-fold)	Histological results	64 (44 to 81)	92 (78 to 100)
Narendra 2011 [30]	Retro, case-control	ML from other STS	38	NR	NR	*DDIT3* gene break-apart by FISH (≥20% rearrangement)	Histological results from resection or core needle biopsy	100 (77 to 100)	100 (86 to 100)
Tap 2011 [34]	Retro, case-control	WDL/DDL from benign tumours (mainly lipoma)	46	Primary or recurrent	58 (32 to 89)	12q13-15 amplification by CMA (DNA copy number gains with $\log_2$ ratio >1)	Histological results from resection	100 (91 to 100)	100 (63 to 100)
Kashima 2012 [3]	Retro, case-control	ALT/DDL from benign tumours (mainly lipoma)	217	NR	NR	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 >2)	Histological results	87 (79 to 93)	98 (93 to 100)
Miura 2012 [12]	Retro, case-control	WDL/DDL from other liposarcoma types	50	NR	NR	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 ≥3)	Histological results	92 (75 to 99)	96 (79 to 100)

		and lipoma							
Cantile 2013 [33]	Retro, case-control	WDL/DDL from lipoma	35	Primary/ Recurrent	60 (17 to 91)	12q13-15 rearrangement by FISH (NR)	Histological results from resection	100 (85 to 100)	100 (74 to 100)
Kimura 2013 [4]	Retro, cohort	ALT/WDL/ DDL from lipoma	79	NR	NR	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 >2)	Histological results from resection	98 (90 to 100)	100 (86 to 100)
		DDL from other STS	81					100 (48 to 100)	95 (87 to 99)
Wang 2014 [31]	Retro, case-control	ML from myxofibro -sarcoma	61	NR	NR (20 to 90)	*DDIT3* translocation by FISH (NR)	Histological results	76 (57 to 90)	100 (89 to 100)
Ware 2014 [13]	Retro, case-control	WDL and DDL from lipoma and other sarcomas	46	NR	59 (22 to 86)	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 >2)	Histological results	94 (81 to 99)	100 (69 to 100)
Creytens 2015 [1]	Retro, case-control	WDL/DDL from lipoma	67	NR	NR	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 >2)	Histological results	100 (91 to 100)	100 (87 to 100)
						MDM2 amplification by MLPA (Peak value >2)		90 (76 to 97)	100 (87 to 100)
						CDK4 amplification by FISH (a ratio of *CDK4* to CEP12 >2)		90 (76 to 97)	100 (87 to 100)
						CDK4 amplification by MLPA (Peak value >2)		80 (64 to 91)	100 (87 to 100)
Mardekian 2015 [5]	Retro, case-control	WDL/DDL from benign tumours (mainly lipoma)	31	NR	54 (25 to 81)	*MDM2* amplification by FISH (a ratio of *MDM2* to CEP12 ≥2)	Histological results	75 (43 to 95)	100 (82 to 100)
						MDM2 amplification by CISH (a ratio of *MDM2* to CEP12 ≥2)		83 (52 to 98)	95 (74 to 100)
Thway 2015 [7]	Retro, case-control	WDL/DDL from benign tumours (mainly lipoma)	297	NR	59 (12 to 95)[j]	*MDM2* amplification by FISH (2-4 CEP12 signals with ≥6extra *MDM2* signals)	Histological results and IHC from resection and biopsy	84 (78 to 89)	93 (87 to 97)
		DDL from other STS	131(53%)					96 (88 to 99)	77 (65 to 87)
Low-grade fibromyxoid sarcoma (LGFM)									
Matsuyama 2006 [60]	Retro, case-control	LGFM from LGFM-like tumours	139	NR	33 (13 to 58)[k]	*FUS-CREB3L2* fusion by RT-PCR (NR)	Histological results	88 (62 to 98)	100 (97 to 100)
Guillou 2007 [59]	Retro, cohort	LGFM from LGFM-like tumours	111	Primary	35 (11 to 75)[l]	*FUS-CREB3L1* or *FUS-CREB3L2* fusion by RT-PCR (NR)	Histological results	81 (69 to 90)	87 (74 to 94)
Malignant peripheral nerve sheath tumour (MPNST)									
Brekke 2010 [35]	Retro, case-control	MPNST from cutaneous neurofibro mas	58[j]	Primary/ Recurrent	33[m] (11 to 79)	DNA copy number changes by CMA (≥2)	Histological results	92 (80 to 98)	90 (56 to 100)
Wallander 2012 [36]	Retro, case-control	MPNST from neurofibro ma and schwanno ma	44	NR	NR	*MDM2* amplification by FISH (A ratio of *MDM2* to CEP12 >2)	Histological results	20 (4 to 48)	100 (88 to 100)
Synovial sarcoma (SS)									
Terry 2005 [17]	Retro, case-control	SS from other sarcomas	46	NR	NR	*SS18* break-apart by FISH (SSR=3.46[l])	Histomorpho logical results +	96 (78 to 100)	100 (85 to 100)

							molecular confirmation		
Thorson 2006 [20]	Retro, case-control	SS from other sarcomas	32	NR	33 (4 to 72)	*SS18-SSX* fusion by RT-PCR (NR)	Histomorphological results	77 (55 to 92)	100 (69 to 100)
Amary and Berisha 2007 [18]	Retro, case-control	SS from normal tissue and other sarcoma	325	NR	35 (5 to 81)[n]	*SS18-SSX* fusion by RT-PCR (NR)	Morphology and IHC	92 (86 to 96)	100 (98 to 100)
Sun 2008 [14]	Retro, case-control	SS from other sarcoma	115	NR	NR	*SS18-SSX* fusion by RT-PCR (NR)	Histological results+ clinical content+IHC	95 (85 to 99)	100 (94 to 100)
			119			*SS18* break-apart by FISH (≥16.39% cell nuclei had orange and green signals)		97 (89 to 100)	100 (94 to 100)
Ten Heuvel 2008 [16]	Retro, case-control	SS from other sarcoma	60	NR	NR	*SS18-SSX* fusion by RT-PCR (NR)	Histological results	98 (89 to 100)	100 (74 to 100)
			53			*SS18* break-apart by FISH (≥15% cell nuclei had orange and green signals)		93 (81 to 99)	100 (66 to 100)
Tanas 2010 [15]	Retro, case-control	SS from other sarcoma	40	NR	NR	*SS18* break-apart by FISH (≥10% of cells showed rearrangement)	Histological results	96 (81 to 100)	100 (75 to 100)

Abbreviations: ALB = albumin gene, ALT = atypical lipomatous tumour, AVL = atypical vascular lesion, CEP = centromere-specific probe for chromosome, CMA = chromosomal microarray, CI = confidence interval, DDL = dedifferentiated liposarcoma, DF = dermatofibromas, DNA = deoxyribonucleic acid, EAS = epithelioid angiosarcoma, ESN = endometrial stromal nodule, FISH = fluorescence in situ hybridization, IHC = immunohistochemistry, IRF = idiopathic retroperitoneal fibrosis, LGFM = low-grade fibromyxoid sarcoma, ML = myxoid liposarcoma, MM = malignant melanoma, MLPA = multiplex ligation-dependent probe amplification (a PCR-based technique), MPNST = malignant peripheral nerve sheath tumor, mRNA = messenger ribonucleic acid, MSRED = mutation-specific restriction enzyme digestion, NA = not applicable, NGS = next-generation sequencing, NR = not reported, PAS = primary angiosarcoma, PCR = polymerase chain reaction, PNST = peripheral nerve sheath tumour, PRS = primary sarcoma (rather thann angiosarcoma), Retro = retrospective, RT-PCR = reverse transcription-PCR, SAS = Breast radiation-induced secondary angiosarcoma, Sen = sensitivity, SFT = solitary fibrous tumour, Spec = specificity, SM = sclerosing mesenteritis, SS = synovial sarcoma, SSR = sample score ratio, ST = soft tissue, STS = soft tissue sarcomas, UUS = undifferentiated uterine sarcoma, VAF = variant allele fraction, WDL = well differentiated liposarcoma.

[a]Thirty-three angiosarcoma patients were overlapped in the Manner 2010 study.

[b]This information came from 98 patients.

[c]Some patients were overlapped with those in the Salgado 2011 study.

[d]This information came from 40 patients

[e]This information came from 254 patients.

[f]This information came from 144 patients

[g]This information came from 52 patients.

[h]This paper may include some patients that were recruited in the Weaver 2010 paper.

[i]Some patients may be overlapped with those in the Sirvent 2007 study.

[j]This information came from 347 patients.

[k]This information came from 63 LGFM patients only.

[l]This study recruited 51 patients with 58 cases, and the age information came from 48 MPNST patients.

[m]Sample score ratio was defined as the ratio of paired signals (an orange and green signal <3 signal diameters apart or a single yellow signal) to unpaired signals (≥3 signal diameters from a oppositely colored signal).
[n]This information came from 134 patients.

Table 4-2. Characteristics and outcomes for prognostic studies (different types of soft tissue sarcomas ordered alphabetically and eligible studies under each type ordered by publication year)

Study	Study design; IC	N	Mean or median age (range), years	Sarcoma type	Median follow-up time (range), months	Molecular alteration; Positive threshold; lab method	Outcome
Angiosarcoma							

Fraga-Guedes 2015 [37]	Retro; IC	37	69 (37 to 88)	Breast radiation-induced secondary angiosarcoma	25 (1 to 159)	*MYC* amplification (with vs. without); *MYC*/CEP8≥2.0; FISH	OS: HR=3.47; 95% CI 1.09 to 11.1; p=0.035 DFS: HR=1.89; 95% CI 0.78 to 4.55; p=0.155
Huang 2016 [38]	Retro; Non-IC	82	58 (13 to 91)	Primary/secondary angiosarcoma	27 (0.3 to 234)	*CIC* alteration (with vs. without); ≥20% of nuclei had a break-apart signal; FISH	DFS: HR=3.46; 95% CI 1.42 to 8.44; p=0.006
Desmoid tumour (fibromatosis)							
Lazar 2008 [22]	Retro; IC	138	32 (0.2 to 78)	Sporadic desmoid tumour	NR	*CTNNB1* gene (T41A, S45F, or S45P vs. WT);NR; PCR	5-year RFS: T41A vs. WT: HR=1.11; 95% CI 0.48 to 2.56; p=0.806 S45F vs. WT: HR=3.50; 95% CI 1.51 to 8.14; p=0.004 S45P vs. WT: HR=1.13; 95% CI 0.33 to 3.86; p=0.850
Domont 2010 [83]	Retro; Non-IC	101	37 (0.1 to 77)	Extra-abdominal fibromatosis	62 (3 to 452)	*CTNNB1* mutation (Mutation status vs. wild-type); NR; PCR	5-year RFS: 49% vs. 75%, p=0.02
Romero 2012 [85]	Retro; IC	69	41 (13 to 94)	Fibromatosis	NR	*CTNNB1* gene (T41A or S45F vs. WT); NR; PCR	5-year RFS: HR=NR, p=NS
Colombo 2013 [21]	Retro; IC	179	39 (IQR 31 to 52)	Desmoid tumour	50 (IQR 28 to 84)	*CTNNB1* gene (S45F vs. T41A/S45P, T41A/S45P vs. WT); NR; PCR	RFS: S45F vs. T41A/S45P: HR=2.59; 1.19 to 5.65; p=0.05 T41A/S45P vs. WT: HR=2.26; 95% CI 1.02 to 5.03; p=0.05
Van Broekhoven 2015 [23]	Retro; IC	101	36 (IQR 28 to 44)	Fibromatosis	41 (IQR 18 to 71)	*CTNNB1* gene (S45F vs.no S45F); NR; PCR	5-year RFS: HR=6.20; 95% CI 2.24 to 17.15; p<0.001
Kim HS 2016 [84]	Retro; IC	159	41 (7 to 83)	Desmoid tumour	NR	*CTNNB1* gene (mutation vs. wild-type); NR; PCR	RFS: HR=1.229; 95% CI 0.659 to 2.289; p=0.517
Liposarcoma							
Oda 2005 [41]	Retro; Non-IC	120	46 (18 to 83)	MLS/RCL	77 (2 to 393)	*MDM2* amplification (with vs. without); >2-fold amplification; PCR	OS: p=0.179
Crago 2012 [43]	Retro; Non-IC	40	60 (41 to 90)[a]	DDL	28 (NR)	19q13 loss (with vs. without); NR; CGH	LRFS: HR=2.99; 95% CI 1.30 to 6.83; p=0.01
Lee S 2014[b] [69]	Retro; Unclear	48	57 (37 to 78)	WDL and DDL	19 (DDL) and 35 (WDL)	*CDK4* amplification (high level vs. low level); high level: copy number of *CDK4* to ALB ≥7.54, low level <7.54 but >2; real-time PCR	LRFS: HR=12.08; 95% CI 1.48 to 98.83; p=0.020
Lee SE 2014 [40]	Retro; Unclear	56	56 (27 to 76)	WDL and DDL	43 (2 to 175)	*MDM2* amplification (high level vs. low level); high level: copy number of *MDM2* to ALB ≥10, low level <10 but >2.2; real-time PCR	DSS: HR=1.35; 95% CI 0.144 to 12.5; p=0.794 PFS: HR=1.22; 95% CI 0.45 to 2.39; p=0.655
						CDK4 amplification (high level vs. low level); high level: copy number of *CDK4* to ALB ≥10, low level <10 but >2.2; real-time PCR	DSS: HR=2.19; 95% CI 1.42 to 23.5; p=0.044 PFS: HR=3.08; 95% CI 1.03 to 10.81; p=0.048
Jour 2015 [39]	Retro; Unclear	50	63 (26 to 88)	DDL	28 (1 to 121)	*MDM2* amplification (high level vs. low level); high level: ≥20 fluorescent	LR: HR=1.92; 95% CI 0.81 to 4.58; p=0.138

						signals/cell, low level: <20 but >5; FISH	
Saada-Bouzid 2015 [42]	Retro; Non-IC	90	66 (38 to 90)[c]	ALT, WDL, and DDL	41 (95% CI 32 to 46)	*CDK4* amplification (Yes vs. No); ≥10 fluorescent signals/cell in ≥1% of cells; FISH	2-year OS: p=NS 2-year RFS: p=NS
		91	66 (38 to 90)[c]	ALT, WDL, and DDL	41 (NR)	*HMGA2* amplification (Yes vs. No); ≥10 fluorescent signals/cell in ≥1% of cells; FISH	2-year RFS: p=NS
Kim JH 2016 [68]	Retro; IC	101	52 (18 to 84)[d]	Primary liposarcoma	51	*PIK3CA* amplification; >4 copies/cell or a ratio of PIK3CA/CEN3 ≥2; FISH	DFS: HR=2.40; 95% CI 1.11 to 5.21; p=0.027 OS: HR=1.63; 95% CI 0.56 to 4.77; p=0.375
Malignant peripheral nerve sheath tumour							
Brekke 2010 [35]	Retro; Non-IC	48	32 (11 to 79)	MPNST	36 (1 to 369)	Loss from Xq or 10q or gain at 16p (Yes vs. No); ≥2 aberrations; CGH	10-year OS: Loss from Xq or 10q or gain at 16p: HR=11.0; 95% CI 3.5 to 35; p<0.001
Yu 2011 [44]	Retro; Non-IC	38	37 (11 to 73)	MPNST	NR	*CDK4* gain (Yes vs. No), *FOXM1* gain (Yes vs. No), *NOL1* gain (Yes vs. No), *SOX5* gain (Yes vs. No), *MYC* gain (Yes vs. No); NR; CGH	OS: CDK4 gain: HR=4.22; 95% CI 1.43 to 12.44; p=0.009 FOXM1 gain: p=NS NOL1 gain: p=NS SOX5 gain: p=NS MYC gain: p=NS
	Retro; Non-IC	87	36 (1 to 86)	MPNST	NR	*CDK4* amplification (Yes vs. No); NR; FISH	OS: CDK4 amplification: HR=2.04; 95% CI 1.03 to 4.04; p=0.041
Synovial sarcoma							
Canter 2008 [61]	Retro; IC	132	39 (16 to 80)	Synovial sarcoma	72 (0 to 287)	*SS18-SSX1* vs. *SS18-SSX2*; NR; RT-PCR	DSS: AR=-0.22; 95% CI -1.87 to 1.43; p=0.794 MFS: AR=-1.15; 95% CI -2.12 to -0.19; p=0.019
Takenaka 2008 [63]	Retro; Non-IC	81	37 (8 to 74)[e]	Synovial sarcoma	54 (4 to 216)	*SS18-SSX1* vs. SS18-SSX2; NR; RT-PCR	OS: RR=1.46; 95% CI 0.64 to 3.28; p=0.36
		67	37 (8 to 74)[e]	Localized synovial sarcoma	54 (4 to 216)	*SS18-SSX1* vs. *SS18-SSX2*; NR; RT-PCR	OS: RR=1.57; 95% CI 0.55 to 4.24; p=0.38 MFS: RR=0.67; 95% CI 0.25 to 1.60; p=0.38
Sun 2009 [62]	Retro; Non-IC	141	37 (4 to 74)	Synovial sarcoma	54 (1 to 246)	*SS18-SSX1* vs. *SS18-SSX2*; NR; RT-PCR	DSS: RR=2.03; 95% CI 1.26 to 3.28; p=0.004 MFS: RR=1.86; 95% CI 1.04 to 3.33; p=0.037
Ren 2013 [70]	Retro; Non-IC	88	33 (11 to 58)	Synovial sarcoma	43 (12 to 110)	*SS18-SSX1* vs. *SS18-SSX2*; NR; RT-PCR	OS: RR=2.34; 95% CI 2.18 to 2.67; p=0.002

Abbreviations: ALT = atypical lipomatous tumour, AR = acceleration rate, CEP = centromere-specific probe for chromosome, CGH = comparative genomic hybridization, CI = confidence interval, DDL = dedifferentiated liposarcoma, DFS = disease-free survival, DSS = disease-specific survival, FISH = fluorescence in situ hybridization, HR = hazard ratio, IC = inception cohort of patients at a similar point in the course of a disease, IQR = interquartile range, KPS = Karnofsky performance status, MFS = metastasis-free survival, MLS = myxoid liposarcoma, LR = local

recurrence, LRFS = local recurrence-free survival, N = sample size, NA = not applicable, NR = not reported, NS = not statistically significantly different, OS = overall survival, PCR = polymerase chain reaction, PFS = progression-free survival, RCLS = round cell liposarcoma, Retro = retrospective study, RFS = recurrence-free survival, RR = relative risk, vs. = versus, RT-PCR = reverse transcription, WDL = well-differentiated liposarcoma, WT = wild type (no mutation).

[a]This information came from 52 patients; 40 of them had final outcomes.

[b]There were 50% of patients overlapped in the Lee SE 2014 paper.

[c]This information came from 116 patients.

[d]This information came from 125 patients.

[e]This information came from 108 patients.

Table 4-3. Meta-analysis results for diagnostic outcomes

Disease	Purpose	Molecular test	Sample size (studies)	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)
Liposarcoma	Differentiating ALT/WDL/DDL from benign tumours (mainly lipoma)	*MDM2* amplification by FISH	971 (10)	95 (89 to 98)	100 (89 to 100)

Disease	Purpose	Molecular test	Sample size (studies)	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)
	Differentiating ALT/WDL/DDL from other sarcomas	*MDM2* amplification by FISH	347 (4)	99 (72 to 100)	90 (78 to 95)
	Differentiating ALT/WDL/DDL from benign tumours (mainly lipoma)	*MDM2* amplification by real-time PCR	346 (4)	88 (70 to 96)	98 (91 to 99)
	Differentiating ML from lipoma and other STS	*DDIT3* rearrangement (FISH)	189[a] (4)	96 (69 to 100)	100 (80 to 100)
Synovial sarcoma	Differentiating synovial sarcoma from other sarcomas	*SS18* break-apart by FISH	258[a] (4)	94 (89 to 97)	97 (60 to 100)
		SYT-SSX fusion by RT-PCR	532[a] (4)	93 (85 to 96)	99 (96 to 100)

Abbreviations: ALT = atypical lipomatous tumour, CI = confidence interval, DDL = dedifferentiated liposarcoma, FISH = fluorescence in situ hybridization, ML = myxoid liposarcoma, PCR = polymerase chain reaction, RT-PCR = reverse transcription-PCR, STS = soft tissue sarcomas, WDL = well-differentiated liposarcoma.

[a]STATA 11 software (TX: StataCorp LP) did not produce an output initially and showed "initial values not feasible", which may be because there were several "0" values in the four studies. Arbitrary values were input as least as possible in only one study in order to generate an output. These would have underestimated the sensitivity or specificity of the test. However, both of the calculated sensitivity and specificity reached the pre-planned threshold of 90%. Therefore, we believe this arbitrary change did not impact the conclusions.

Table 4-4. Main findings for molecular tests for diagnosis and prognosis in Non-GIST STS[a]

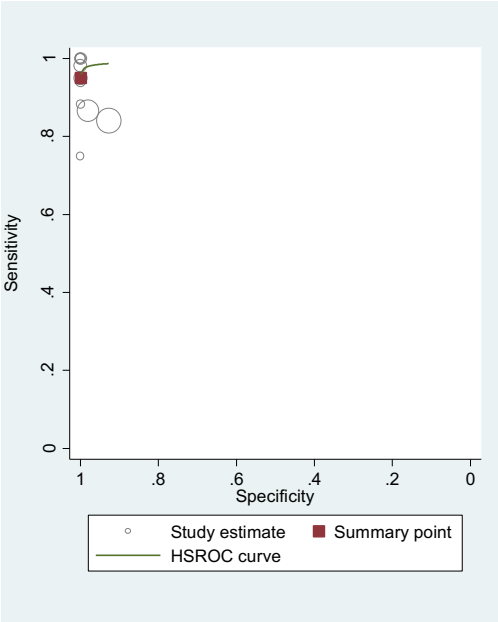
Type of STS	Molecular test	Purpose	Findings[b]
Angiosarcoma	*MYC* amplification (FISH)	SAS from PAS and/or AVL	Diagnosis: Maybe Yes Prognosis: Maybe Yes for OS, Maybe No for DFS
	CIC alteration (FISH)	SAS/PAS	Prognosis: Maybe Yes for DFS

Desmoid tumors	*CTNNB1* mutation (PCR/NGS/direct Sanger sequencing)	Demoid tumor from histological mimics	Diagnosis: Maybe Yes ***Prognosis: CTNNB1 S45F mutation: Yes***; other CTNNB1 gene mutations: Uncertain
Liposarcoma	*MDM2* amplification (FISH)	ALT/WDL/DDL from benign tumors (mainly lipoma) or other STS	***Diagnosis: Yes*** Prognosis: Maybe No for LR
	MDM2 amplification (real-time PCR)	ALT/WDL/DDL from benign tumors (mainly lipoma) or other STS	Diagnosis: Maybe Yes Prognosis: Maybe No for DSS and PFS
		ML/RCL	Prognosis: Maybe No for OS
	CDK4 amplification (FISH/real-time PCR/CMA)	ALT/WDL/DDL from benign tumors (mainly lipoma) or other STS	Diagnosis: Maybe Yes Prognosis: Uncertain
	HMGA2 rearrangement (FISH/real-time PCR)	WDL/DDL from lipoma	Diagnosis: Uncertain Prognosis: Maybe No for RFS
	DDIT3 rearrangement (FISH)	ML from other STS and lipoma	Diagnosis: Maybe Yes
	12q13-15 amplification/ rearrangement (CMA/FISH)	WDL/DDL from benign tumors (mainly lipoma)	Diagnosis: Maybe Yes
	CPM amplification (FISH)	ALT/WDL from lipoma	Diagnosis: Maybe Yes
	FUS rearrangement (FISH)	ML from other myxoid soft tissue tumors	Diagnosis: Maybe Yes
	19q13 loss (with vs. without, CMA)	DDL	Prognosis: Maybe Yes for local RFS
	PIK3CA amplification (FISH)	Liposarcoma	Prognosis: Uncertain
MPNST	DNA copy number changes (CMA)	MPNST from cutaneous neurofibroma	Diagnosis: Maybe Yes
	MDM2 amplification (FISH)	MPNST from neurofibroma and schwannoma	Diagnosis: Maybe No
	Loss from Xq or 10q, gain at 16p, *CDK4* gain or amplification, *FOXM1* gain, *NOL1* gain, *SOX5* gain, *MYC* gain (CMA)	MPNST	Prognosis: Maybe Yes for loss from Xq or 10q, or gain at 16p on OS. Maybe Yes for CDK4 gain or amplification on OS. Maybe No for FOXM1 gain, NOL1 gain, SOX5 gain, MYC gain on OS.
Synovial sarcoma	*SS18* break-apart (FISH) or *SS18-SSX* fusion (RT-PCR)	Synovial sarcoma from other sarcomas	***Diagnosis: Yes*** Prognosis: Maybe Yes for MFS, but uncertain for OS and DSS
Clear cell sarcoma (CCS)	*EWSR1* rearrangement (FISH)	CCS from MM	Diagnosis: Maybe Yes
DFSP	*PDGFB* break-apart (FISH) or *COL1A1/PDGFB* fusion (RT-PCR)	DFSP from DF	Diagnosis: Maybe Yes
Epithelioid sarcoma (ES)	*SMARCB1* deletion (FISH)	ES from ES mimickers	Diagnosis: Maybe Yes
Endometrial stromal sarcoma (ESS)	*JAZF1* rearrangement (FISH)	ESS from ESN+UUS	Diagnosis: Maybe No
EHE	*WWTR1/CAMTA1* rearrangement (FISH)	EHE from EAS	Diagnosis: Uncertain
HPC	*NAB2*ex6-*STAT6*ex16/17 fusion (RT-PCR)	HPC from SFT	Diagnosis: Maybe Yes

Hybrid HFLT-MIFS	*TGFBR3/MGEA5* rearrangement (FISH)	Hybrid HFLT-MIFS from MIFS (21%)	Diagnosis: Uncertain
LGFM	*FUS-CREB3L1* or *FUS-CREB3L2* fusion (RT-PCR)	LGFM from LGFM-like tumors	Diagnosis: Maybe Yes

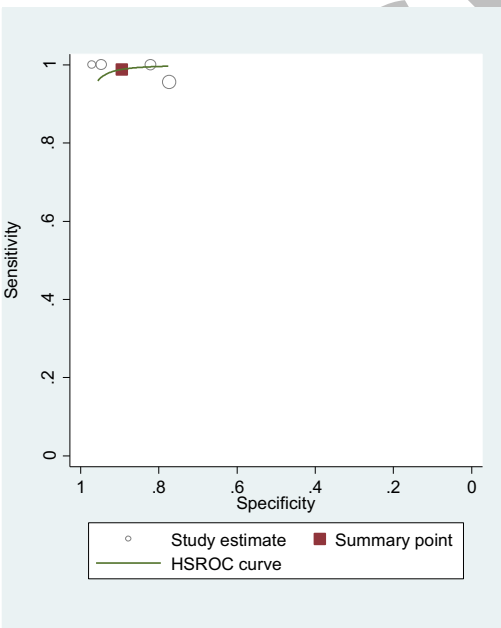
Abbreviation: ALT = atypical lipomatous tumour, AVL = atypical vascular lesion, CMA = chromosomal microarray, DF = dermatofibromas, DFSP = dermatofibrosarcoma protuberans, DDL = dedifferentiated liposarcoma, DFS = disease-free survival, DSS = disease-specific survival, EAS = epithelioid angiosarcoma, EHE = epithelioid hemangioendothelioma, ESN = endometrial stromal nodule, FISH = fluorescence in situ hybridization, HFLT = hemosiderotic fibrolipomatous tumour, HPC = hemangiopericytoma, LGFM = Low-grade fibromyxoid sarcoma, LR = local recurrence, MFS = metastasis-free survival, MIFS = myxoinflammatory fibroblastic sarcoma, ML = myxoid liposarcoma, MM = malignant melanoma, MPNST = malignant peripheral nerve sheath tumor, NGS = next generation sequencing, OS = overall survival, PAS = primary angiosarcoma, PCR = polymerase chain reaction, PFS = progression-free survival, RFS = recurrence-free survival, RT-PCR = reverse transcription-PCR, SAS = breast radiation-induced secondary angiosarcoma, SFT = solitary fibrous tumour, STS = soft tissue sarcomas, UUS = undifferentiated uterine sarcoma, WDL = well-differentiated liposarcoma.
[a]For prognostic research question, patients with the molecular alteration is compared with patient without the molecular alteration.
[b]"Maybe" means that we are not very confident to support the finding for this molecular test in diagnosis or predicting prognosis due to low or very low quality of evidence.

Figure 4-1. HSROC curve for *MDM2* amplification test by FISH to differentiate ALT/WDL/DDL from benign tumours



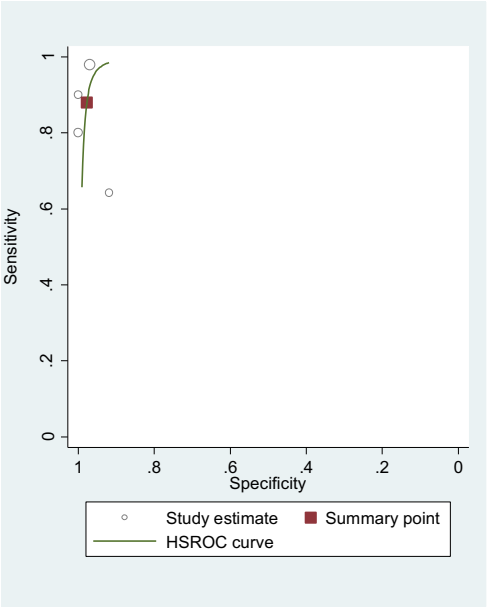
ALT, atypical lipomatous tumor; DDL, dedifferentiated liposarcoma; FISH, fluorescence in situ hybridization; HSROC, hierarchical summary receiver operating characteristic; WDL, well-differentiated liposarcoma.

Figure 4-2. HSROC curve for *MDM2* amplification test by FISH to differentiate ALT/WDL/DDL from other sarcomas



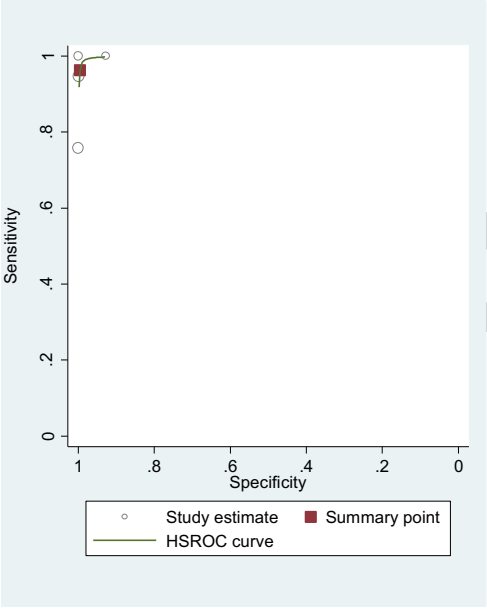
ALT, atypical lipomatous tumor; DDL, dedifferentiated liposarcoma; FISH, fluorescence in situ hybridization; HSROC, hierarchical summary receiver operating characteristic; WDL, well-differentiated liposarcoma.

Figure 4-3. HSROC curve for *MDM2* amplification test by real-time PCR to differentiate ALT/WDL/DDL from benign tumours



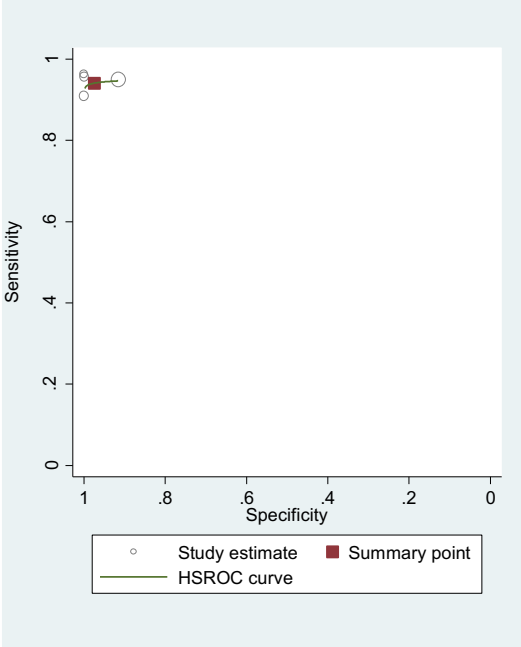
ALT, atypical lipomatous tumor; DDL, dedifferentiated liposarcoma; PCR, polymerase chain reaction; HSROC, hierarchical summary receiver operating characteristic; WDL, well-differentiated liposarcoma.

Figure 4-4. HSROC curve for *DDIT3* amplification test by FISH to differentiate ML from other STS



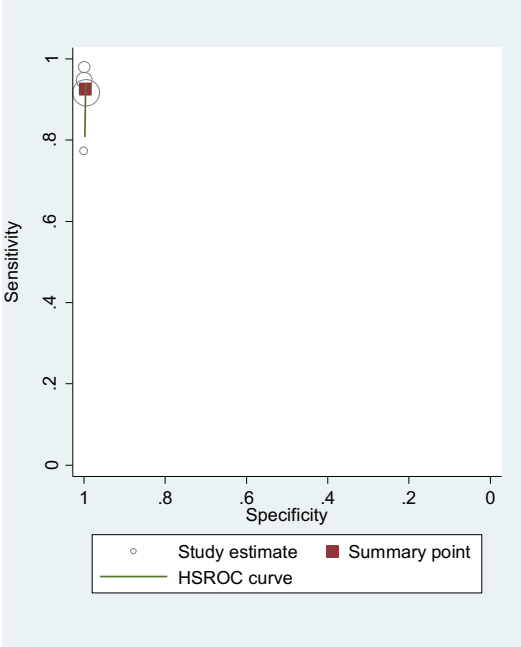
FISH, fluorescence in situ hybridization; HSROC, hierarchical summary receiver operating characteristic; ML, myxoid liposarcoma.

Figure 4-5. HSROC curve for *SYT-SSX* fusion test by FISH to differentiate synovial sarcoma from other sarcomas



FISH, fluorescence in situ hybridization; HSROC, hierarchical summary receiver operating characteristic.

Figure 4-6. HSROC curve for *SYT-SSX* fusion test by RT-PCR to differentiate synovial sarcoma from other sarcomas



HSROC, hierarchical summary receiver operating characteristic; RT-PCR, reverse transcription-polymerase chain reaction.

Molecular Analyses in the Diagnosis, Prognosis, and Selection of Therapy in non-GIST Soft Tissue Sarcomas

Section 5: Internal and External Review

INTERNAL REVIEW

The guideline was evaluated by the Expert Panel and the PEBC Report Approval Panel (RAP) (Appendix 2). The results of these evaluations and the Working Group's responses are described below.

Expert Panel Review and Approval

Of the seven members of the Expert Panel, six cast votes and one abstained, for a total of 86% response in November 2017. Of those that cast votes, six approved the document (100%). The main comments from the Expert Panel and the Working Group's responses are summarized in Table 5-1.

Table 5-1. Summary of the Working Group's responses to comments from the Expert Panel.

Comments	Responses
1. Is immunohistochemistry (IHC) for *MDM2* not presently recommended?	This guideline focuses on molecular analysis, so IHC examination results are beyond the scope.
2. In Recommendation Part II, the molecular tests that were recommended and those that were not recommended are mixed, which makes it hard for readers to follow.	We have classified recommendations into two categories: **"Recommend to USE these gene tests",** and **"Recommend NOT to USE these gene tests"** under every Recommendation Part**.** Also, we have specified which recommendation is for diagnostic or prognostic purpose.
3. "Comparative genomic hybridization (CGH)" refers to a specific type of array technology. A more generic term is preferable, such as chromosomal microarray (CMA).	We have used "CMA" instead of "CGH".
4. I think that the positive thresholds in these tables were used in supporting evidence and may vary from established in laboratory cut-offs developed via an approved validation for a laboratory developed tests. Also, depending on probe/primer design or assay strategy, these values serve only as a reference to the validity of the test in the context of disease.	We discussed this point under ***Interpretation of Evidence for Recommendation Part II and Qualifying Statements***. But we have added more explanation base on the reviewer's comments.
5. The Erickson-Johnson 2009 paper reported that *CPM* amplification test by FISH might be a sensitive and specific test to differentiate patients with ALT/WDL from patients with lipoma (both sensitivity and specificity are 100%). Does anybody actually do this in Ontario?	The Working Group members agreed with the reviewer's comment that no one in Ontario tests for *CPM* amplification. As *CPM* is in the same area as MDM2 in the diagnosis of liposarcomas, which is recommended in this guideline, this recommendation regarding *CPM* amplification test has been removed from **Sections 1 and 2**.

RAP Review and Approval

Four RAP members, including the PEBC Director, reviewed and approved this document in November 2017 after the following modifications in Table 5-2. The main comments from the RAP and the Working Group's responses are summarized in Table 5-2.

Table 5-2. Summary of the Working Group's responses to comments from RAP.

Comments	Responses
1. What are the definitions for "Strong Recommendations", "Recommendations", and "No Recommendations"?	We have discussed with the PEBC director, Melissa Brouwers, and added the definitions for the strength of recommendations in **Section 1**.
2. "Data were not extracted from the diagnostic paper if it reported the sensitivity of a molecular test as less than 50% (i.e. lower than the probability of either heads or tails resulting from a coin toss), suggesting that the index test is not useful in clinical practice." I think these papers should be reported and the tests from these papers may be recommended not to be used in the clinical practice at present.	We have carefully reviewed these papers and added the appropriate information into the document.
3. I am not sure whether it is necessary to add the follow-up time information into the prognostic recommendations.	We have removed all the follow-up information from recommendations to make the recommendations more concise, and also the key evidence showed this follow-up information.
4. I agree with the idea of not extracting data from the studies that had sensitivity of <50% for a molecular test. But would it not be useful to provide a list of these tests as things NOT to use?	We have reviewed these studies carefully and added the appropriate data and recommendations in this document
5. "To differentiate ALT/WDL/DDL from other STS, four studies were pooled in a meta-analysis with a pooled sensitivity of 99% (95% CI, 72% to 100%) and a pooled specificity of 90% (95% CI, 78% to 95%) with a combined sample size of 347 (Table 4-3)." I would like to avoid using the word "pooled" because it may let the readers think that you used the incorrect methods to pool the sensitivity and specificity from these four studies respectively, rather than a bivariate random-effects meta-regression model to obtain sensitivity and specificity at the same time.	We have changed the wording based on the reviewer's comments.
6. In DISCUSSION: "Two additional overall limitations include that we required that a study could only be included if it had a sample size of ≥30 patients analyzed from a statistical perspective consideration (the Central Limit Theory)." I would argue this is strength, not a limitation, of your review.	We have changed the wording based on the reviewer's comments.
7. The 95% confidence region and 95% prediction region of Figures 4-2 to 4-6 for the meta-analyses of diagnostic studies	A confidence interval is usually for individual parameter and is considered as a point estimate; a confidence region usually covers the complete

seem HUGE. Also, it seems there is no relation to the values reported in the Table 4-3. For example, the 95% confidence region of Figure 4-2 would suggest that the confidence region was about 30% to 100% for the sensitivity and about 20% to 100% for the specificity. While the calculated sensitivity was 99% (95% CI, 72% to 100%) and the calculated specificity was 90% (95% CI, 78% to 95%).	range of data and incorporates both uncertainty in the parameter estimate and prediction error [98]. A prediction region contains a future observation during a specified percentage of the time [99], and therefore it should be wider than confidence region. Except for Figure 4-1, other Figures only have four studies to perform the mate-analysis respectively, which is the minimal study requirement for STATA software version 11. to conduct a meta-analysis. That may be the reason why their 95% confidence regions and 95% prediction regions are very wide. In order not to confuse the readers, we have removed 95% confidence region and 95% prediction region from all the Figures. We believe that study estimate, summary point, and HSROC curve in each Figure demonstrate enough information for readers. Also, STATA software version 11. did not produce an output initially and showed "initial values not feasible" for three meta-analyses in Table 4-3, which may be because there were several "0" values in the four studies for each meta-analysis. Arbitrary values were input as least as possible in only one study in order to generate an output. These would have underestimated the sensitivity or specificity of the test. However, both of the calculated sensitivity and specificity reached the pre-planned threshold of 90% for these three meta-analyses. Therefore, we believe this arbitrary change did not impact the conclusions.
8. Table 4-2 was easy to follow, but I got confused in Table 4-1 until I realized it was alphabetical. Maybe state this in brackets after the table title.	We have added brackets in the titles of Tables 4-1 and 4-2: "(different types of soft tissue sarcoma ordered alphabetically and eligible studies under each type ordered by publication year)".
9. Will this be updated as it is now Oct 2017?	The first literature search was conducted in January 2016 and we updated the search in October 2016. We do not have resource to update again at this moment. However, every PEBC guideline is assessed for its relevance and usefulness annually after it is published on the CCO website.

EXTERNAL REVIEW

External Review by Ontario Clinicians and Other Experts

Targeted Peer Review

Nine targeted international peer reviewers who are considered to be clinical and/or methodological experts on the topic were identified by the Working Group and contacted. Three agreed to be reviewers, and the remaining six automatically became Professional Consultation reviewers (See details in ***Professional Consultation*** part below). Two responses were received by February 9 2018 (Appendix 2). Results of the feedback survey are summarized in Table 5-3. The main comments from targeted peer reviewers and the Working Group's responses are summarized in Table 5-4.

Table 5-3. Responses to nine items on the targeted peer reviewer questionnaire.

	Reviewer Ratings (N=2)				
Question	Lowest Quality (1)	(2)	(3)	(4)	Highest Quality (5)
1. Rate the guideline development methods.				1	1
2. Rate the guideline presentation.			1	1	
3. Rate the guideline recommendations.				2	
4. Rate the completeness of reporting.				1	1
5. Does this document provide sufficient information to inform your decisions? If not, what areas are missing?				2	
6. Rate the overall quality of the guideline report.			1	1	
	Strongly Disagree (1)	(2)	Neutral (3)	(4)	Strongly Agree (5)
7. I would make use of this guideline in my professional decisions.		1		1	
8. I would recommend this guideline for use in practice.		1		1	
9. What are the barriers or enablers to the implementation of this guideline report?	• Funding may potentially be a barrier for some molecular testings; if a diagnosis can be made confidently via hematoxylin & eosin (H&E) and IHC, in the absence of other prognostic or therapeutic information that may be gained by molecular testing, the referring lab may not wish to pursue molecular testing as the expense will come out of the referring institutions' budget.				

Table 5-4. Responses to comments from targeted peer reviewers.

Comments	Responses
1. It is very thorough (perhaps even too inclusive in terms of molecular data), which may be at the risk of neglecting reliable and cheaper testing in some instances (e.g., use of IHC).	The objectives of this guideline focused on molecular analysis in the diagnosis, prognosis, and selection of therapy in non-STS rather than comparing the diagnostic and prognostic value with other non-molecular tests or predictive risk factors.
2. Consider page breaks to better separate strong recommendations from recommendations, etc. for easier reading.	We have changed the fonts and distance among the three Recommendation Parts (Strong Recommendations, Recommendations, No Recommendations), and also added colour to make them easy to follow.
3. It is well organized but there are almost too many data, not all of which are of equally high quality. It is not always easy to follow what are being recommended and what are not. For example, it seems that only the "Strong Recommendations" are intended to be followed - so what is the significance of the ordinary "Recommendations" (see page 6, Part II) - which are not convicing/do not have good evidence	For the diagnostic research question, we set up a high preplanned threshold for a molecular test with both sensitivity and specificity of 90% to make recommendations. Since there is a trade-off relationship between sensitivity and specificity (When the sensitivity increases, the specificity decreases), the genetic tests that could reach this threshold would be accurate with only a few false-positive and false-negative results. Thus, we believed

base and likely do not merit recommendation - as simple examples, IHC staining for beta catenin and SMARCB1/INI-1 are easier/quicker than molecular tests for diagnosing desmoid tumors and epithelioid sarcoma. Simple morphology is much easier (and generally perfectly reliable) in distinguishing MPNST form neurofibroma in the vast majority of cases - so molecular testing is unnecessary. Fusion genes were discredited as being prognostic in synovial sarcoma more than 10 years ago and these data are not generally used in major sarcoma centres in USA.	that the data might be useful in clinical practice although the quality of eligible studies to support the Recommendation Part II was low or very low. Additionally, we used "may be" for all the Recommendation statements in Part II. This guideline did not provide information on comparison of the diagnostic accuracy of the genetic tests with other lab tests (e.g., IHC staining), which could be one topic of the next sarcoma guideline options in the future. Based on the reviewer's comments, we have moved the Recommendation Statement of synovial sarcoma regarding *SS18-SSX1* as a prognostic factor for a poor metastasis-free survival to Qualifying Statement section. Please see the details under ***Interpretation of Evidence for Recommendation Part II and Qualifying Statements*** in Section 2.
4. The document does not indicate which of the three expert centres in Ontario performs which of the molecular tests listed throughout the document; this information should be disseminated to pathology laboratories across Ontario in order to facilitate and expedite ordering of molecular tests for sarcomas.	This information was partially discussed under **IMPLEMENTATION CONSIDERATIONS** at the end of Section 2. How to implement this guideline and the cost consideration is beyond the scope of this guideline.
5. The document analyzed the existing evidence to determine which molecular tests should be recommended for the diagnosis, prognosis and selection of therapy for non-GIST STS. The document does not provide guidelines on which cases to request molecular testing, ie., should MDM2 analysis be performed on all lipomatous lesions? Just lipomatous lesions with equivocal cytological atypia, recurrent lipomas, deep lipomas without atypia that exceed 15 cm, all retroperitoneal/intra-abdominal lipomatous lesions; should SYT molecular testing be performed on synovial sarcoma that is readily diagnosed by H&E and IHC, or DDIT3 for myxoid liposarcoma diagnostic on H&E, etc.	Please see the response for comment 1 in this table. The guideline is meant to guide clinicians with respect to the available and recommended molecular tests that can aid clinical decision making. It was not meant to guide clinical care on a case-by-case basis, which should be done by multidisciplinary sarcoma teams at the host sites.

Professional Consultation

Feedback was obtained through a brief online survey of healthcare professionals and other stakeholders who are the intended users of the guideline. All oncologists and pathologists in the PEBC database who showed interest in sarcoma, and the clinical experts whom the Working Group members recommended were contacted by email to inform them of the survey. Fifty-five professionals were contacted; 46 practice in Ontario versus nine who practice outside Ontario. Fifteen (27%) responses were received. Five stated that they did not have interest in this area or were too busy to participate in this survey. The results of the feedback survey from 10 people are summarized in Table 5-5. The main comments from the consultation and the Working Group's responses are summarized in Table 5-6.

Table 5-5. Responses to four items on the professional consultation survey.

	Number (%)

General Questions: Overall Guideline Assessment	Lowest Quality (1)	(2)	(3)	(4)	Highest Quality (5)
1. Rate the overall quality of the guideline report.				2 (20)	8 (80)
	Strongly Disagree (1)	(2)	(3)	(4)	Strongly Agree (5)
2. I would make use of this guideline in my professional decisions.			1 (10)	2 (20)	7 (70)
3. I would recommend this guideline for use in practice.				3 (30)	7 (70)
4. What are the barriers or enablers to the implementation of this guideline report?	• Potential barriers might be the lack of available tools at various institutions. • Excellent guideline - pathology needs to be reviewed at a 'sarcoma' pathology centre so availability of the testing should not be an issue, however the time to get the results may be a barrier. • a. Funding: For the best practice, we need to follow the guideline to test these evidence-proven molecular markers in patients with certain types of STS. Funding is the main barrier. b. Testing centres: A few specialized centres will be better than multiple centres. c. Human rsources: Need to recruit a group of skilled personnel, including healthcare professionals to conduct/interpret test results. d. Primary care physician/non-centralized patient care centres' healthcare professionals: Need to educate them on how to refer these patients to the centre for the best care. e. Specimen handling at the primary care sites: Need to educate the non-centralized centres' healthcare personnel on how to handle sarcoma specimens on site and how to send the specimens to the regional centres. f. Public (patient) awareness: need to educate patients that it is their right to ask for these molecular tests. • a. As we all understand that the pathology or laboratory budget is quite tight throughout the country, priority of new pathology, especially molecular tests is often placed on common cancers such as breast and non-small cell lung cancer. Implementation of these tests in sarcoma will need some intense advocacy. b. As the incidences of the various types of sarcoma are very low, initial validation may take some time to perform. An ongoing quality assurance program must be devised through cross-country collaboration. c. The turnaround time of these test results is very important. Unlike breast and non-small cell lung cancer, the molecular tests may be run no more than once every 1-2 weeks due to the rarity of various types of sarcomas.				

Table 5-6. Modifications/Actions taken/Responses regarding main written comments from professional consultants.

Comments	Responses
1. The guideline is excellent, but their presentation is a bit unusual in that the	All the PEBC guidelines are required to have five sections: Recommendations, Recommendations and

	methods are only presented at the end. The guidelines contribute to filling a void in the diagnostic arena. The thoroughness of their documentation makes them particularly worthwhile.	Key Evidence, Guideline Methods Overview, Systematic Review, and Internal and External Review (Please see the Table of Contents on Page 1).
2.	Tables with supporting evidence are not easy to follow.	Based on 70 eligible articles, we made three Strong Recommendations, 14 Recommendations, and 10 Qualifying Statements, which covered 11 types of STS for diagnosis and/or prognosis (**Part I** and **Part II** in **Section 1**). We have changed some format to make the recommendations and evidence easy to follow. Please see the response for comment 2 in Table 5-4.
3.	We are in the era of precision medicine in cancer care, so we need to provide molecular tests to archive accurate diagnosis, which ultimately will have impact on patients' prognosis and therapy.	We are calling more high-quality studies to reach this goal.

CONCLUSION

The final guideline recommendations contained in Section 2 and summarized in Section 1 reflect the integration of feedback obtained through the external review processes with the document as drafted by the GDG Working Group and approved by the GDG Expert Panel and the PEBC RAP.

References

1. Creytens D, van Gorp J, Ferdinande L, Speel EJ, Libbrecht L. Detection of MDM2/CDK4 amplification in lipomatous soft tissue tumors from formalin-fixed, paraffin-embedded tissue: comparison of multiplex ligation-dependent probe amplification (MLPA) and fluorescence in situ hybridization (FISH). Appl Immunohistochem Mol Morphol. 2015;23:126-33.
2. Erickson-Johnson MR, Seys AR, Roth CW, King AA, Hulshizer RL, Wang X, et al. Carboxypeptidase M: a biomarker for the discrimination of well-differentiated liposarcoma from lipoma. Mod Pathol. 2009;22:1541-7.
3. Kashima T, Halai D, Ye H, Hing SN, Delaney D, Pollock R, et al. Sensitivity of MDM2 amplification and unexpected multiple faint alphoid 12 (alpha 12 satellite sequences) signals in atypical lipomatous tumor. Mod Pathol. 2012;25:1384-96.
4. Kimura H, Dobashi Y, Nojima T, Nakamura H, Yamamoto N, Tsuchiya H, et al. Utility of fluorescence in situ hybridization to detect MDM2 amplification in liposarcomas and their morphological mimics. Int J Clin Exp Pathol. 2013;6:1306-16.
5. Mardekian SK, Solomides CC, Gong JZ, Peiper SC, Wang ZX, Bajaj R. Comparison of Chromogenic In Situ Hybridization and Fluorescence In Situ Hybridization for the Evaluation of MDM2 Amplification in Adipocytic Tumors. J Clin Lab Anal. 2015;29:462-8.
6. Sirvent N, Coindre JM, Maire G, Hostein I, Keslair F, Guillou L, et al. Detection of MDM2-CDK4 amplification by fluorescence in situ hybridization in 200 paraffin-embedded tumor samples: utility in diagnosing adipocytic lesions and comparison with immunohistochemistry and real-time PCR. Am J Surg Pathol. 2007;31:1476-89.
7. Thway K, Wang J, Swansbury J, Min T, Fisher C. Fluorescence In Situ Hybridization for MDM2 Amplification as a Routine Ancillary Diagnostic Tool for Suspected Well-Differentiated and Dedifferentiated Liposarcomas: Experience at a Tertiary Center. Sarcoma. 2015;2015:812089.
8. Weaver J, Downs-Kelly E, Goldblum JR, Turner S, Kulkarni S, Tubbs RR, et al. Fluorescence in situ hybridization for MDM2 gene amplification as a diagnostic tool in lipomatous neoplasms. Mod Pathol. 2008;21:943-9.
9. Weaver J, Goldblum JR, Turner S, Tubbs RR, Wang WL, Lazar AJ, et al. Detection of MDM2 gene amplification or protein expression distinguishes sclerosing mesenteritis and retroperitoneal fibrosis from inflammatory well-differentiated liposarcoma. Mod Pathol. 2009;22:66-70.
10. Weaver J, Rao P, Goldblum JR, Joyce MJ, Turner SL, Lazar AJ, et al. Can MDM2 analytical tests performed on core needle biopsy be relied upon to diagnose well-differentiated liposarcoma? Mod Pathol. 2010;23:1301-6.
11. Shimada S, Ishizawa T, Ishizawa K, Matsumura T, Hasegawa T, Hirose T. The value of MDM2 and CDK4 amplification levels using real-time polymerase chain reaction for the differential diagnosis of liposarcomas and their histologic mimickers. Hum Pathol. 2006;37:1123-9.
12. Miura Y, Keira Y, Ogino J, Nakanishi K, Noguchi H, Inoue T, et al. Detection of specific genetic abnormalities by fluorescence in situ hybridization in soft tissue tumors. Pathol Int. 2012;62:16-27.
13. Ware PL, Snow AN, Gvalani M, Pettenati MJ, Qasem SA. MDM2 copy numbers in well-differentiated and dedifferentiated liposarcoma. Am J Clin Pathol. 2014;141:334-41.
14. Sun B, Sun Y, Wang J, Zhao X, Zhang S, Liu Y, et al. The diagnostic value of SYT-SSX detected by reverse transcriptase-polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH) for synovial sarcoma: a review and prospective study of 255 cases. Cancer Sci. 2008;99:1355-61.
15. Tanas MR, Rubin BP, Tubbs RR, Billings SD, Downs-Kelly E, Goldblum JR. Utilization of fluorescence in situ hybridization in the diagnosis of 230 mesenchymal neoplasms: an institutional experience. Arch Pathol Lab Med. 2010;134:1797-803.
16. Ten Heuvel SE, Hoekstra HJ, Suurmeijer AJ. Diagnostic accuracy of FISH and RT-PCR in 50 routinely processed synovial sarcomas. Appl Immunohistochem Mol Morphol. 2008;16:246-50.

17. Terry J, Barry TS, Horsman DE, Hsu FD, Gown AM, Huntsman DG, et al. Fluorescence in situ hybridization for the detection of t(X;18)(p11.2;q11.2) in a synovial sarcoma tissue microarray using a breakapart-style probe. Diagn Mol Pathol. 2005;14:77-82.
18. Amary MF, Berisha F, Bernardi Fdel C, Herbert A, James M, Reis-Filho JS, et al. Detection of SS18-SSX fusion transcripts in formalin-fixed paraffin-embedded neoplasms: analysis of conventional RT-PCR, qRT-PCR and dual color FISH as diagnostic tools for synovial sarcoma. Mod Pathol. 2007;20:482-96.
19. Amary MF, Pauwels P, Meulemans E, Roemen GM, Islam L, Idowu B, et al. Detection of beta-catenin mutations in paraffin-embedded sporadic desmoid-type fibromatosis by mutation-specific restriction enzyme digestion (MSRED): an ancillary diagnostic tool. Am J Surg Pathol. 2007;31:1299-309.
20. Thorson JA, Weigelin HC, Ruiz RE, Howard JK, Lucas DR. Identification of SYT-SSX transcripts from synovial sarcomas using RT-multiplex PCR and capillary electrophoresis. Mod Pathol. 2006;19:641-7.
21. Colombo C, Miceli R, Lazar AJ, Perrone F, Pollock RE, Le Cesne A, et al. CTNNB1 45F mutation is a molecular prognosticator of increased postoperative primary desmoid tumor recurrence: an independent, multicenter validation study. Cancer. 2013;119:3696-702.
22. Lazar AJ, Tuvin D, Hajibashi S, Habeeb S, Bolshakov S, Mayordomo-Aranda E, et al. Specific mutations in the beta-catenin gene (CTNNB1) correlate with local recurrence in sporadic desmoid tumors. Am J Pathol. 2008;173:1518-27.
23. van Broekhoven DL, Verhoef C, Grunhagen DJ, van Gorp JM, den Bakker MA, Hinrichs JW, et al. Prognostic value of CTNNB1 gene mutation in primary sporadic aggressive fibromatosis. Ann Surg Oncol. 2015;22:1464-70.
24. GRADEpro GDT. GRADE's software for summary of findings tables, health technology assessment and guidelines. https://gradepro.org/; 2017 [accessed 22 November 2017].
25. Guyatt GH, Oxman AD, Kunz R, Brozek J, Alonso-Coello P, Rind D, et al. GRADE guidelines 6. Rating the quality of evidence--imprecision. J Clin Epidemiol. 2011;64:1283-93.
26. Aitken SJ, Presneau N, Kalimuthu S, Dileo P, Berisha F, Tirabosco R, et al. Next-generation sequencing is highly sensitive for the detection of beta-catenin mutations in desmoid-type fibromatoses. Virchows Arch. 2015;467:203-10.
27. Hodge JC, Bedroske PP, Pearce KE, Sukov WR. Molecular Cytogenetic Analysis of JAZF1, PHF1, and YWHAE in Endometrial Stromal Tumors: Discovery of Genetic Complexity by Fluorescence in Situ Hybridization. J Mol Diagn. 2016;18:516-26.
28. Le Loarer F, Zhang L, Fletcher CD, Ribeiro A, Singer S, Italiano A, et al. Consistent SMARCB1 homozygous deletions in epithelioid sarcoma and in a subset of myoepithelial carcinomas can be reliably detected by FISH in archival material. Genes Chromosomes Cancer. 2014;53:475-86.
29. Downs-Kelly E, Goldblum JR, Patel RM, Weiss SW, Folpe AL, Mertens F, et al. The utility of fluorescence in situ hybridization (FISH) in the diagnosis of myxoid soft tissue neoplasms. Am J Surg Pathol. 2008;32:8-13.
30. Narendra S, Valente A, Tull J, Zhang S. DDIT3 gene break-apart as a molecular marker for diagnosis of myxoid liposarcoma--assay validation and clinical experience. Diagn Mol Pathol. 2011;20:218-24.
31. Wang T, Goodman MA, McGough RL, Weiss KR, Rao UN. Immunohistochemical analysis of expressions of RB1, CDK4, HSP90, cPLA2G4A, and CHMP2B is helpful in distinction between myxofibrosarcoma and myxoid liposarcoma. Int J Surg Pathol. 2014;22:589-99.
32. Willmore-Payne C, Holden J, Turner KC, Proia A, Layfield LJ. Translocations and amplifications of chromosome 12 in liposarcoma demonstrated by the LSI CHOP breakapart rearrangement probe. Arch Pathol Lab Med. 2008;132:952-7.

33. Cantile M, Galletta F, Franco R, Aquino G, Scognamiglio G, Marra L, et al. Hyperexpression of HOXC13, located in the 12q13 chromosomal region, in well-differentiated and dedifferentiated human liposarcomas. Oncol Rep. 2013;30:2579-86.
34. Tap WD, Eilber FC, Ginther C, Dry SM, Reese N, Barzan-Smith K, et al. Evaluation of well-differentiated/de-differentiated liposarcomas by high-resolution oligonucleotide array-based comparative genomic hybridization. Genes Chromosomes Cancer. 2011;50:95-112.
35. Brekke HR, Ribeiro FR, Kolberg M, Agesen TH, Lind GE, Eknaes M, et al. Genomic changes in chromosomes 10, 16, and X in malignant peripheral nerve sheath tumors identify a high-risk patient group. J Clin Oncol. 2010;28:1573-82.
36. Wallander ML, Tripp S, Layfield LJ. MDM2 amplification in malignant peripheral nerve sheath tumors correlates with p53 protein expression. Arch Pathol Lab Med. 2012;136:95-9.
37. Fraga-Guedes C, Andre S, Mastropasqua MG, Botteri E, Toesca A, Rocha RM, et al. Angiosarcoma and atypical vascular lesions of the breast: diagnostic and prognostic role of MYC gene amplification and protein expression. Breast Cancer Res Treat. 2015;151:131-40.
38. Huang SC, Zhang L, Sung YS, Chen CL, Kao YC, Agaram NP, et al. Recurrent CIC Gene Abnormalities in Angiosarcomas: A Molecular Study of 120 Cases With Concurrent Investigation of PLCG1, KDR, MYC, and FLT4 Gene Alterations. Am J Surg Pathol. 2016;40:645-55.
39. Jour G, Gullet A, Liu M, Hoch BL. Prognostic relevance of Federation Nationale des Centres de Lutte Contre le Cancer grade and MDM2 amplification levels in dedifferentiated liposarcoma: a study of 50 cases. Mod Pathol. 2015;28:37-47.
40. Lee SE, Kim YJ, Kwon MJ, Choi DI, Lee J, Cho J, et al. High level of CDK4 amplification is a poor prognostic factor in well-differentiated and dedifferentiated liposarcoma. Histol Histopathol. 2014;29:127-38.
41. Oda Y, Yamamoto H, Takahira T, Kobayashi C, Kawaguchi K, Tateishi N, et al. Frequent alteration of p16(INK4a)/p14(ARF) and p53 pathways in the round cell component of myxoid/round cell liposarcoma: p53 gene alterations and reduced p14(ARF) expression both correlate with poor prognosis. J Pathol. 2005;207:410-21.
42. Saada-Bouzid E, Burel-Vandenbos F, Ranchere-Vince D, Birtwisle-Peyrottes I, Chetaille B, Bouvier C, et al. Prognostic value of HMGA2, CDK4, and JUN amplification in well-differentiated and dedifferentiated liposarcomas. Mod Pathol. 2015;28:1404-14.
43. Crago AM, Socci ND, DeCarolis P, O'Connor R, Taylor BS, Qin LX, et al. Copy number losses define subgroups of dedifferentiated liposarcoma with poor prognosis and genomic instability. Clin Cancer Res. 2012;18:1334-40.
44. Yu J, Deshmukh H, Payton JE, Dunham C, Scheithauer BW, Tihan T, et al. Array-based comparative genomic hybridization identifies CDK4 and FOXM1 alterations as independent predictors of survival in malignant peripheral nerve sheath tumor. Clin Cancer Res. 2011;17:1924-34.
45. Ginter PS, Mosquera JM, MacDonald TY, D'Alfonso TM, Rubin MA, Shin SJ. Diagnostic utility of MYC amplification and anti-MYC immunohistochemistry in atypical vascular lesions, primary or radiation-induced mammary angiosarcomas, and primary angiosarcomas of other sites. Hum Pathol. 2014;45:709-16.
46. Kacker C, Marx A, Mossinger K, Svehla F, Schneider U, Hogendoorn PC, et al. High frequency of MYC gene amplification is a common feature of radiation-induced sarcomas. Further results from EORTC STBSG TL 01/01. Genes Chromosomes Cancer. 2013;52:93-8.
47. Lae M, Lebel A, Hamel-Viard F, Asselain B, Trassard M, Sastre X, et al. Can c-myc amplification reliably discriminate postradiation from primary angiosarcoma of the breast? Cancer Radiother. 2015;19:168-74.
48. Manner J, Radlwimmer B, Hohenberger P, Mossinger K, Kuffer S, Sauer C, et al. MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema. Am J Pathol. 2010;176:34-9.

49. Mentzel T, Schildhaus HU, Palmedo G, Buttner R, Kutzner H. Postradiation cutaneous angiosarcoma after treatment of breast carcinoma is characterized by MYC amplification in contrast to atypical vascular lesions after radiotherapy and control cases: clinicopathological, immunohistochemical and molecular analysis of 66 cases. Mod Pathol. 2012;25:75-85.
50. Patel RM, Downs-Kelly E, Weiss SW, Folpe AL, Tubbs RR, Tuthill RJ, et al. Dual-color, break-apart fluorescence in situ hybridization for EWS gene rearrangement distinguishes clear cell sarcoma of soft tissue from malignant melanoma. Mod Pathol. 2005;18:1585-90.
51. Yang L, Chen Y, Cui T, Knosel T, Zhang Q, Geier C, et al. Identification of biomarkers to distinguish clear cell sarcoma from malignant melanoma. Hum Pathol. 2012;43:1463-70.
52. Salgado R, Llombart B, R MP, Fernandez-Serra A, Sanmartin O, Toll A, et al. Molecular diagnosis of dermatofibrosarcoma protuberans: a comparison between reverse transcriptase-polymerase chain reaction and fluorescence in situ hybridization methodologies. Genes Chromosomes Cancer. 2011;50:510-7.
53. Segura S, Salgado R, Toll A, Martin-Ezquerra G, Yebenes M, Saez A, et al. Identification of t(17;22)(q22;q13) (COL1A1/PDGFB) in dermatofibrosarcoma protuberans by fluorescence in situ hybridization in paraffin-embedded tissue microarrays. Hum Pathol. 2011;42:176-84.
54. Le Guellec S, Soubeyran I, Rochaix P, Filleron T, Neuville A, Hostein I, et al. CTNNB1 mutation analysis is a useful tool for the diagnosis of desmoid tumors: a study of 260 desmoid tumors and 191 potential morphologic mimics. Mod Pathol. 2012;25:1551-8.
55. Huss S, Nehles J, Binot E, Wardelmann E, Mittler J, Kleine MA, et al. beta-catenin (CTNNB1) mutations and clinicopathological features of mesenteric desmoid-type fibromatosis. Histopathology. 2013;62:294-304.
56. Barthelmess S, Geddert H, Boltze C, Moskalev EA, Bieg M, Sirbu H, et al. Solitary fibrous tumors/hemangiopericytomas with different variants of the NAB2-STAT6 gene fusion are characterized by specific histomorphology and distinct clinicopathological features. Am J Pathol. 2014;184:1209-18.
57. Binh MB, Sastre-Garau X, Guillou L, de Pinieux G, Terrier P, Lagace R, et al. MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: a comparative analysis of 559 soft tissue neoplasms with genetic data. Am J Surg Pathol. 2005;29:1340-7.
58. Ito M, Barys L, O'Reilly T, Young S, Gorbatcheva B, Monahan J, et al. Comprehensive mapping of p53 pathway alterations reveals an apparent role for both SNP309 and MDM2 amplification in sarcomagenesis. Clin Cancer Res. 2011;17:416-26.
59. Guillou L, Benhattar J, Gengler C, Gallagher G, Ranchere-Vince D, Collin F, et al. Translocation-positive low-grade fibromyxoid sarcoma: clinicopathologic and molecular analysis of a series expanding the morphologic spectrum and suggesting potential relationship to sclerosing epithelioid fibrosarcoma: a study from the French Sarcoma Group. Am J Surg Pathol. 2007;31:1387-402.
60. Matsuyama A, Hisaoka M, Shimajiri S, Hayashi T, Imamura T, Ishida T, et al. Molecular detection of FUS-CREB3L2 fusion transcripts in low-grade fibromyxoid sarcoma using formalin-fixed, paraffin-embedded tissue specimens. Am J Surg Pathol. 2006;30:1077-84.
61. Canter RJ, Qin LX, Maki RG, Brennan MF, Ladanyi M, Singer S. A synovial sarcoma-specific preoperative nomogram supports a survival benefit to ifosfamide-based chemotherapy and improves risk stratification for patients. Clin Cancer Res. 2008;14:8191-7.
62. Sun Y, Sun B, Wang J, Cai W, Zhao X, Zhang S, et al. Prognostic implication of SYT-SSX fusion type and clinicopathological parameters for tumor-related death, recurrence, and metastasis in synovial sarcoma. Cancer Sci. 2009;100:1018-25.
63. Takenaka S, Ueda T, Naka N, Araki N, Hashimoto N, Myoui A, et al. Prognostic implication of SYT-SSX fusion type in synovial sarcoma: a multi-institutional retrospective analysis in Japan. Oncol Rep. 2008;19:467-76.

64. Straus SE, Richardson S, Glasziou P, Haynes RB. Evidence-base medicine: How to practice and teach it. 4th ed, Edinburgh: Churchill Livingstone/Elsevier; 2016.
65. Anderson T, Zhang L, Hameed M, Rusch V, Travis WD, Antonescu CR. Thoracic epithelioid malignant vascular tumors: a clinicopathologic study of 52 cases with emphasis on pathologic grading and molecular studies of WWTR1-CAMTA1 fusions. Am J Surg Pathol. 2015;39:132-9.
66. Zreik RT, Carter JM, Sukov WR, Ahrens WA, Fritchie KJ, Montgomery EA, et al. TGFBR3 and MGEA5 rearrangements are much more common in "hybrid" hemosiderotic fibrolipomatous tumor-myxoinflammatory fibroblastic sarcomas than in classical myxoinflammatory fibroblastic sarcomas: a morphological and fluorescence in situ hybridization study. Hum Pathol. 2016;53:14-24.
67. Bianchini L, Saada E, Gjernes E, Marty M, Haudebourg J, Birtwisle-Peyrottes I, et al. Let-7 microRNA and HMGA2 levels of expression are not inversely linked in adipocytic tumors: analysis of 56 lipomas and liposarcomas with molecular cytogenetic data. Genes Chromosomes Cancer. 2011;50:442-55.
68. Kim JH, Lee JS, Kim EJ, Park KH, Kim KH, Yi SY, et al. Prognostic implications of PIK3CA amplification in curatively resected liposarcoma. Oncotarget. 2016;7:24549-58.
69. Lee S, Park H, Ha SY, Paik KY, Lee SE, Kim JM, et al. CDK4 amplification predicts recurrence of well-differentiated liposarcoma of the abdomen. PLoS ONE [Electronic Resource]. 2014;9:e99452.
70. Ren T, Lu Q, Guo W, Lou Z, Peng X, Jiao G, et al. The clinical implication of SS18-SSX fusion gene in synovial sarcoma. Br J Cancer. 2013;109:2279-85.
71. Browman GP, Newman TE, Mohide EA, Graham ID, Levine MN, Pritchard KI, et al. Progress of clinical oncology guidelines development using the Practice Guidelines Development Cycle: the role of practitioner feedback. J Clin Oncol. 1998;16:1226-31.
72. Browman GP, Levine MN, Mohide EA, Hayward RS, Pritchard KI, Gafni A, et al. The practice guidelines development cycle: a conceptual tool for practice guidelines development and implementation. J Clin Oncol. 1995;13:502-12.
73. Brouwers MC, Kho ME, Browman GP, Burgers JS, Cluzeau F, Feder G, et al. AGREE II: advancing guideline development, reporting and evaluation in health care. CMAJ. 2010;182:E839-42.
74. Canadian Cancer Society's Steering Committee on Cancer Statistics. Soft tissue sarcoma statistics. Toronto, ON: Canadian Cancer Society. http://www.cancer.ca/en/cancer-information/cancer-type/soft-tissue-sarcoma/statistics/?region=bc; 2013 [accessed 30 October 2017].
75. College of American Pathologists. Protocol for the examination of specimens from patients with tumours of soft tissue. Based on AJCC/UICC TNM, 7th ed. 2013. Available from: http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/softtissue-13protocol-3120.pdf [accessed 30 November, 2017].
76. NCCN National Comprehensive Cancer Network. Soft tissue sarcoma. Version 1.2016. Available from: http://www.nccn.org/index.asp. [accessed 30 November 2017].
77. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155:529-36.
78. Hayden JA, van der Windt DA, Cartwright JL, Cote P, Bombardier C. Assessing bias in studies of prognostic factors. Ann Intern Med. 2013;158:280-6.
79. Higgins JPT, Green S (editors). Cochrane handbook for systematic reviews of interventions. Version 5.1.0. The Collaboration, 2011. Available from: www.cochrane-handbook.org. Accessed November 30, 2017.
80. StataCorp. Stata Statistical Software: Release 11. 2009. College Station, TX: StataCorp LP.

81. Harbord RM, Deeks JJ, Egger M, Whiting P, Sterne JA. A unification of models for meta-analysis of diagnostic accuracy studies. Biostatistics (Oxford, England). 2007;8:239-51.
82. Yao X, Vella E, Brouwers M. How to conduct a high-quality systematic review on diagnostic research topics. Surg Oncol. 2018;27:70-5.
83. Domont J, Salas S, Lacroix L, Brouste V, Saulnier P, Terrier P, et al. High frequency of beta-catenin heterozygous mutations in extra-abdominal fibromatosis: a potential molecular tool for disease management. Br J Cancer. 2010;102:1032-6.
84. Kim HS, Kim J, Nam KH, Kim WH. Clinical significance of midkine expression in sporadic desmoid tumors. Oncol Lett. 2016;11:1677-84.
85. Romero S, Szafranska J, Cabrera E, Gonzalez A, Peiro A, Llauger J, et al. Role of tumor-associated macrophages and angiogenesis in desmoid-type fibromatosis. Virchows Arch. 2012;461:117-22.
86. Bartel F, Schulz J, Bohnke A, Blumke K, Kappler M, Bache M, et al. Significance of HDMX-S (or MDM4) mRNA splice variant overexpression and HDMX gene amplification on primary soft tissue sarcoma prognosis. Int J Cancer. 2005;117:469-75.
87. Kubo T, Shimose S, Fujimori J, Furuta T, Ochi M. Prognostic value of SS18-SSX fusion type in synovial sarcoma; systematic review and meta-analysis. Springerplus. 2015;4:375.
88. Mullen JT, DeLaney TF, Rosenberg AE, Le L, Iafrate AJ, Kobayashi W, et al. beta-Catenin mutation status and outcomes in sporadic desmoid tumors. Oncologist. 2013;18:1043-9.
89. Ohnstad HO, Castro R, Sun J, Heintz KM, Vassilev LT, Bjerkehagen B, et al. Correlation of TP53 and MDM2 genotypes with response to therapy in sarcoma. Cancer. 2013;119:1013-22.
90. Palmerini E, Benassi MS, Quattrini I, Pazzaglia L, Donati D, Benini S, et al. Prognostic and predictive role of CXCR4, IGF-1R and Ezrin expression in localized synovial sarcoma: is chemotaxis important to tumor response? Orphanet J Rare Dis. 2015;10:6.
91. ten Heuvel SE, Hoekstra HJ, Bastiaannet E, Suurmeijer AJ. The classic prognostic factors tumor stage, tumor size, and tumor grade are the strongest predictors of outcome in synovial sarcoma: no role for SSX fusion type or ezrin expression. Appl Immunohistochem Mol Morphol. 2009;17:189-95.
92. Teng HW, Wang HW, Chen WM, Chao TC, Hsieh YY, Hsih CH, et al. Prevalence and prognostic influence of genomic changes of EGFR pathway markers in synovial sarcoma. J Surg Oncol. 2011;103:773-81.
93. Palmerini E, Benassi MS, Benini S, Pazzaglia L, Eric L. Staals EL, Gamberi G, et al. Synovial sarcoma: Is chemotaxis important to tumor progression? J Clin Oncol 31, 2013 (Suppl; abstr 10571).
94. Kimura H, Yamamoto N, Shirai T, Nishida H, Hayashi K, Takeuchi A, et al. Detection of MDM2 gene amplification in soft tissue sarcoma by FISH. J Clin Oncol 32:5s, 2014 (Suppl; abstr 10562).
95. Kimura H, Yamamoto N, Nishida H; Hayashi K; Takeuchi A, Igarashi K, et al. MDM2 amplification in soft tissue sarcoma by FISH. Poster 3. Connective Tissue Oncology Society 20th Annual Meeting; 2014 Oct 15-18.
96. WHO Classification of Tumours of Soft Tissue and Bone. 4th ed. 2013. Available from: http://publications.iarc.fr/Book-And-Report-Series/Who-Iarc-Classification-Of-Tumours/Who-Classification-Of-Tumours-Of-Soft-Tissue-And-Bone-2013. Accessed November 30 2017.
97. Mendenhall W, Beaver RJ, Beaver BM. Introduction to probability and statistics. 11th ed. Brooks/Cole-Thomson Learning: Pacific Grove; 2003.
98. Draper NR, Guttman I. Confidence intervals versus regions. J R Stat Soc.1995;44(3):399-403.
99. PROPHARMA Group News and Blog. Understanding statistical intervals: part 2 – prediction intervals. http://www.propharmagroup.com/blog/understanding-statistical-intervals-part-2-prediction-intervals. Accessed November 21, 2017.

Appendix 1. Molecular tests' full names

Molecular test abbreviation	Full name
CAMTA1	Calmodulin Binding Transcription Activator 1
CDK4	Cyclin-dependent kinase 4
DDIT3	DNA Damage Inducible Transcript 3
CIC	Capicua Transcriptional Repressor
COL1A1/PDGFB	Collagen Type I Alpha 1 Chain/ Platelet Derived Growth Factor Subunit B
CPM	Carboxypeptidase M
CTNNB1	Catenin Beta 1
EWSR1/ATF1	EWS RNA Binding Protein 1/ Activating Transcription Factor 1
FUS	FUS RNA Binding Protein
CREB3L1	CAMP Responsive Element Binding Protein 3 Like 1
HMGA2	High Mobility Group AT-Hook 2
JAZF1	JAZF Zinc Finger 1
MDM2	Murine double-minute type 2
MGEA5	Meningioma Expressed Antigen 5 (Hyaluronidase)
MYC	MYC proto-oncogene, bHLH transcription factor
NAB2	NGFI-A binding protein 2
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
SMARCB1	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily B, Member 1
SS18-SSX (SYT-SSX)	SS18, NBAF Chromatin Remodeling Complex Subunit-SSX Family Member (Synaptotagmin-SSX Family Member)
STAT6	Signal transducer and activator of transcription 6
TGFBR3	Transforming Growth Factor Beta Receptor 3
WWTR1	WW Domain Containing Transcription Regulator 1

Appendix 2. Affiliations and conflict of interest declarations

(1). Members of the Working Group

Name	Affiliation	Declarations of interest
Rita Kandel, Pathologist	Department of Pathology & Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada	None declared
Xiaomei Yao, Health Research Methodologist	Program in Evidence-Based Care, Cancer Care Ontario, Department of Oncology, McMaster University, Hamilton, Ontario, Canada	None declared
Brendan Dickson, Pathologist	Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada	Receiving molecular test kits from Illumina
Michelle Ghert, Orthopaedic Oncologist	Division of Orthopaedic Surgery, Juravinski Cancer Centre, Hamilton, Ontario, Canada	$5,000 or more in 2017 to act in a consulting capacity from Wright Medical and Kuros Bioscience
Snezana Popovic, Pathologist	Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada	None declared
Bibianna Purgina, Pathologist	Department of Pathology and Laboratory Medicine, The Ottawa Hospital, Ottawa, Ontario, Canada	None declared
Shailendra Verma, Medical Oncologist	Department of Medical Oncology, The Ottawa Hospital Regional Cancer Centre, Ottawa, Ontario, Canada	None declared
Joel Werier, Orthopaedic Oncologist	Division of Orthopaedic Surgery, The Ottawa Hospital Regional Cancer Centre, Ottawa, Ontario, Canada	None declared

(2). Expert panel members for this guideline

Name, Expertise	Affiliation	Declarations of interest
Charles Catton, Radiation Oncologist	Division of Radiation Oncology, The Princess Margaret Hospital, Toronto, Ontario, Canada	None declared
Susan Crocker	Department of Pathology and Molecular Medicine, Queen's	None declared

	University, Kingston, Ontario, Canada	
Jay Engel, Surgical Oncologist	Department of Surgical Oncology, Cancer Centre of Southeastern Ontario, Kingston, Ontario, Canada	None declared
Rebecca Gladdy, Surgical Oncologist	Department of Surgical Oncology, Mount Sinai Hospital, Toronto, Ontario, Canada	None declared
Abha Gupta, Medical Oncologist	Division of Haematology/Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada	None declared
Albiruni Razak, Medical Oncologist	Division of Haematology/Oncology, The Princess Margaret Hospital and Mount Sinai Hospital, Toronto, Ontario, Canada	None declared
Adam Smith	Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada	None declared

(3). Members of the Report Approval Panel

Name, Expertise	Affiliation	Declarations of interest
Melissa Brouwers, Health Research Methodologist	Cancer Care Ontario, Toronto, Ontario, Canada; McMaster University, Hamilton, Ontario, Canada	None declared
Laurie Elit, Gynecologist	Juraviniski Cancer Centre, Hamilton, Ontario, Canada	None declared
Bill Evans, Medical Oncologist	Retired from Juraviniski Cancer Center, Hamilton, Ontario, Canada	None declared
Hans Messersmith, Health Research Methodologist	McMaster University, Hamilton, Ontario, Canada	None declared

(4). Members of the Targeted Peer Reviewers

Name, Expertise	Affiliation	Declarations of interest
Chris Fletcher, Pathologist	Brigham and Women's Hospital, Boston, Massachusetts, United States	None declared
Bret Wehrli	Department of Pathology and Laboratory Medicine, Western University, Ontario, Canada	None declared

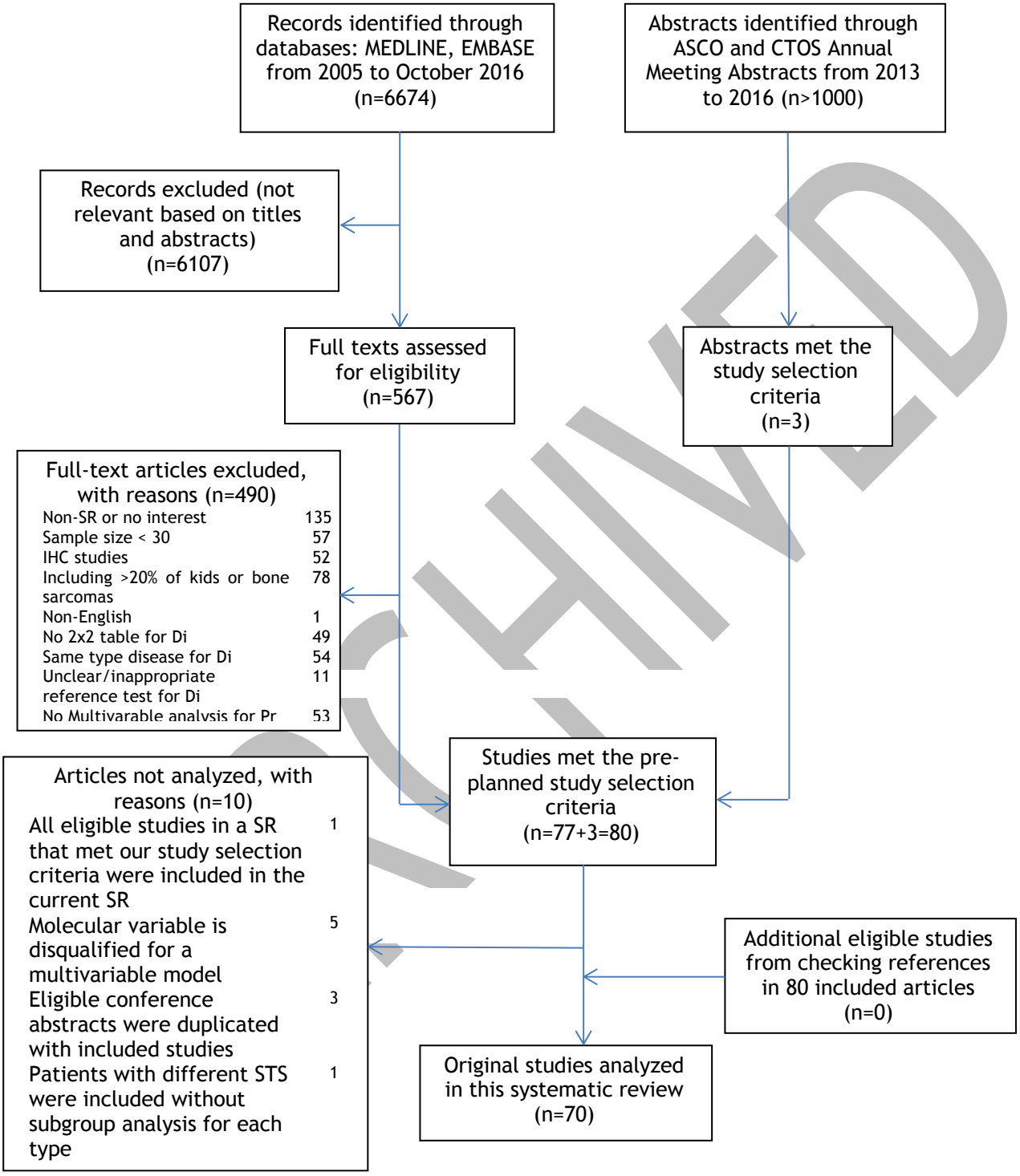
Appendix 3. Literature search strategies

#	Searches
1	("t(16;17)(q22;p13)" or "t(1;17)(p34.3;p13)" or "t(3;17)(q21;p13)" or "t(9;17)(q22;p13)" or "t(17;17)(q21;p13)" or "t(5;8)(p15;q13)" or "t(12;22)(q13;q12)" or "t(2;22)(q33;q12)").mp.
2	("t(2;13)(q35;q14)" or "t(1;13)(p36;q25)" or "t(11;16)(q13;p13)" or "t(12;15)(p13;q25)" or "t(11;22)(p13;q12)" or "t(7;17)(p15;q11)" or "t(6;7)(p21;p15)" or "t(6;10)(p21;p11)").mp.
3	("t(10;17)(q22;p13)" or "t(1;6)(p34;p21)" or "t(1;6)(p33;p21)" or "t(1;6)(p32;p21)" or "der(22)t(X;22)" or "t(1;3)(p36.3;q25)" or "t(1;3)(p36;q25)" or "t(X;11)(p11.2;q22.1)" or "t(11;12)(q24;q12)" or "t(21;22)(q22;q12)" or "t(7;22)(p22;q12)").mp.
4	("t(17;22)(q12;q12)" or "inv(22)(q21;q12)" or "t(16;21)(p11;q22)" or "inv(X)(p11.4;p11.22)" or "t(20;22)(q13;q12)" or "t(9;22)(q22;q12)" or "t(9;17)(q22;q11)" or "t(9;15)(q22;q21)" or "t(3;9)(q11;q22)").mp.
5	("t(1;2)(q22;p23)" or "t(2;19)(p23;p13)" or "t(2;17)(p23;q23)" or "t(2;2)(p23;q13)" or "t(2;11)(p23;p15)" or "inv(2)(p23;q35)" or "t(2;4)(p23;q21)" or "t(7;8)(p22;q13)" or "t(3;12)(q27-28;q14-15)" or "t(7;16)(q33;p11)" or "t(11;16)(p11;p11)").mp.
6	("t(2;4)" or "t(8;8)(q13;q21)" or "t(6;22)(p21;q12)" or "t(19;22)(q13;q12)" or "t(1;22)(q23;q12)" or "t(1;10)(p22;q24)" or "t(17;22)(p13;q13)" or "t(7;12)(p22;q13)" or "t(7;19)(q22;q13)" or "t(2;22)(q34;q12)").mp.
7	("t(7;16)(p22;q24)" or "t(11;16)(p13;p11.2)" or "inv(12)(q13;q13)" or "t(6;8)(p21;q13)" or "t(8;11)(q13;p15)" or "t(X;18)(p11;q11)" or "t(X;18)(p11.2;q11.2)" or "t(1;2)(p13;q35-37)" or "t(1;2)(p13;q35)" or "t(4;19)(q35;q13)" or "t(10;19)(q26;q13)" or "t(X;2)(q13;q35)" or "t(17;22)(q21;q13)").mp.
8	("t(X;17)(p11;q25)" or "t(12;16)(q13;p11)" or "t(11;22)(q24;q12)" or "t(21;22)(q12;q12)" or "t(12;15)(p13;q26)" or (trisom$ adj ("8" or "11" or "17" or "20" or "2q"))).mp.
9	("t(1;13)(p36;q14)" or "t(2;2)(q35;p23)" or inversion chromosome 12 or "t(2;16)(q35;p11)" or "t(4;22)(q31;q12)" or "t(1;22)(p36.1;q12)" or "t(2;22)(q31;q12)").mp.
10	((copy number? and (variation or loss or gain or alter$ or variation$ or chang$)) or activating kinase mutation$ or krebs cycle mutation$).tw.
11	*copy number variation/ or next generation sequenc$.tw.
12	((CDH11 adj2 USP6) or ((TAF2N or TCF12 or TFG or TAF15) adj2 NR4A3) or (CIC adj2 DUX4) or (COL6A3 adj2 CSF1) or (COLIA1 adj2 PDGFB) or ((CARS or ATIC or SEC31A) adj2 ALK)).mp.
13	("CDH11/USP6" or "TAF2N/NR4A3" or "TCF12/NR4A3" or "TFG/NR4A3" or "CIC/DUX4" or "COL6A3/CSF1" or "CLIA1/PDGFB" or "CARS/ALK" or "ATIC/ALK" or "SEC31A/ALK" or "SYT/SSX1" or "SYT/SSX2" or "SYT/SSX4").mp.

14	((EWS$ adj2 (ATF1 or CREB1 or WT1 or FLI1 or ERG or FEV or ETV1 or E1AF or ZSG or DDIT3 or NFATC2 or POU5F1 or SMARCA5 or PATZ or SP3 or NR4A3 or PBX1 or ZNF444)) or (ZEP36 adj2 FOSB) or (FOS adj2 LMNA) or (FN1 adj2 FGFR1) or (SYT adj2 SSX$)).tw.
15	("ZEP36/FOSB" or "FOS/LMNA" or "FN1/FGFR1" or "SYT/SSX" or "EWSR1/ATF1" or "EWS/NR4A3" or "EWSR1/CREB1" or "EWSR1/WT1" or "EWSR1/FLI1" or "EWSR1/ERG" or "EWSR1/FEV" or "EWSR1/ETV1" or "EWSR1/E1AF" or "EWSR1/ZSG" or "EWSR1/DDIT3" or "EWSR1/NFATC2" or "EWSR1/POU5F1" or "EWSR1/SMARCA5" or "EWSR1/PATZ" or "EWSR1/SP3" or "EWSR1/NR4A3" or "EWSR1/PBX1" or "EWSR1/ZNF444").mp.
16	((THRAP3 adj2 USP6) or (CNBP adj2 USP6) or (OMD adj2 USP6) or (COL1A1 adj2 USP6) or (COL1A1 adj2 PDGFB) or (AHRR adj2 NCOA2) or (JAZF1 adj2 JJAZ1) or (JAZF1 adj2 SUZ12) or (JAZF1 adj2 PHF1) or (EPC$ adj2 PHF1) or (YWHAE adj2 FAM22$) or (MEAF6 adj2 PHF1) or (ZC3H7B adj2 BCOR)).mp.
17	("THRAP3/USP6" or "CNBP/USP6" or "OMD/USP6" or "COL1A1/USP6" or "COL1A1/PDGFB" or "AHRR/NCOA2" or "JAZF1/JJAZ1" or "JAZF1/SUZ12" or "JAZF1/PHF1" or "EPC/PHF1" or "EPC1/PHF1" or "YWHAE/FAM22" or "MEAF6/PHF1" or "ZC3H7B/BCOR").mp.
18	((ETV6 adj2 NTRK3) or (TPM3 adj2 ALK) or (TPM4 adj2 ALK) or (CLTC adj2 ALK) or (RANBP2 adj2 ALK) or (TLS adj2 DDIT3) or (WWTR1 adj2 CAMTA1) or (BCOR adj2 CCNB3) or (TGFBR3 adj2 MGEA5)).mp.
19	("ETV6/NTRK3" or "TPM3/ALK" or "TPM4/ALK" or "CLTC/ALK" or "RANBP2/ALK" or "TLS/DDIT3" or "WWTR1/CAMTA1" or "BCOR/CCNB3" or "TGFBR3/MGEA5").mp.
20	((ASPSCR1 adj2 TFE3) or (C11orf95 adj2 MKL2) or (MBTD1 adj2 Cxorf67) or (NAB2 adj2 STAT6) or "ASPSCR1/TFE3" or "C11orf95/MKL2" or "MBTD1/Cxorf67" or "NAB2/STAT6").mp.
21	((COL1A2 adj2 PLAG1) or (LPP adj2 HMGA2) or (HEY1 adj2 NCOA2) or (MYH9 adj2 USP6) or (ACTB adj2 GLI1) or (FUS adj2 CREB3L$) or (FUS adj2 DDIT3) or (SRF adj2 NCOA2) or (TEAD adj2 NCOA2) or giant marker chromosome$ or (SERPINE1 adj2 FOSB) or (ZC3H7B adj2 BCOR) or (factor 6 and PHF1)).mp.
22	("COL1A2/PLAG1" or "LPP/HMGA2" or "HEY1/NCOA2" or "MYH9/USP6" or "ACTB/GLI1" or "FUS/CREB3L1" or "FUS/CREB3L2" or "FUS/DDIT3" or "SRF/NCOA2" or "TEAD/NCOA2" or "SERPINE1/FOSB" or "ZC3H7B/BCOR").mp.
23	or/1-22
24	(IDH1$ or IDH2$ or VGLL3$ or CHMP2B$ or CDK4$ or MDM2$ or HMGA2$ or SAS or GL1$ or beta-catenin or CTNNB1 or APC or GNAS or FGF-23$ or MYOD1$ or PDGFRA$ or BRAF or TSC1$ or TSC2$ or INI1$ or CSF1 or PHF1 or SMARCB-1 or ROS1).tw.
25	(Xp11 or 1p or 22q or 12q13-15 or 12q14-15 or 11p15 or 5q21 or 22q13 or 16p11 or 1p13 or 19q13 or cmyc$ or c-myc$ or myc or CPM or carboxypeptidase M or ring chromosome$ or TFE3$ or SDH? or HOXC13 or FOXM1 or NOL1 or SOX5 or EWS1 or SYT or DDIT3 or PAX3$ or PAX7$ or SS18$).tw.

26	(mutat$ or rearrangemant$ or translocat$ or amplif$ or overexpression$ or expression$ or deficien$ or aberration$ or variation$ or hyperexpress$ or inversion$ or insertion$ or deletion$ or inactivation$ or loss$ or gain$ or fusion$).tw.
27	(24 or 25) and 26
28	(FUS and (mutat$ or rearrangemant$ or translocat$ or amplif$ or overexpression$ or expression$ or deficien$ or aberration$ or variation$ or hyperexpress$ or inversion$ or insertion$ or deletion$ or inactivation$ or loss$ or gain$)).tw.
29	23 or 27 or 28
30	exp sarcoma/ or sarcoma$.mp. or (PNET$ or botryoid$ or primitive neuroectodermal tumo?r$ or dermatofibrosarcoma$ or chondrosarcoma$ or osteosarcoma$ or giant cell fibroblastoma$ or oligosarcoma$).tw.
31	(hemangioendothelioma$ or malignant peripheral nerve sheath tumo?r$ or MPNST$ or neurofibrosarcoma$ or neurosarcoma$ or inflammatory myofibroblastic tumo?r$ or lipoblastoma$).tw.
32	(angiosarcoma$ or cystsarcoma$ or desmoid tumo?r$ or fibromatos$ or fibrosarcoma$ or hemosiderotic fibrolipomatous tumo?r$ or pecoma$ or perivascular epithelioid tumo?r$ or myoepithelial tumo?r$).tw.
33	(h?emangiopericytoma$ or h?emangiosarcoma$ or leiomyosarcoma$ or liposarcoma$ or histiocytoma$ or lymphangiosarcoma$ or lymphosarcoma$ or pericytoma$ or solitary fibrous tumo?r$ or ossifying fibromyxoid tumo?r$ or epitheliod h?emangioma$ or phosphaturic mesenchymal tumo?r$).tw.
34	(rhabdomyosarcoma$ or rhabdoid tumo?r$ or (small round$ adj3 tumo?r$) or (clear cell adj3 tumo?r$) or myxofibrosarcoma$ or MFH$).tw.
35	or/30-34
36	Animal/ not Human/
37	in vitro/
38	cell line/
39	(comment or letter or editorial or note or erratum or short survey or news or newspaper article or patient education handout or case reports or historical article).pt.
40	or/36-39
41	(29 and 35) not 40
42	limit 41 to (english language and yr="2005 -Current")
43	remove duplicates from 42

Appendix 4. PRISMA flow diagram.



Abbreviations: ASCO = American Society of Clinical Oncology, CTOS = Connective Tissue Oncology Society, Di = diagnostic studies, Pr = prognostic studies, SR = systematic review.

Appendix 5. Quality assessment for diagnostic studies[a]

Study	Risk of Bias				Applicability Concerns			Overall
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard	
Angiosarcoma								
Manner 2010 [48]	H	U	L	U	H	U	L	H
Mentzel 2012 [49]	H	U	L	U	H	U	L	H
Kacker 2013 [46]	H	U	L	U	H	U	L	H
Ginter 2014 [45]	U	U	L	L	U	U	L	U
Fraga-Guedes 2015 [37]	U	L	L	H	U	L	L	U
Lae 2015 [47]	U	U	L	L	U	U	L	U
Clear cell Sarcoma								
Patel 2005 [50]	U	U	L	H	U	U	L	U
Yang 2012 [51]	H	U	L	H	H	U	L	H
Dermatofibrosarcoma protuberans								
Salgado 2011 [52]	H	U	L	H	H	U	L	H
Segura 2011 [53]	H	U	L	H	H	U	L	H
Desmoid tumour (fibromatosis)								
Amary and Pauwels 2007 [19]	H	U	L	U	H	U	L	H
Le Guellec 2012 [54]	H	U	L	U	H	U	L	H
Huss 2012 [55]	H	U	L	U	H	U	L	H
Aitken 2015 [26]	H	U	L	U	H	U	L	H
Epithelioid sarcoma								
Le Loarer 2014 [28]	H	U	L	U	H	U	L	H
Endometrial stromal sarcoma								
Hodge 2016 [27]	H	U	L	U	H	U	L	H
Epithelioid vascular tumors								
Anderson 2015 [65]	H	U	L	U	H	U	L	H
Hemangiopericytoma								
Barthelmeß 2014 [56]	H	L	L	L	H	L	L	H
Hybrid hemosiderotic fibrolipomatous tumour-myxoinflammatory fibroblastic sarcoma								
Zreik 2016 [66]	H	U	L	L	H	U	L	H
Liposarcoma								
Binh 2005 [57]	H	L	L	U	H	L	L	H
Shimada 2006 [11]	H	U	L	L	H	U	L	H

Sirvent 2007 [6]	H	U	L	L	H	U	L	H
Downs-Kelly 2008 [29]	H	L	L	L	H	L	L	H
Willmore-Payne 2008 [32]	H	U	L	L	H	U	L	H
Weaver 2008[h] [8]	U	L	L	H	U	L	L	U
Erickson-Johnson 2009 [2]	U	U	L	U	U	U	L	U
Weaver 2009 [9]	H	U	L	H	H	U	L	H
Weaver 2010 [10]	U	L	L	H	U	L	L	U
Bianchini 2011 [67]	H	L	L	L	H	L	L	H
Narendra 2011 [30]	H	U	L	H	H	U	L	H
Ito 2011 [58]	H	L	L	L	H	L	L	H
Tap 2011 [34]	H	L	L	L	H	L	L	H
Kashima 2012 [3]	H	U	L	U	H	U	L	H
Miura 2012 [12]	H	U	L	L	H	U	L	H
Cantile 2013 [33]	H	U	L	L	H	U	L	H
Kimura 2013 [4]	H	U	L	L	H	U	L	H
Wang 2014 [31]	H	U	L	U	H	U	L	H
Ware 2014 [13]	H	U	L	U	H	U	L	H
Creytens 2015 [1]	H	U	L	U	H	U	L	H
Mardekian 2015 [5]	H	U	L	U	H	U	L	H
Thway 2015 [7]	U	U	L	L	U	U	L	U
Low-grade fibromyxoid sarcoma								
Matsuyama 2006 [60]	H	L	L	L	H	L	L	H
Guillou 2007 [59]	H	U	L	H	H	U	L	H
Malignant peripheral nerve sheath tumours								
Brekke 2010 [35]	H	L	L	L	H	L	L	H
Wallander 2012 [36]	H	U	L	L	H	U	L	H
Synovial sarcoma								
Terry 2005 [17]	H	U	L	L	H	U	L	H
Thorson 2006 [20]	H	L	L	U	H	L	L	H
Amary and Berisha 2007 [18]	H	L	L	U	H	L	L	H
Sun 2008 [14]	H	L	L	U	H	L	L	H
Ten Heuvel 2008 [16]	H	L	L	L	H	L	L	H
Tanas 2010 [15]	H	U	L	L	H	U	L	H

Abbreviations: H = high risk, L = low risk, U = unclear.

[a]The QUADAS-2 tool was used and it is arbitrary to think that if any two or more items are "H", the overall risk of bias of the study is considered as "H"; if one item is "H" and less than or equal to two

items are “U”, or any three or more items are “U”, the study is considered as “Unclear”; for the rest of studies, the overall risk of bias is considered as “L”.

Appendix 6. Assessing risk of bias for prognostic studies[a]

Study	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis and reporting	Funding[b]	Overall
Angiosarcoma								
Fraga-Guedes 2015 [37]	M	H	L	L	M	L	L	M
Huang 2016 [38]	M[c]	H	M	L	L	M	L	M
Desmoid tumour (fibromatosis)								
Lazar 2008 [22]	H	H	L	L	L	M	M	H
Domont 2010 [83]	H	H	L	L	M	L	M	H
Romero 2012 [85]	M	H	L	L	L	M	L	M
Colombo 2013 [21]	M	H	L	L	L	L	L	M
Van Broekhoven 2015 [23]	M	H	L	M	M	L	L	M
Kim HS 2016 [84]	M	H	L	M	H	L	M	H
Liposarcoma								
Oda 2005 [41]	H	H	M	NI	L	M	M	H
Crago 2012 [43]	H	H	M	L	M	H	L	H
Lee SE 2014 [40]	H	H	L	L	M	L	L	H
Lee S 2014 [69]	H	H	L	L	H	L	L	H
Jour 2015 [39]	H	H	L	L	L	M	M	H
Saada-Bouzid 2015 [42]	H	H	H	L	M	H	L	H
Kim JH 2016 [68]	M	H	M	L	H	M	L	H

Malignant peripheral nerve sheath tumour								
Brekke 2010 [35]	H	H	L	L	L	M	L	H
Yu 2011 [44]	M	H	L	M	M	M	L	H
Synovial sarcoma								
Canter 2008 [61]	H	H	M	L	M	L	L	H
Takenaka 2008 [63]	H	H	M	L	M	M	M	H
Sun 2009 [62]	H	H	L	L	L	H	M	H
Ren 2013 [70]	H	H	L	L	L	L	L	H

Abbreviations: L = Low risk, M = Moderate risk, H = High risk, NI = no information.

[a]The QUIPs tool was used and it was arbitrary to think that if any two or more items of a study were "H", the overall risk of bias for the study was considered as "H"; if one item was "H" or ≥ two items were "M" or if ≥ three items were "No information", the overall risk of bias of this study was considered as "M"; for the rest of studies, the overall risks of bias were considered as "L".

[b]The definitions of overall funding risks are defined as followings: Low risk means that all of the authors declared no financial conflict of interest and the study funding was from non-industry resource; Moderate risk means that either all of the authors declared no financial conflict of interest or the study funding was from non-industry resource; High risk means that main authors declared financial conflict of interest plus the study funding was from industry resource, main authors declared financial conflict of interest plus no information for the study funding, or no information for the authors' financial conflict of interest plus the study funding was from industry resource; and no information means that there was no information from both study funding and from authors' financial conflict of interest.

[c]Although this study included primary and secondary angiosarcoma patients, the authors treated sarcoma type as a confounder and put it in the multivariable model to control. Thus, the risk of bias for this item is "M" instead of "H".

Appendix 7. Ongoing trials.

Primary investigator (country)	Title	Study design, sample size (age)	Protocol ID	Estimated study completion date
Razelle Kurzrock (United States)	Patients Diagnosed With Advanced Malignancy or Myelodysplasia, Tested by Standardized Sequencing, and Treated by Physician-Determined Care Plan: A MED-C Observational Registry (N1)	Prospective cohort, 100000 (≥18 years)	NCT02900248	October 2019
Phillipe Maingon (France)	Predictive Study of Radiation Induced Sarcoma From the GSF-GETO Data Base	Non-randomized parallel assignment, 360 (≥ 18 years)	NCT01504360	December 2017
Funda Meric-Bernstam (United States)	Molecular Testing for the MD Anderson Cancer Center Personalized Cancer Therapy Program	Observational study, 8000 (Child, Adult, and Senior)	NCT01772771	March 2032