Mismatch repair deficiency is rare in bone and soft tissue tumors

Suk Wai Lam,1 Marie Kostine,2 Noel F C C de Miranda,1 Patrick Schöffski,3,4 Che-Jui Lee,3,4 Hans Morreau1 & Judith V M G Bovée1
1Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands, 2Department of Rheumatology, Centre Hospitalier Universitaire de Bordeaux Groupe hospitalier Pellegrin, Bordeaux, France, 3Department of General Medical Oncology, University Hospitals Leuven, Leuven Cancer Institute, Leuven, and 4Department of Oncology, KU Leuven, Laboratory of Experimental Oncology, Leuven, Belgium

Introduction: There has been an increased demand for mismatch repair (MMR) status testing in sarcoma patients after the success of immune checkpoint inhibition (ICI) in MMR deficient tumors. However, data on MMR deficiency in bone and soft tissue tumors is sparse, rendering it unclear if routine screening should be applied. Hence, we aimed to study the frequency of MMR deficiency in bone and soft tissue tumors after we were prompted by two (potential) Lynch syndrome patients developing sarcomas.

Methods: Immunohistochemical expression of MLH1, PMS2, MSH2 and MSH6 was assessed on tissue micro arrays (TMAs), and included 353 bone and 539 soft tissue tumors. Molecular data was either retrieved from reports or microsatellite instability (MSI) analysis was performed. In MLH1 negative cases, additional MLH1 promoter hypermethylation analysis followed. Furthermore, a systematic literature review on MMR deficiency in bone and soft tissue tumors was conducted.

Results: Eight MMR deficient tumors were identified (1%), which included four leiomyosarcoma, two rhabdomyosarcoma, one malignant peripheral nerve sheath tumor and one radiation-associated sarcoma. Three patients were suspected for Lynch syndrome. Literature review revealed 30 MMR deficient sarcomas, of which 33% were undifferentiated/unclassifiable sarcomas. 57% of the patients were genetically predisposed.

Conclusion: MMR deficiency is rare in bone and soft tissue tumors. Screening focusing on tumors with myogenic differentiation, undifferentiated/unclassifiable sarcomas and in patients with a genetic predisposition / co-occurrence of other malignancies can be helpful in identifying patients potentially eligible for ICI.

Keywords: bone and soft tissue tumors, immune checkpoint inhibitors, immunohistochemistry, mismatch repair deficiency

Introduction

Immune checkpoint inhibitors (ICI’s) have proven their utility in the past several years across many cancer subtypes. Particularly, antibodies blocking the programmed death (PD-1) pathway have been approved as second-line or first-line therapies for melanomas and an ever-growing list of mostly epithelial malignancies. Activation of the PD-1 pathway in T cells represses Th1 and cytotoxic responses in the presence of its ligands (PD-L1 or PD-L2). The former can be
abundantly expressed in tumor microenvironments by both cancer and immune cells. The blocking of this pathway with therapeutic antibodies reinvigorates anti-tumor immune responses and elimination of cancer cells.\textsuperscript{1,2} Since the success of checkpoint blockade immunotherapy, the identification of predictive biomarkers for ICI response has been the subject of investigation. Although no single biomarker can predict which patients will likely benefit from immunotherapy, PD-L1, with its limitations, has been identified as a predictive biomarker. However, in contrast to other solid cancers the predictive value of PD-L1 for ICI response is limited in sarcomas.\textsuperscript{3–5} Another strong association of ICI response is the tumor mutational burden (TMB), where a high TMB leads to production of more neo-antigens that might be recognized by the immune system, thereby eliciting an anti-tumor response. This partially explains why therapy response is more often seen in tumors with a high TMB, e.g., lung carcinomas and melanomas, than in tumors with a low mutational burden, such as sarcomas, which are mostly refractory to ICI.\textsuperscript{6} This theory is further supported by the finding that mismatch repair (MMR) deficiency is associated with a high sensitivity to ICI, as the defect in the MMR machinery leads to a high mutational load.\textsuperscript{7,8} The tumor agnostic approach of some clinical trials, the so-called basket trials, has led to an increased demand for MMR status testing in advanced cancer patients, irrespective of the tumor type, and thus also including advanced sarcoma patients.\textsuperscript{9} However, in contrast to other cancer types, such as colon- and endometrial carcinoma, MMR deficiency does not seem to play a major role in sarcomagenesis and only anecdotal cases of sarcomas have been reported in Lynch syndrome patients.\textsuperscript{10–12} Yet, we were prompted by two (potential) Lynch syndrome patients with leiomyosarcoma and pleomorphic rhabdomyosarcoma, respectively. Since reliable data on MMR deficiency in soft tissue sarcomas is sparse, and almost absent for bone sarcomas, we aimed to study the frequency of MMR deficiency in sarcomas by immunohistochemical testing of MMR proteins in a large cohort of different bone and soft tissue tumors and by systematically reviewing the literature in order to determine if there is a rationale for routine MMR testing in advanced sarcoma patients.

**Material and methods**

**Sample collection**

Two index cases displaying MMR deficiency were identified. In addition, tissue microarrays (TMAs) of two institutions (Leiden University Medical Center (LUMC) and UZ Leuven) were used to assess MMR deficiency. For most of the LUMC TMAs, clinicopathological data were previously published, and the series included conventional chondrosarcoma (\(n = 137\)), dedifferentiated chondrosarcoma (\(n = 28\)), mesenchymal chondrosarcoma (\(n = 21\)), clear cell chondrosarcoma (\(n = 20\)), leiomyosarcoma (\(n = 87\)), angiosarcoma (\(n = 60\)), different subtypes of liposarcoma (\(n = 42\)), undifferentiated pleomorphic sarcoma (\(n = 22\)), vestibular schwannoma (\(n = 22\)), Ewing sarcoma (\(n = 19\)), malignant peripheral nerve sheath tumor (MPNST) (\(n = 19\)), myxofibrosarcoma (\(n = 17\)), enchondroma (\(n = 11\)), neurofibroma (\(n = 10\)), osteochondroma (\(n = 9\)), undifferentiated spindle cell sarcoma (\(n = 7\)), radiation-associated sarcoma (\(n = 4\)), rhabdomyosarcoma (\(n = 2\)) and osteosarcoma (\(n = 2\)).\textsuperscript{13–20} In addition, TMA’s of synovial sarcoma (\(n = 69\)), osteosarcoma (\(n = 65\)), MPNST (\(n = 20\)), rhabdomyosarcoma (\(n = 13\)), dedifferentiated liposarcoma (\(n = 20\)), radiation-associated tumors (\(n = 11\)) were constructed as previously described.\textsuperscript{21} Samples were handled according to the ethical guidelines described in “code for Proper Secondary Use of Human Tissue in the Netherlands” in a coded (pseudonymized) manner, as approved by the Leiden University Medical Center ethical board (B17.020, B17.036, B17.030, and B20.064). Furthermore, previously constructed TMAs from the UZ Leuven institute included alveolar soft part sarcoma (\(n = 59\)), different subtypes of liposarcoma (\(n = 42\)), inflammatory myofibroblastic tumor (\(n = 33\)) and alveolar rhabdomyosarcoma (\(n = 21\)).\textsuperscript{22} The analysis of anonymized data and use of archival FFPE tumor samples were approved by the Medical Ethics Committee, UZ Leuven (S51495, S59181). All tumors were classified according to the WHO classification of bone and soft tissue tumors, fifth edition.

**Immunohistochemistry**

Immunohistochemistry was performed with commercially available antibodies using a standard lab protocol, as described previously.\textsuperscript{21} In short, microwave antigen retrieval in either TRIS-EDTA (pH 9.0) or Citrate (pH 6.0) was performed using deparaffinized sections, followed by overnight incubation with the primary antibody. Details of antibodies are summarized in Supplementary Table 1. The following day, detection using power vision poly-HRP (Immunologic, the Netherlands) and visualization with a DAB+...
Table 1. Mismatch repair deficiency in bone and soft tissue tumors

| Tumor type                        | n  | MMRd | %  |
|-----------------------------------|----|------|----|
| Enchondroma                        | 11 | 0/8  | 0  |
| Osteochondroma                     | 9  | 0/5  | 0  |
| Chondrosarcoma                     | 206| 0/181| 0  |
| Subtypes: conventional             | 137| 0/118| 0  |
| Dedifferentiated                   | 28 | 0/26 | 0  |
| Clear cell                        | 20 | 0/18 | 0  |
| Mesenchymal                       | 21 | 0/19 | 0  |
| Osteosarcoma                      | 67 | 0/65 | 0  |
| Angiosarcoma of bone              | 37 | 0/24 | 0  |
| Ewing sarcoma                     | 19 | 0/18 | 0  |
| Radiation-associated bone sarcoma | 4  | 0/3  | 0  |
| Schwannoma                        | 22 | 0/22 | 0  |
| Neurofibroma                      | 10 | 0/10 | 0  |
| Inflammatory myofibroblastic tumor| 33 | 0/29 | 0  |
| Liposarcoma                       | 104| 0/101| 0  |
| Subtypes: myxoid                  | 49 | 0/48 | 0  |
| Dedifferentiated                  | 31 | 0/30 | 0  |
| Well differentiated               | 13 | 0/12 | 0  |
| Pleomorphic                       | 11 | 0/11 | 0  |
| Leiomyosarcoma                    | 88 | 4/88 | 5  |
| Synovial sarcoma                  | 69 | 0/65 | 0  |
| Alveolar soft part sarcoma        | 59 | 0/31 | 0  |
| Malignant peripheral nerve sheath tumor | 35 | 1/32 | 3  |
| Rhabdomyosarcoma                  | 37 | 2/33 | 6  |
| Subtypes: alveolar                | 22 | 0/18 | 0  |
| Pleomorphic                       | 3  | 1/3  | 33 |
| Embryonal                         | 4  | 1/4  | 25 |
| Spindle cell                      | 6  | 0/6  | 0  |
| NOS                               | 2  | 0/2  | 0  |
| Undifferentiated soft tissue sarcoma | 29 | 0/29 | 0  |
| Angiosarcoma of soft tissue       | 23 | 0/19 | 0  |
| Myxofibrosarcoma                  | 17 | 0/17 | 0  |
| Radiation-associated soft tissue sarcoma | 15 | 1/14 | 7  |

MMRd, mismatch repair deficient; NOS, not otherwise specified.

Substrate chromogen system (Dako, Glostrup, Denmark) followed. Finally, slides were counterstained with haematoxylin, dehydrated and mounted.

Nuclear expression of the MMR proteins was scored as positive, heterogeneous or negative. If heterogeneous or negative, the expression of the internal control was evaluated and staining was repeated using a whole slide section of the same tumor. Subsequently, immunohistochemistry for PD-1, PD-L1 and CD3 was performed on MMR deficient tumors. The scoring system was adapted from previous studies: PD-L1: negative: <1%, +: 1–49% and ++: ≥50%. The degree of T cell infiltration was graded as low if ≤5 T cells/HPF or high if >5 T cells/HPF. PD-1 expression was assessed on T cells and was considered positive if membranous staining was present.

MLH1 PROMOTER METHYLATION ASSAY

Since the loss of MLH1 and PMS2 expression is commonly caused by somatic promoter hypermethylation of MLH1, MLH1 promoter status was analysed in MLH1/PMS2 negative cases using methylation specific PCR. Briefly, using the EZ DNA methylation Gold kit (Zymo Research, Orange, US) bisulfite conversion of tumor DNA was performed. Bisulfite-converted DNA was amplified using specific methylated and unmethylated primers in a PCR reaction, as described previously.24,25

MICROSATELLITE INSTABILITY (MSI) ANALYSIS

MSI analysis was performed using MSI analysis system, version 1.2 (Promega), according to the manufacturer’s instructions. In short, PCR using five MSI Markers (BAT26-, BAT-25, NR-24, NR21, MONO-27) was performed and PCR products were analyzed using the SeqStudio genetic analyzer (ThermoFisher, Waltham, Massachusetts, U.S.). Samples were classified as microsatellite stable (MSS) if none of the markers were altered, MSI-Low if 1 out of 5 markers was unstable and MSI-High if ≥2 out of 5 markers were unstable.

LITERATURE SEARCH

A Pubmed search matching the terms of HNPCC, Lynch syndrome, mismatch repair deficiency, microsatellite instability and sarcoma(s), soft tissue tumor(s), bone tumor(s) was conducted. Studies were included if the full text was available and if reference to an internal control was made in case no expression of MMR proteins detected in tumor cells.
Results

INDEX CASES

The first index patient was a 55-year-old male presenting with a pleomorphic rhabdomyosarcoma in the lower extremity (Figure 1). Subsequently, he developed a pancreatic adenocarcinoma at the age of 60 and two years later an urothelial carcinoma of the ureter. He was referred to the clinical geneticist, where a germline mutation in \textit{MSH2} (p. Cys697Tyr) was found. The second index patient involved a male of 42 years presenting with a leiomyosarcoma of the psoas (Figure 2). Seven years later, the patient developed acute myeloid leukaemia, a sebaceous gland carcinoma and adenocarcinoma of the coecum. Although no mutation analysis was performed, the leiomyosarcoma showed a MSI-high phenotype (instability of three out of five microsatellite markers) and the coecum tumor a MSI-low phenotype (instability of one maker). Combined with the loss of MSH2/MSH6 expression, it is highly suspicious that this

\textbf{Figure 1.} Pleomorphic rhabdomyosarcoma of first index patient with a \textit{MSH2} germline mutation. H&E staining showing numerous lymphocytes intermingled between tumor cells. Cells are pleomorphic with enlarged nuclei, prominent nucleoli and surrounded by abundant eosinophilic cytoplasm, resembling rhabdomyoblasts (insert) (A). Immunohistochemistry for MyoD1 confirms skeletal muscle differentiation (B). Loss of expression of MSH2 (C) and MSH6 (D) is seen in tumor cells, while expression in immune and stromal cells is retained. Expression of PD-L1 is seen on tumors cells (E). Note the abundance of T cells in the CD3 immunohistochemical detection (F). Scale bar: 50 µm.
patient developed diverse tumors in the context of Muir-Torre syndrome, a variant of Lynch syndrome.

**PROTEIN EXPRESSION**

The index cases showed loss of expression of both MSH2 and MSH6, while MLH1 and PMS2 were retained (Figure 1 and 2). In addition, a total of six other tumors (three leiomyosarcomas, one embryonal rhabdomyosarcoma, one MPNST and one radiation-associated soft tissue sarcoma) showed loss of expression of one or more MMR proteins, leading to a total of eight cases with potential MMR defects (1%) (Table 1). Loss of MLH1 and PMS2 was seen in three cases (two leiomyosarcomas and one radiation-associated sarcoma), loss of MSH2 and MSH6 was present in one embryonal rhabdomyosarcoma and one MPNST. Isolated loss of PMS2 was seen in one leiomyosarcoma (Table 2). In the remaining 786 bone and soft tissue tumors, no loss of expression was observed (Table 1).

Five out of eight tumors with loss of MMR protein expression displayed expression of PD-L1 and a high influx of T cells. In two of these cases expression of
PD-1 was observed. Among the three PD-L1 negative tumors, the majority showed a low amount of tumor-infiltrating T cells (Table 2).

**Clinical and Genotypic Analysis**

In addition to the index cases, molecular information was available for one other leiomyosarcoma, which showed a **MLH1** mutation (p. Val7Argfs*18) in the tumor sample. Clinical data of this patient revealed a breast tumor and a rectal carcinoma. The patient was referred to the clinical genetics, though additional information could not be retrieved. Among the other MMR deficient sarcoma patients, one was known with neurofibromatosis type 1 and one developed adenocarcinoma of the prostate, while the remaining patients had no other tumors. The MMR deficient radiation-associated sarcoma occurred 10 years after radiation therapy of a liposarcoma. None of the examined **MLH1** negative tumors (n = 3) showed **MLH1** promoter hypermethylation. MSI analysis revealed one microsatellite stable tumor, while analysis failed on the remaining tumors due to insufficient quality of the DNA (Table 2).

**Mismatch Repair Deficient Bone and Soft Tissue Sarcomas in Literature**

A total of 30 MMR deficient bone and soft tissue sarcomas were encountered in literature (details are summarized in Table 3). Histologically classifiable tumors included liposarcoma (n = 5), osteosarcoma (n = 5), rhabdomyosarcoma (n = 4), alveolar soft part sarcoma (n = 3), clear cell sarcoma (n = 2), leiomyosarcoma (n = 1), PEComa (n = 1). Undifferentiated pleomorphic/unclassifiable sarcoma accounted for eight cases and in one cases the subtype was not specified.4,26-42 While most studies referred to case reports or case series, Doyle and colleagues investigated the frequency of MMR deficiency in a cohort of 279 cases and identified 6 MMR deficient cases (2%).26 In all these studies, seventeen patients had a germline mutation in one of the mismatch repair genes (Lynch syndrome n = 13; Muir-Torre syndrome n = 2; Constitutional Mismatch Repair Deficiency n = 2). A predominance of **MSH2** mutations, either germline or somatic, was found in MMR deficient sarcomas.

**Discussion**

This study provides a comprehensive immunohistochemical evaluation of MMR protein expression in a large series of bone and soft tissue tumors. We show that MMR deficiency is a rare phenomenon in bone and soft tissue tumors but can be relatively more frequent in soft tissue sarcomas with myogenic differentiation and in patients with a genetic predisposition/co-occurrence of other malignancies. MMR deficiency was detected in 1% of the total bone and soft tissue tumor cohort and was enriched to up to 5% in tumors with myogenic differentiation. The only non-myogenic MMR deficient tumors were a radiation-associated bone sarcoma and a MPNST. Notably, MMR deficiency was completely absent in a relatively large series of osteosarcomas and

| Histology                        | Grade* | MSH2 | MSH6 | MLH1 | PMS2 | Molecular data                  | PD-1 (%) | PD-L1 (%) | T cells/HPF |
|----------------------------------|--------|------|------|------|------|---------------------------------|----------|-----------|-------------|
| Pleomorphic rhabdomyosarcoma     | N/A    | –    | –    | +    | +    | MSH2 p. Cys697Tyr               | 64       | 80        | 140         |
| Embryonal rhabdomyosarcoma      | N/A    | –    | –    | +    | +    | NA                              | –        | 40        | 50          |
| Leiomyosarcoma                   | 1      | –    | –    | +    | +    | MSI-High                        | 23       | 40        | 47          |
| Leiomyosarcoma                   | 1      | +    | +    | –    | –    | **MLH1** p. Val7Argfs*18        | –        | 90        | 60          |
| Leiomyosarcoma                   | 1      | +    | +    | –    | –    | failed                          | –        | –         | <5          |
| Leiomyosarcoma                   | 1      | +    | +    | –    | –    | MSS                             | –        | –         | <5          |
| Radiation-associated sarcoma     | N/A    | +    | Weak | –    | –    | failed                          | –        | –         | <5          |
| MPNST                            | N/A    | –    | –    | +    | +    | NA                              | –        | 5         | 18          |

HPF, high-power field; het, heterogenous; +, positive; –, negative; N/A, not applicable; NA, not assessed; MPNST, malignant peripheral nerve sheath tumor.

*Grading according to FNCLCC.
| Authors                  | Year | Sarcoma          | Associated tumors/syndrome | MMR loss IHC on sarcoma | Genotypic analysis                                      |
|-------------------------|------|------------------|-----------------------------|-------------------------|--------------------------------------------------------|
| De Angelis de Carvalho, et al. | 2020 | Liposaroma       | Lynch syndrome              | MSH2 and MSH6           | MSH2 c.2152C>T                                         |
|                         |      | Osteosarcoma     | Lynch syndrome              | MSH2 and MSH6           | MSH2 c.1661+1G>A                                      |
| Doyle L, et al.        | 2019 | PEComa           | NA                          | MSH2 and MSH6           | MSH2 Y678*                                             |
|                         |      | Rhabdomyosarcoma | Lynch syndrome              | MSH2 and MSH6           |                                                        |
|                         |      | UPS              | NA                          | MSH2 and MSH6           | MSH2 R389*                                             |
|                         |      | Undifferentiated sarcoma | NA                  | PMS2                    | PMS2 R315*                                             |
|                         |      | Undifferentiated sarcoma | NA                  | MSH6                    | MSH6 F1088Sfs*2                                       |
| Kim S, et al.          | 2017 | Sarcoma NOS      | NA                          | MSH2 or MLH1            |                                                        |
| Daou B, et al.         | 2015 | Osteosarcoma     | CMMRD                       | PMS2                    | PMS2 c.400C>T                                          |
|                         |      |                  | Colorectal adenocarcinoma   |                         | PMS2 c.1579del                                        |
|                         |      |                  | Anaplastic ganglioglioma    |                         |                                                        |
|                         |      |                  | Acute myeloid leukemia      |                         |                                                        |
| Cranmer L, et al.      | 2013 | Pleomorphic      | Lynch syndrome              | MLH1 and PMS2           | PMS2 G857A                                             |
|                         |      | rhabdomyosarcoma |                             |                         |                                                        |
|                         |      |                  | Colorectal adenocarcinoma   |                         |                                                        |
| Lee N, et al.          | 2013 | UPS              | Cutaneous sebaceous tumor   | MSH2                    | NA                                                     |
|                         |      |                  | Muir-Torre syndrome         |                         |                                                        |
| Yozu M, et al.         | 2013 | Pleomorphic      | Colorectal cancer           | MSH2 and MSH6           | MSH2 mutation                                          |
|                         |      | liposarcoma      |                             |                         |                                                        |
|                         |      |                  | Sebaceous neoplasm          |                         |                                                        |
|                         |      |                  | Muir-Torre syndrome         |                         |                                                        |
| Urso E, et al.         | 2012 | Leiomyosarcoma   | Lynch syndrome              | MSH2 and MSH6           | MSH2; deletion of exon 1-16                            |
|                         |      |                  |                              |                         |                                                        |
|                         |      |                  | Mucinous adenocarcinoma     |                         |                                                        |
|                         |      |                  | colon                       |                         |                                                        |
|                         |      |                  | Kidney cancer               |                         |                                                        |
| Ahmed H, et al.        | 2012 | Osteosarcoma     | Invasive duct carcinoma     | NA                      | MSH2 mutation and MLH1 mutation                        |
| Brieger A, et al.      | 2011 | UPS              | Lynch syndrome              | MSH2                    | MSH2 c.2038C>T                                        |

© 2021 The Authors. Histopathology published by John Wiley & Sons Ltd., Histopathology
Among the MMR deficient tumors, three patients were suspected to have or had an established diagnosis of Lynch syndrome / Muir-Torre syndrome. Our findings are in keeping with the study of Doyle et al., who also reported an overall frequency of 2% but a marked enrichment (10%) among undifferentiated/unclassifiable sarcomas using parallel sequencing followed by immunohistochemical evaluation of MMR protein expression. The fact that the frequency of MMR deficiency is comparable between their study, starting with an NGS approach, and the present study, starting with immunohistochemistry, suggests that immunohistochemistry could serve as a cost-effective surrogate marker for MMR deficiency.

The current study includes a relatively large cohort of bone sarcomas, including osteogenic, chondrogenic tumors and Ewing sarcoma, thereby representing the three most common bone sarcomas. Among the soft tissue sarcomas, also the most common subtypes are included (liposarcoma, leiomyosarcoma and undifferentiated soft tissue sarcoma). However, given the high amount of sarcoma subtypes it is not possible to evaluate a completely representative cohort. In addition, some tumor types are overrepresented, including those with myogenic differentiation (leiomyosarcoma, rhabdomyosarcoma and inflammatory myofibroblastic tumor), which was based on the myogenic differentiation in the tumors of our two index patients.

Table 3. (Continued)

| Authors          | Year | Sarcoma          | Associated tumors/syndrome | MMR loss IHC on sarcoma | Genotypic analysis               |
|------------------|------|------------------|----------------------------|------------------------|----------------------------------|
| UPS              |      | Gliosarcoma      | Lynch syndrome             | MSH2                   | \(MSH2 \text{ c.942 + 3A>T}\)  |
|                  |      | Breast cancer     | \text{CMMRD}               | NA                     | \( PMS2 \text{ p. Cys73}^* \)  |
|                  |      | Cervix carcinoma  | \text{Muir-Torre syndrome} | \text{MSH2}            |                                  |
| Kratz CP, et al. | 2009 | Embryonal Rhabdomyosarcoma | \text{CMMRD} | NA | \( PMS2 \text{ p. Cys73}^* \)  |
|                  |      | Adenocarcinoma    | colo                       | \text{PMS2}            | NA                               |
| Nilbert M, et al.| 2009 | Liposarcoma       | Lynch syndrome             | MSH2 and MSH6          | \(MSH2 \text{ c.942 + 3A>T}\)  |
|                  |      | Liposarcoma       | Lynch syndrome             | MSH2 and MSH6          | \(MSH2 \text{ c.1-7_366 +?del}\) |
| Hirata K, et al. | 2006 | Liposarcoma       | Lynch syndrome             | MSH2                   | AT deletion at codon 677 in exon 13 of MSH2 |
| Garcia I, et al. | 2006 | Clear cell sarcoma| MSH6                      | NA                     |                                  |
| Lynch HT, et al. | 2003 | Osteosarcoma      | Lynch syndrome             | NA                     | \(MSH2 \text{ mutation in exon 4}\) |
| den Bakker MA, et al. | 2003 | Pleomorphic Rhabdomyosarcoma | Lynch syndrome | MSH2 | \(MSH2 \text{ mutation}\)  |
| Saito T, et al.  | 2003 | ASPS \((n = 3)\)  | MSH2 and MLH1 in 2 cases   | NA                     |                                  |
| Sijmons R, et al.| 2000 | UPS               | Lynch syndrome             | MSH6                   | \(MSH6 \text{ mutation}\)  |

ASPS, alveolar soft part sarcoma; CMMRD, constitutional mismatch repair deficiency; LMS, leiomyosarcoma; NA, not available; NOS, not otherwise specified; UPS, undifferentiated pleomorphic sarcoma.

chondrosarcomas. Among the MMR deficient tumors, three patients were suspected to have or had an established diagnosis of Lynch syndrome / Muir-Torre syndrome. Our findings are in keeping with the study of Doyle et al., who also reported an overall frequency of 2% but a marked enrichment (10%) among undifferentiated/unclassifiable sarcomas using parallel sequencing followed by immunohistochemical evaluation of MMR protein expression. The fact that the frequency of MMR deficiency is comparable between their study, starting with an NGS approach, and the present study, starting with immunohistochemistry, suggests that immunohistochemistry could serve as a cost-effective surrogate marker for MMR deficiency.
addition, we included a series of alveolar soft part sarcomas which was based on data from literature. In contrast to our findings and those from Doyle et al., two other groups reported a higher frequency of MMR deficiency varying between 23% and 85% in soft tissue sarcoma and osteosarcoma, respectively.41,44 In our series, none of the 65 osteosarcomas investigated demonstrated loss of MMR protein expression. Since MMR deficient sarcomas often show a significantly elevated TMB relative to MMR proficient sarcomas,45 and the TMB in osteosarcoma is reportedly low,46 with low to moderate response to ICI,46–48 it seems very unlikely that the majority of osteosarcomas would be MMR deficient. Given the lack of reporting on a positive internal control and the lack of molecular validation in these publications, these cases were not taken along in Table 3.

This is the first systematic analysis of MMR deficiency in cartilaginous tumors, which showed complete absence of MMR deficiency in 181 patients. Based on this specific biomarker, these patients would not be eligible to ICI therapy. We previously also showed the absence of PD-L1 expression in conventional, clear cell and mesenchymal chondrosarcoma. However, PD-L1 expression and the presence of an immune infiltrate were found in 52% of the dedifferentiated chondrosarcomas, which were also included in the current study, and PD-L1 expression was restricted to the dedifferentiated component.23 Response to immunotherapy in clinical trials was observed in few (dedifferentiated) chondrosarcoma patients.47,49 This again illustrates that in the current era of immunotherapy, with the lack of definitive biomarkers, evaluation of tumors based on both their immune phenotype and genomic mutation profile is needed to determine which patients would likely be responsive to ICI treatment.

For alveolar soft part sarcoma, loss of expression of MSH2 and MLH1 was previously reported in two (18.2%) and three (27.3%) of eleven cases, respectively.40 Hypermethylation of MSH2 and MLH1 promoter region was absent, but three of eight (37.5%) cases were found to be MSI-low. Moreover, alveolar soft part sarcoma, despite a low mutational load and lack of inflammatory infiltrate, was observed to be able to respond to immune checkpoint inhibitors.50 Two patients with sustained partial response showed a MMR mutational signature after sequencing, however, staining for MMR protein expression was intact.51 This led us to include a relatively large series of this very rare sarcoma subtype in our studies, as tissue microarrays were previously constructed and available from the EORTC-CREATE study.22,52 We did not find loss of MMR protein expression in 31 evaluable cases. Thus, we cannot confirm previous results of MMR deficiency in alveolar soft part sarcoma, and other mechanisms underlying sensitivity to immune checkpoint inhibitors in these tumors seem more likely.

Despite the selection bias in our cohort, both tumors of the index patients demonstrated myogenic differentiation, most of the other MMR deficient sarcomas also displayed myogenic differentiation. Notably, all MMR deficient leiomyosarcoma were low-grade (grade 1). Since leiomyosarcomas often show a poor response to chemotherapy, it would be worthwhile to examine MMR status in this selected tumor group, ultimately providing these patients novel treatment options. Moreover, we previously showed PD-L1 expression together with high T cell infiltrate and HLA class I expression in around 30% of high grade leiomyosarcoma, reflecting an active immune microenvironment.53 Thus far, results of clinical trials of PD-1 blockade therapy in leiomyosarcoma patients are diverse. Single reports with successful treatment or a mixed partial response or stable disease are described, while others report no effect to treatment.5,49,54

Of note, one of the leiomyosarcomas with loss of PMS2 expression showed a microsatellite stable phenotype. Although MSI analysis kit is commonly used in colorectal cancer, it is not widely applicable in other tumors. In addition, concordance between MMR protein expression and MSI is variable between tumor types with percentages varying between 68% in epithelial ovarian tumors to 97% in colorectal carcinomas.56 However, no data is available for sarcoma. It would be highly interesting to see whether this patient is carrying a germline variant in one of the mismatch repair genes, however germline analysis was not covered by the IRB approval.

Most of the MMR deficient bone and soft tissue sarcomas in the current study showed presence of infiltrating immune cells and five cases also showed expression of PD-L1 on the tumor cells. This may indicate that these patients could benefit from ICI. Thus far, effectiveness of ICI in sarcoma patients has only been studied in limited trials with variable results. In the SARC028 study, Pembrolizumab showed promising results in patients with undifferentiated pleomorphic sarcoma and dedifferentiated liposarcoma, while in the PEMBROSARC and Alliance A091401 trial no response was observed. Also, PD-L1 expression alone was not a predictive biomarker.47,48,57 Clearly, there is an urgent need for predictive biomarkers, and it remains to be answered if the MMR status contributes to the selection of patients who will respond to ICI.
To conclude, MMR deficiency is rare in bone and soft tissue tumors. Screening focusing on tumors with myogenic differentiation, undifferentiated/unclassifiable sarcomas and in patients with a genetic predisposition / co-occurrence of other malignancies can be helpful identifying patients potentially eligible for ICI, while for other bone and soft tissue tumors reflex testing remains debatable.

Acknowledgments

We thank Arnoud Boot for providing excellent technical assistance while performing the MLH1 promoter hypermethylation assay. We also acknowledge Anne Jansen for the fruitful discussion. We also thank the European Organization for Research and Treatment of Cancer for permission to use the data for this research. The contents of this publication and methods used are solely the responsibility of the authors and do not necessarily represent the official views of the EORTC. I. Briaire-de Bruijn, L.G. Sand, P.C.W. Hogendoorn, M.A. de Graaff, M. de Vries, S.J. Luk, S. Verbeke, D. Meijer and Y.T. Sundara are acknowledged for contributing to construction of TMAs.

Author contributions

The study was designed, written and reviewed by S.W. Lam, M. Kostine and J.V.M.G. Bovée. All authors contributed to the data collection, data analysis and interpretation. The manuscript was approved by all authors.

Conflict of interest

None declared.

Funding

Leiden University Medical Center

References

1. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer 2019; 19: 133–150.
2. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med 2018; 50: 1–11.
3. D’Angelo SP, Shoushtari AN, Agaram NP et al. Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. Hum Pathol 2015; 46: 357–365.
4. Kim ST, Klemperner SJ, Park SH et al. Correlating programmed death ligand 1 (PD-L1) expression, mismatch repair deficiency, and outcomes across tumor types: implications for immunotherapy. Oncotarget 2017; 8: 77415–77421.
5. Carbognin L, Pilotto S, Milella M et al. Differential activity of Nivolumab, Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity analysis of trials in Melanoma, Lung and Gastrointestinal Cancers. PLoS One 2015; 10: e0130142.
6. Maleki VS. High and low mutational burden tumors versus immunologically hot and cold tumors and response to immune checkpoint inhibitors. J Immunother Cancer 2018; 6: 157.
7. Le DT, Durham JN, Smith KN et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017; 357: 409–413.
8. Le DT, Uram JN, Wang H et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015; 372: 2509–2520.
9. Qin B-D, Jiao X-D, Liu KE et al. Basket trials for intractable cancer. Front Oncol 2019; 9: 229.
10. Sinicrope FA. Lynch syndrome-associated colorectal cancer. N Engl J Med 2015; 372: 193–201.
11. Engel C, Loeffler M, Steinke V et al. Risks of less common cancers in proven mutation carriers with Lynch syndrome. J Clin Oncol 2012; 30: 4409–4415.
12. Baglietto L, Lindor NM, Dowty JG et al. Risks of Lynch syndrome cancers for MSH6 mutation carriers. J Natl Cancer Inst 2010; 102: 193–201.
13. van Oosterwijk JG, Meijer D, van Ruler MA et al. Screening for potential targets for therapy in mesenchymal, clear cell, and dedifferentiated chondrosarcoma reveals Bcl-2 family members and TGFbeta as potential targets. Am J Pathol 2013; 182: 1347–1356.
14. van Oosterwijk JG, van Ruler M, Briaire-de Bruijn IH et al. Src kinases in chondrosarcoma chemoresistance and migration: Dusartnib sensitises to doxorubicin in TP53 mutant cells. Br J Cancer 2013; 109: 1214–1222.
15. de Graaff MA, Cleton-Jansen AM, Szuikai K, Bovee JV. Mediator complex subunit 12 exon 2 mutation analysis in different subtypes of smooth muscle tumors confirms genetic heterogeneity. Hum Pathol 2013; 44: 1597–1604.
16. van IJzendoorn DGP, Szuikai K, Briaire-de Bruijn IH, Kostine M, Kuijjer ML, Bovee JMV. Machine learning analysis of gene expression data reveals novel diagnostic and prognostic biomarkers and identifies therapeutic targets for soft tissue sarcomas. PLoS Comput Biol 2019; 15:e1006826.
17. Endo M, de Graaff MA, Ingram DR et al. NY-ESO-1 (CTAG1B) expression in mesenchymal tumors. Mod Pathol 2015; 28: 587–595.
18. de Vries M, van Tellingen O, van der Mey AG, Bunt AMG, Bruin PB, Hogendoorn PCW. BCRP expression in schwannoma, plexiform neurofibroma and MPNST. Oncotarget 2017; 8: 88751–88759.
19. Verbeke SLJ, de Jong D, Bertioli F et al. Array CGH analysis identifies two distinct subgroups of primary angiosarcoma of bone. Genes Chromosomes Cancer 2015; 54: 72–81.
20. Sand LG, Berghuis D, Szuikai K, Hogendoorn PC. Expression of CCL21 in Ewing sarcoma shows an inverse correlation with metastases and is a candidate target for immunotherapy. Cancer Immunol Immunother 2016; 65: 995–1002.
MMR deficiency in bone and soft tissue tumors

21. Cleven AH, Hocker S, Briare-de Bruijn I, Szuhaï K, Cleton-Jansen AM, Bovee JV. Mutation analysis of H3F3A and H3F3B as a diagnostic tool for giant cell tumor of bone and chondroblastoma. Am J Surg Pathol 2015; 39: 1576–1583.

22. Lee N, Luthra R, Lopez-Terrada D, Wang WL, Lazar AJ. Characteristics of mismatch repair deficiency in sarcomas. Mod Pathol 2019; 32: 977–987.

23. Dong J, Zanello M, Varlet P et al. An unusual case of constitutional mismatch repair Deficiency Syndrome with Anaplastic Ganglioglioma, Colonic Adenocarcinoma, Osteosarcoma, Acute Myeloid Leukemia, and signs of Neurofibromatosis Type 1: case report. Neurosurgery 2015; 77(1): E145–E152; discussion E152.

24. Cramer LD, Chen CC, Morgan S, Martino G, Ray J. Pleomorphic rhabdomyosarcoma in a patient with hereditary non-polyposis colorectal cancer. J Clin Oncol 2013; 31: e108–110.

25. Lee N, Luthra R, Lopez-Terrada D, Wang WL, Lazar AJ. Retropertioneal undifferentiated pleomorphic sarcoma having microsatellite instability associated with Muir-Torre syndrome: case report and review of literature. J Cutan Pathol 2013; 40; 730–733.

26. Zou M, Symmans P, Dray M et al. Muir-torre syndrome-associated pleomorphic liposarcoma arising in a previous radiation Beld. Virchows Arch 2013; 462; 357–360.

27. Ueno E, Agostini M, Pucciarrelli S et al. Soft tissue sarcoma and the hereditary non-polyposis colorectal cancer (HNPCC) syndrome: Formulation of an hypothesis. Mol Biol Rep 2012; 39; 9307–9310.

28. Ahmed H, Salama A, Salem SE, Bahnassy AA. A case of synchronous double primary breast carcinoma and osteosarcoma: Mismatch repair gene mutations as a possible cause for multiple early onset malignant tumors. Am J Case Rep 2012; 13: 218–223.

29. Bronner E, Hocker S, Schaefer D et al. Malignant fibrous histiocytoma is a rare Lynch syndrome-associated tumor in two German families. Fam Cancer 2011; 10: 591–595.

30. Krieger C, Balzer S, Mitterer P et al. Malignant fibrous histiocytoma in patients with constitutional mismatch-repair-deficiency syndrome. J Med Genet 2009; 46: 418–420.

31. Nilbert M, Therkildsen C, Nissen A, Akerman M, Bernstein I. Sarcomas associated with hereditary nonpolyposis colorectal cancer: Broad anatomical and morphological spectrum. Fam Cancer 2009; 8: 209–213.

32. Hirata K, Kanemitsu S, Nakayama Y et al. A novel germline mutation of MSH2 in a hereditary nonpolyposis colorectal cancer patient with liposarcoma. Am J Gastroenterol 2006; 101; 191–196.

33. Garcia JJ, Kramer MJ, O’Donnell RJ, Horvai AE. Mismatch repair protein expression and microsatellite instability: A comparison of clear cell sarcoma of soft parts and metastatic melanoma. Mod Pathol 2006; 19: 950–957.

34. Lynch HT, Deters CA, Hogg D, Lynch JF, Kinarsky Y, Gatalica Z. Familial sarcoma: Challenging pedigrees. Cancer 2003; 98; 1947–1957.

35. den Bakker MA, Seynaeve C, Klijn M, Dinjens WN. Microsatellite instability in a pleomorphic rhabdomyosarcoma in a patient with hereditary non-polyposis colorectal cancer. Histopathology 2003; 43: 297–299.

36. Saito T, Oda Y, Kawaguchi K-I et al. Possible association between tumor-suppressor gene mutations and hMSH2/ hMLH1 inactivation in alveolar soft part sarcoma. Hum Pathol 2003; 34: 841–849.

37. Sijmons R, Hofstra R, Hollema H et al. Inclusion of malignant fibrous histiocytoma in the tumour spectrum associated with hereditary non-polyposis colorectal cancer. Genes Chromosomes Cancer 2000; 29: 353–355.

38. de Angelis de Carvalho N, Nitsuma BN, Kozak VN, et al. Clinical and molecular assessment of patients with Lynch Syndrome and Sarcomas underpinning the association with MSH2 Germline Pathogenic Variants. Cancers (Basel) 2020; 12(7): 1848.

39. Jentzsch T, Rohb H, Husmann M, Bode-Lehniewska B, Fuchs B. Expression of MSH2 and MSH6 on a tissue microarray in patients with osteosarcoma. Anticancer Res 2014; 34; 6961–6972.

40. Kawaguchi K, Oda Y, Takahira T et al. Microsatellite instability and hMLH1 and hMSH2 expression analysis in soft tissue sarcomas. Oncol Rep 2005; 13; 241–246.

41. Doyle LA, Fletcher CD, Hornick JL. Nuclear expression of CAMTA1 distinguishes epithelioid hemangiendothelioma from histologic mimics. Am J Surg Pathol 2016; 40: 94–102.

42. Wu C-C, Beird HC, Andrew Livingston J et al. Immuno-genomic landscape of osteosarcoma. Nat Commun 2020; 11; 1008.

43. Tawbi HA, Burgess M, Bolejjack V et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol 2017; 18: 1493–1501.

44. D’Angelo SP, Mahoney MB, Van Tine BA et al. Nivolumab with or without ipilimumab treatment for metastatic sarcoma (alliance A091401): two open-label, non-comparative, randomised, phase 2 trials. Lancet Oncol 2018; 19: 416–426.

45. Paoluzzi L, Cucavio A, Ghisani M et al. Response to anti-PD1 therapy with nivolumab in metastatic sarcomas. Clin Sarcoma Res 2016; 6: 24.

46. Paoluzzi L, Mak GJ, Diagnosis, prognosis, and treatment of alveolar soft-part sarcoma: a review. JAMA Oncol 2019; 5: 254–260.

47. Lewin J, Davidson S, Anderson ND et al. Response to immune checkpoint inhibition in two patients with alveolar soft-part sarcoma. Cancer Immunol Res 2018; 6: 1001–1007.

48. Schöffski P, Wozniak A, Kasper B et al. Activity and safety of crizotinib in patients with alveolar soft part sarcoma with rearrangement of TFE3: European organization for research and treatment of cancer (EORTC) phase ii trial 90101 ‘CREATE’. Ann Oncol 2018; 29: 758–765.

49. Kostine M, Briare-de Bruijn IH, Cleven AHG et al. Increased infiltration of M2-macrophages, T-cells and PD-L1 expression in high grade leiomyosarcomas supports immunotherapeutic strategies. Oncoimmunology 2018; 7: e1386828.

50. Quiroga D, Liebner DA, Philpion JS et al. Activity of PD1 inhibitor therapy in advanced sarcoma: a single-center retrospective analysis. BMC Cancer 2020; 20: 527.

© 2021 The Authors. Histopathology published by John Wiley & Sons Ltd., Histopathology
55. Burgess MA, Bolejack V, Van Tine BA et al. Multicenter phase ii study of pembrolizumab (P) in advanced soft tissue (STS) and bone sarcomas (BS): final results of SARC028 and biomarker analyses. J. Clin. Oncol. 2017; 35; 11008–11008.

56. Lee J-H, Cragun D, Thompson Z et al. Association between IHC and MSI testing to identify mismatch repair-deficient patients with ovarian cancer. Genet Test Mol Biomarkers 2014; 18; 229–235.

57. Toulmonde M, Penel N, Adam J et al. Use of PD-1 targeting, macrophage infiltration, and IDO pathway activation in Sarcomas: a phase 2 clinical trial. JAMA Oncol 2018; 4; 93–97.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Details of antibody.