CHARLES UNIVERSITY
MEDICAL FACULTY IN PILSEN
ŠIKL'S DEPARTMENT OF PATHOLOGY

RARE TUMORS OF SOFT TISSUES AND OTHER SITES

HABILITATION THESIS

MUDr. Michael Michal, Ph.D.

PILSEN 2022

DECLARATION

Hereby I declare that this habilitation thesis is based on research work performed at the Department of Pathology of the Faculty of Medicine in Pilsen, Charles University in Prague, during the period 2014-2022 and was written by me. All sources of information are reported in the list of references.

I agree with permanent deposition of my thesis at the Centre for Scientific Information in Faculty of Medicine in Pilsen, Charles University in Prague. I also agree with permanent deposition of my habilitation thesis in the database of the inter-university project Theses.cz for the purposes of a continuous surveillance of comparability of degree dissertations.

CONTENT

INTRODUCTION

The habilitation thesis named “Rare tumors of soft tissues and other sites” represents a commentary to my articles that have been published from 2015 - 2022. My research work has been mainly focused on very rare tumors, in keeping with the long-term focus of our department. That has included the identification of novel tumor entities as well as a better characterization of the existing ones in terms of their clinical, histopathological, molecular and/or prognostic features

Overall, this work includes 55 English articles on which I participated either as the main author (13x), senior author (2x) or as the co-author (40x). Apart from one study, all were published in journals with impact factor. These journals include various American surgical pathology journals (43x), the leading European surgical pathology journal “Virchows Archive” (6x; IF 4.5), the leading British surgical pathology journal “Histopathology” (4x; IF 7.78) or the main Japanese surgical pathology journal “Pathology International” (1x; IF 2.1). Twenty-two articles were published in three of the leading journals in the field of surgical pathology, namely Modern Pathology (IF 8.25), Histopathology (IF 7.78) and American Journal of Surgical Pathology (IF 6.3), I was the first author of 5 of these articles.

The habilitation thesis is thematically divided into 5 parts:

1. Bone, soft tissue and other mesenchymal tumors
2. Tumors of the lymphoid system and tumors of the spleen
3. Head and neck tumors
4. Testicular and penile tumors
5. The rest

Each part is further subdivided into subchapters that are either formed by a single article or - when more articles relate to the same topic/entity - by a group of articles. Each subchapter is provided with a commentary. Each article is also provided at least with a title page but in order to limit the extent of this thesis to a reasonable number of pages, only first and senior author articles are provided with a full text.

1. PART:

BONE, SOFT TISSUE AND OTHER MESENCHYMAL TUMORS

a. ADIPOCYTIC TUMORS

i. Spindle cell/pleomorphic lipoma

1. Lipoblasts in spindle cell and pleomorphic lipomas: A close scrutiny

Spindle cell and pleomorphic lipomas (SCL/PL) are benign lipomatous tumors considered to represent a morphological spectrum of one single entity. In our first study in this area, we investigated the presence and frequency of lipoblasts, an immature form of fat cell, in these tumors. By many general pathologists, lipoblasts are incorrectly considered as a diagnostic criterion for atypical lipomatous tumor/well differentiated liposarcoma (ALT/WDL) – an intermediate to malignant adipocytic tumor - where they very often occur but their presence does not automatically make the diagnosis of ALT/WDL. The presence of lipoblasts in SCL/PL has been reported in the past (1-3), but contemporary reports often fail to mention this frequent and important feature of SCL/PL which often leads to diagnostic difficulties or errors. Our goal was to illustrate how common lipoblasts in these benign tumors are. Overall, we collected 129 cases of SCL/PL. Then we started to morphologically review all the cases again, searching for lipoblasts. When more than 3 unequivocal fat cells having hyperchromatic, indented, or sharply scalloped nuclei (i.e. lipoblasts) were found, the case was regarded as positive for the presence of lipoblasts. The 129 cases selected consisted of 91 SCL and 38 PL. Lipoblasts were found in 37 cases (41%) of SCL. Of the 38 PL cases, 25 (66%) contained lipoblasts. While in the majority of SCL cases, only a few lipoblasts were found after a meticulous search, PL showed single or several lipoblasts in a much more diffuse manner. Nevertheless, a significant subset of cases of both SCL and PL contained several foci with a highly prominent lipoblastic admixture, which was easy to be found.

After this step, we stained all the lipoblasts containing cases with CD34 and RB protein antibodies, both of which yielded results compatible with the diagnosis of SCL/PL. Then we performed FISH analysis using probes for *FUS* gene to exclude myxoid liposarcoma and *MDM2* and *CDK4* genes to rule out the possibility of ALT/WDL. Since many of these cases were sent to us for consultation, we also collected the referring diagnoses. For all the cases we also obtained follow up information. Overall, the most common referring diagnosis was that of ALT or another type of liposarcoma, which was listed in 6 of 15 SCL and in 8 of 13 PL cases with known submitting diagnosis. Follow-up was available for 44 patients, of whom only 2 suffered recurrence of SCL/PL, further underscoring their completely benign nature.

Summarizing our findings, this study emphasized the fact that lipoblasts are very frequent finding in both SCL (41%) and PL (66%), and it shows that their presence is not a distinguishing feature of these tumors from ALT/WDL. Even more importantly, in many SCL/PL, lipoblasts constitute a component so prominent as to cause diagnostic difficulties to the unaware as illustrated by the referral diagnoses which commonly listed liposarcoma as the possibility. In order to prevent a misdiagnosis, it is therefore important to base the diagnosis on different, diagnostically more relevant clinicopathological features such as the typical location of SCL/PL in the subcutis of the upper back/neck/shoulder region or histological features such as the presence of ropey collagen bundles in SCL/PL. A useful byproduct of our study was also the confirmed low recurrence rate of immunohistochemically and molecular genetically validated set of SCL/PL.

2. A report of a patient presenting with three metachronous 13q14LOH mesenchymal tumours: spindle cell lipoma, cellular angiofibroma and mammary myofibroblastoma

SCL/PL, cellular angiofibroma and mammary myofibroblastoma represent a group of closely related tumors which share somewhat similar morphological and immunohistochemical features (4). Moreover, they also share very similar molecular background characterized by the frequent presence of *RB1* gene deletions which is located at chromosomal region 13q14. In this paper, we reported the first patient who, over the years, presented with all three of these lesions, further confirming that these tumors are closely related on molecular level as this patient had to be genetically predisposed. Unfortunately, we did not have the possibility to perform germline testing from a nonneoplastic patient tissue.

3. Spindle Cell Predominant Trichodiscoma or Spindle Cell Lipoma With Adnexal Induction? A Study of 25 Cases, Revealing a Subset of Cases With *RB1* Heterozygous Deletion in the Spindle Cell Stroma

Trichodiscoma is a cutaneous adnexal lesion with predominantly sebaceous differentiation. In trichodiscoma, the predominant tissue element is centrally located variably myxoid spindle cell stroma admixed with collagen bundles surrounded by prominent clusters of sebaceous glands. We noticed that a relatively high proportion of trichodiscomas demonstrates a remarkable stromal "lipomatous metaplasia," which along with spindle cells, collagen bundles, and myxoid stroma closely resembles or seems identical to SCL. This finding prompted us to collect more such cases to investigate whether they exhibit the typical immunohistochemical and cytogenetic features of SCL. This study is based on the hypothesis that the trichodiscoma with "lipomatous metaplasia" might represent cutaneous SCL with reactive changes in overlying adnexal structures.

To clarify their possible relationship to SCL, 25 cases of trichodiscoma with stromal "lipomatous metaplasia" were collected and examined using immunohistochemical stains (CD34 and RB1 protein) and fluorescence in situ hybridization for the detection of *RB1* deletion. The spindle cell stroma was immunohistochemically positive for CD34 in 16 of 20 cases, and 18 of 19 cases showed loss of RB1 staining in lesional spindle cells which would be compatible with SCL. However, fluorescence in situ hybridization analysis detected *RB1* gene heterozygous deletion only in 6 of 20 cases.

We concluded that despite the SCL-like appearance of the investigated cases, the majority of them supposedly represent genuine spindle cell predominant trichodiscomas with adipose tissue admixture. However, there was a subset of histopathologically indistinguishable cases with proved *RB1* deletion, which likely represent SCL with trichodiscoma-like epithelial/adnexal induction rather than spindle cell predominant variant of trichodiscoma.

ii. Dysplastic lipoma

1. Dysplastic Lipoma: A Distinctive Atypical Lipomatous Neoplasm With Anisocytosis, Focal Nuclear Atypia, p53 Overexpression, and a Lack of *MDM2* Gene Amplification by FISH

In our routine and consultation practice, we have repeatedly encountered a distinctive subcutaneous fatty neoplasm with notable adipocytic size variation, focal (usually mild) adipocytic atypia and patchy, often single-cell, fat necrosis. Because of the size variation and atypia, these tumors were usually received with a submitting diagnosis of atypical lipomatous tumor, i.e. a lipomatous tumor with borderline/intermediate malignancy which is in most cases characterized by *MDM2* gene amplification. However, these tumors were consistently negative for *MDM2* gene amplification by fluorescence in situ hybridization (FISH). Similar tumors have been reported previously in small series under the designations "subcutaneous minimally atypical lipomatous tumor" and "anisometric cell lipoma" (5, 6).

We undertook a study to analyze a large cohort of these lesions. Sixty-six cases of this tumor type were collected, and morphology was carefully reviewed. Immunohistochemistry with multiple markers was performed and cases were tested for *MDM2* gene amplification and *RB1* gene deletion with FISH and for *TP53* mutations by Sanger sequencing. Next-generation sequencing analysis using a panel of 271 cancer-related genes, including *TP53*, *RB1*, and *MDM2*, was also carried out in selected cases. Our patient cohort included 57 male patients, 8 female patients, and 1 patient of unstated sex, who ranged in age from 22 to 87 years (mean: 51.2 y). All tumors were subcutaneous, with most examples occurring on the upper back, shoulders, or posterior neck (86.4%). Ten patients had multiple (2 to 5) lipomatous tumors, and the histology was confirmed to be similar in the different sites in 4 of them, including 1 patient who had a retinoblastoma diagnosed at age 1. The tumors were generally well circumscribed. All tumors were managed by simple local excision. Follow-up was available for 47 patients (range: 1 to 192 mo; mean: 27 mo) and revealed 2 local recurrences and no metastases. In all cases, p53 immunoexpression was noted (range: 2% to 20% of adipocytic nuclei), characteristically highlighting the most atypical cells. Twenty of 50 cases had MDM2 immunoreactivity, usually in <1% of the neoplastic cells, but in 4 cases, up to 10% of the cells were positive. Of 32 cases tested, 22 showed a near total loss of *RB1* immunoexpression, and the remainder showed partial loss. Three of 13 cases showed *RB1* gene deletion in >45% of the cells by FISH (our threshold value for reporting a positive result) with an additional 3 cases being very close to the required cutoff value. *MDM2* gene amplification was absent in all 60 cases tested, including those with the greatest *MDM2* immunoexpression and most pronounced atypia. All 5 tested cases showed no *TP53* mutation with Sanger sequencing.

Our results indicated this fatty tumor variant, for which we proposed the designation "dysplastic lipoma," is a distinctive and relatively common clinicopathologic entity that has a strong male predominance, a characteristic anatomic distribution, and reproducible histologic, immunohistochemical, and molecular genetic findings. This tumor warrants separation from ordinary lipoma with fat necrosis, fat-rich spindle cell lipoma and mainly from the conventional form of atypical lipomatous tumor that features *MDM2* gene amplification. The distinction from the latter is particularly clinically relevant as atypical lipomatous tumors are tumors of intermediate malignancy and have a capacity to progress to high-grade sarcomas.

2. Dysplastic lipoma: potential diagnostic pitfall of using *MDM2* RNA in situ hybridization to distinguish between lipoma and atypical lipomatous tumor

The molecular background of the vast majority of ALT is characterized by *MDM2* gene amplification. Detection of this abnormality by FISH serves as a very useful diagnostic tool in distinguishing ALT from benign mimics including dysplastic lipoma as these tumors consistently lack *MDM2* gene amplification. RNA in situ hybridization (RNA-ISH) has been found to be a good alternative method for detecting *MDM2* gene amplification in clinical practice (7). However, our group found that *MDM2* RNA-ISH is routinely positive in cases of dysplastic lipoma. Of 11 studied cases, ten (91%) showed positive *MDM2* RNA-ISH. The reason for *MDM2* RNA transcript upregulation in dysplastic lipoma remains unclear; however, given the frequent p53 overexpression and complete Rb loss in dysplastic lipoma, one may speculate that p53 and/or Rb could be involved as there is a close interplay between these proteins (8).

Nevertheless, positive RNA-ISH in cases of dysplastic lipoma is an important diagnostic pitfall that might lead to overdiagnosis of this benign tumor as ALT and needs to be remembered when using this assay.

iii. Lipoblastoma-like tumor

1. **Lipoblastoma-like tumor of the vulva: a clinicopathologic, immunohistochemical, fluorescence in situ hybridization and genomic copy number profiling study of seven cases**
2. **A lipoblastoma-like tumour of the testicular cord: male counterpart of lipoblastoma-like tumour of the vulva**

(Joint commentary to both publications)

Lipoblastoma-like tumor of the vulva is a benign mesenchymal neoplasm of adipocytic differentiation occurring almost exclusively in anogenital region of adult females. It has overlapping features with other 3 adipocytic tumors, namely lipoblastoma, myxoid liposarcoma, and spindle cell lipoma. Prior studies of lipoblastoma-like tumor have evaluated PLAG1, HMGA2, and RB1 immunohistochemistry and *DDIT3* rearrangement status, with results supporting its distinction from lipoblastoma and myxoid liposarcoma. However, absent RB1 expression was reported in a majority of tested cases, suggesting that lipoblastoma-like tumor may have underlying 13q alterations and therefore might be related to *RB1*-deleted soft tissue tumors such as spindle cell lipoma (9).

To further understand the molecular genetics of lipoblastoma-like tumor, in our study **iii.1.,** we examined 7 cases by RB1 immunohistochemistry, *DDIT3* and *PLAG1* break apart FISH probes, *RB1* enumeration FISH probe, and genomic copy number analysis by microarray. Patient age ranged from 21 to 56 years (median 35 years). Clinical follow up was available for 5 patients (71%) ranging 3–264 months (median 74 months). Microscopically, lipoblastoma-like tumor formed large lobules separated by thin and/or thick bands of fibrous tissue and had a prominent network of thin-walled vessels. Each tumor was predominantly composed of spindle cells and lipoblasts with variable quantities of mature adipocytes. RB1 immunohistochemistry exhibited a heterogeneous or “mosaic” pattern of weak and negative nuclear expression in all seven cases. *DDIT3* and *PLAG1* FISH were negative in each case. No evidence of *RB1* regional gain or loss was identified by FISH. Genomic copy number analysis by chromosomal microarray showed a normal diploid profile in six tumors (86%). One tumor had copy number abnormalities consisting of an 11.9 megabase deletion from 1p13.3 to 1p11.2 and monosomy 14.

Although lipoblastoma-like tumor has certain morphological features of lipoblastoma and myxoid liposarcoma, we confirmed it is genetically different from these tumors. Lipoblastoma-like tumor also shares overlapping morphological features with spindle cell lipoma but for the first time we also found that lipoblastoma-like tumor does not appear to have structural abnormalities of 13q resulting in deletion of *RB1* and is therefore genetically distinct from this tumor as well.

While ordinary lipoblastoma-like tumor occurs almost exclusively in anogenital region of adult females, particularly on the vulva, our study **iii.2.** has presented the first case of this tumor in a male patient. Our publication was followed by another case report confirming occasional occurrence of this tumor in males (10) which might eventually lead to dropping out of the words “of the vulva” in the name of this tumor.

iv. Others

1. Dedifferentiated liposarcoma composed predominantly of rhabdoid/epithelioid cells: a frequently misdiagnosed highly aggressive variant

After the recognition that most malignant fibrous histiocytomas of the retroperitoneum contain *MDM2* gene amplification most characteristic of (but not exclusive to) well differentiated liposarcomas, most of these tumors started to be classified as dedifferentiated liposarcomas (DDL) (11). Today DDLs are considered to be the most frequent undifferentiated sarcomas in the retroperitoneum. They also occur in other sites including the paratesticular area, the extremities, the head and neck and the trunk (12). DDL is defined as a non-lipogenic sarcoma usually with spindled morphology that develops within a WDL as a recurrence of the former or, more commonly (90% of cases), de novo as an undifferentiated sarcoma showing amplification of *MDM2* and *CDK4* (both mapping to chromosome 12q14-15) and occurring at anatomic sites where DDL typically occurs (12). Since FISH testing for *MDM2* amplification became widely available, numerous morphological patterns of DDLs have been reported.

In this study, we described our experience with DDL showing a striking predominance of small to medium-sized cells with rhabdoid, epithelioid or non-descript round cell morphology. They closely mimicked a variety of other neoplasms, in particular undifferentiated carcinoma, mesothelioma, malignant melanoma, anaplastic large cell lymphoma, epithelioid leiomyosarcoma, epithelioid variant of pleomorphic rhabdomyosarcoma, PEComa, high grade epithelioid myxofibrosarcoma and others. Five of our 6 cases originated in the retroperitoneum (3 in the psoas muscle) and one in the deep soft tissue of the thigh. All 3 patients with follow-up died of metastatic disease within 4 to 8 months.

One of the aims of this study was to analyze their IHC profile, in order to find staining patterns overlapping with the differential diagnostic entities. Our cohort showed expression of MDM2 (6/6), CDK4 (5/6) but also of pancytokeratin AE/1AE3 (4/6) and diffuse desmin and myogenin (2/6) reactivity. Therefore, these tumors might potentially mimic undifferentiated carcinoma or pleomorphic rhabdomyosarcoma unless one thinks of DDL as a possible diagnosis. On the other hand, the SWI/SNF complex components (SMARCB1, SMARCA2, SMARCA4, ARID1A and PBRM1) were intact in all cases, therefore excluding the possibility of an alternative diagnosis from the heterogeneous spectrum of SWI/SNF-deficient malignancies (13). Most importantly, all cases showed high-level co-amplification of *MDM2/CDK4* by FISH.

As our follow-up shows, it is a very aggressive liposarcoma variant with a very short median survival. The molecular background of these tumors may also be a potential target to novel therapeutic modalities (i.e. Nutlins (14)) in a close future. Therefore, the separation from other differential diagnostic entities is warranted.

b. FIBROBLASTIC/MYOFIBROBLASTIC AND FIBROHISTIOCYTIC TUMORS

i. Myxoinflammatory fibroblastic sarcoma

1. **Myxoinflammatory fibroblastic sarcoma: an immunohistochemical and molecular genetic study of 73 cases**
2. **RNA-sequencing of myxoinflammatory fibroblastic sarcomas reveals a novel *SND1::BRAF* fusion and 3 different molecular aberrations with the potential to upregulate the TEAD1 gene including *SEC23IP::VGLL3* and *TEAD1::MRTFB* gene fusions**
3. **High-grade myxoinflammatory fibroblastic sarcoma: a report of 23 cases**
4. **Pleomorphic hyalinizing angiectatic tumor revisited: all tumors manifest typical morphologic features of myxoinflammatory fibroblastic sarcoma, further suggesting 2 morphologic variants of a single entity**

(Joint commentary to all 4 publications)

Myxoinflammatory fibroblastic sarcoma (MIFS) is an entity simultaneously described in 1998 by 3 different groups of authors under various names as a low-grade soft tissue tumor of distal extremities with a high propensity for local recurrence (15-17). As further studies have shown, this tumor may not be restricted only to acral sites (10), and apart from very frequent local recurrences which typify these tumors, 10 cases with metastatic disease have been documented in the literature so far (18). Nonetheless, despite these publications, MIFS is still generally considered a low-grade neoplasm with a very low metastatic potential.

The last decade has brought a significant increase in our understanding of the molecular genetic background of MIFS. Using FISH, two studies have reported *BRAF* rearrangements in a subset of MIFS with the following frequency: 0/7 cases and 5/19 (26%, this study analyzed a cohort from which all t(1;10) positive cases were excluded) (19, 20). RNA-seq of a single *BRAF* rearranged case revealed *TOM1L2::BRAF* fusion (19). The *BRAF* abnormalities seem to be mutually exclusive with a t(1;10) translocation with breakpoints mapping to *TGFBR3* in 1p22 and *OGA* (formerly known as *MGEA5*) in 10q24 (19). For unclear reasons, possibly related to varying diagnostic criteria and/or different sensitivity of diagnostic assays, the frequency of this translocation extremely varies between published reports. In several smaller studies, FISH analysis revealed the t(1;10) in 0/6 (21), 5/7 (22), and 3/4 (23) cases, respectively. Nevertheless, the largest FISH study to date by Zreik et al. revealed this aberration in 0/31 cases and only demonstrated 2/31 cases with OGA rearrangements, while a *TGFRB3* rearrangement was not identified in any case (24). The most frequent molecular aberration in MIFS is therefore a *VGLL3* gene amplification which, in larger studies analyzing more than 4 cases, has been detected in 5/7 (20), and 14/26 (19)

cases, respectively. Overall, the *VGLL3* amplification was found in 57.6 % of 33 reported cases.

In the publication **b.i.1.** we contributed almost one half of cases into the largest molecular and immunohistochemical study of MIFS to date in order to search for diagnostically helpful immunohistochemical markers and to provide more data about the molecular genetic background of this tumor as the studies to date have provided disparate results.

While the study did not identify any single immunohistochemical marker that would be specific for this neoplasm, it confirmed the frequent expression of cyclin D1(bcl-1), Factor XIIIa, CD10 and D2-40 already reported previously in smaller series. These markers were in our study expressed in 94.5%, 89%, 80% and 56% of conventional MIFS cases, respectively. While all these markers are relatively non-specific by themself, their use in combination may help to support the diagnosis. Regarding the molecular analysis, FISH and array comparative genomic hybridization were performed in a large subset of cases to investigate the utility for detecting the *TGFBR3* and *OGA* t(1;10) rearrangement and *BRAF* abnormalities. Using a combination of FISH and/or aCGH, t(1;10) was detected in only 3 of 54 cases (5.5%). The array comparative genomic hybridization study also demonstrated amplification of *VGLL3* on chromosome 3 that was detected in 8 of 20 cases (40%). *BRAF* alterations were observed by FISH in 4 of 70 cases (5.7%) and correlated with gain of chromosome 3p12 (*VGLL3*). A novel fusion transcript involving exon 6 of *ZNF335* and exon 10 of *BRAF* was identified in one case.

Our study confirmed that recurrent molecular alterations, with the exception of *VGLL3* amplification, are relatively uncommon in MIFS. Given the low rate of t(1;10) and *BRAF* gene rearrangements in our study, we believe neither of these is cost-effective or sensitive enough to serve as routine diagnostic markers for MIFS. While *VGLL3* amplification has been identified in various soft tissue neoplasms (25), it appears to be present quite commonly in MIFS, occurring in approximately half of all MIFS cases studied to date. Demonstration of amplification of *VGLL3* on chromosome 3 in combination with expression of bcl-1 and FXIIIa may help support the diagnosis, however, due to their low specificity these markers are not sufficient for a definitive diagnosis in the absence of the appropriate clinical-pathological context. Therefore, until a more robust genetic or immunohistochemical signature is identified, the diagnosis of MIFS rests on its characteristic clinicopathological features.

In the publication **b.i.2.** we performed a smaller but more throughout molecular analysis of 14 cases of MIFS using FISH and RNA sequencing. Using these techniques, we detected three novel gene fusion, namely *SND1::BRAF*, *SEC23IP::VGLL3* and *TEAD1::MRTFB* and also confirmed the frequent presence of *VGLL3* gene amplification which was detected in 5/11 cases tested. Both *VGLL3* gene amplification and the novel *SEC23IP::VGLL3* and *TEAD1::MRTFB* gene fusions have the ability to switch on the TEAD1 signaling cascade and may result in cancerogenesis and tumor progression. The *SND1::BRAF* fusion likely causes an increased activity of the MEK/ERK signaling cascade as has been published previously (19).

The presented cases broaden the molecular genetic spectrum of MIFS and highlight the importance of the VGLL3-TEAD1 interaction, while further confirming the role of *BRAF*

deregulation in the pathogenesis of this neoplasm. Future studies may uncover further genetic aberrations within the network, either as part of the Hippo or MEK/ERK pathways, or within a different signaling cascade.

While most MIFS are low-grade neoplasms, we have noticed that some cases show very similar to identical morphological features but also exhibit high-grade cytological traits in the form of increased mitotic activity, necrosis or extreme atypia with or without transformation into undifferentiated high-grade sarcoma already lacking MIFS features. Twenty-three examples of such tumors were presented in the publication **b.i.3**. Clinically, the 23 cases of HG MIFS affected mostly older people, with a mean age of 64.3 years (age ranged from 39-93 years). Some of these tumors were located at the acral sites, but the majority affected the proximal parts of the extremities, especially the thigh. Follow-up was available for 18 patients, of whom 9 developed metastatic disease and 7 of these died. Therefore, it confirmed that this high-grade variant has a much more ominous prognosis that the classical MIFS. As the genetic background of MIFS was largely unknown at the time of publication (except for the occasional presence of t(1;10) in a small subset of cases), we did not include molecular genetic analysis in our study. However, we are currently preparing a molecular study on this topic that confirmed *VGLL3* gene amplification in about half of the cases (same ratio as in conventional low-grade MIFS) and occasional presence of *BRAF* amplification. Preliminary results were presented at a platform presentation in March 2022 at the Annual meeting of United Stated and Canadian Academy of Pathology in Los Angeles.

Pleomorphic hyalinizing angiectatic tumor (PHAT) is locally aggressive sarcoma (21) that is generally considered to be closely related to MIFS. The progression of PHAT to a myxofibrosarcoma-like sarcoma has been described in several cases (26). Both PHAT and MIFS have a very similar clinical presentation, and prognosis and the morphological features of both tumors are also considered to be very much alike. However, these morphological similarities have never been precisely studied and defined in the literature. For this purpose, we review all 9 cases filed as PHATs from our files and published our findings in publication **b.i.4**.. All the 9 lesions contained cells morphologically indistinguishable from MIFS on the background of predominant features of PHAT characterized by thin-walled angular ectatic vessels surrounded by perivascular hyaline material. This vascular change can be theoretically initiated by the tumor growth itself, possibly being more likely to occur at the lower extremities (which is a typical location of PHAT), where high hydrostatic pressure and often concomitant venous insufficiency are present.

The possibility that vascular changes in PHAT represent solely a histologic pattern and not a true neoplasm or that MIFS and most of the cases of PHAT represent a different morphologic manifestation of a single entity has already been raised by other authors (21). That would also explain why some authors described cases showing features of PHAT recurring as myxofibrosarcomas because the recurrence in most cases might, in fact, be high-grade MIFS, and most of these reported lesions might have represented primarily a low-grade MIFS associated with morphologically prominent ectatic hyalinized vessels.

t(1;10) leading to identical gene rearrangement of *TGFBR3* and *MGEA5* in a subset of cases of PHAT and MIFS lends further support to the proposition that these 2 entities are likely morphologic variants of the same tumor (21).

ii. Solitary fibrous tumor

1. Misleading Morphologic and Phenotypic Features (Transdifferentiation) in Solitary Fibrous Tumor of the Head and Neck: Report of 3 Cases and Review of the Literature

2. Lipomatous solitary fibrous tumors harbor rare *NAB2-STAT6* fusion variants and show upregulation of the gene Peroxisome Proliferator Activated Receptor Gamma (PPARG) encoding for a regulator of adipocyte differentiation

(Joint commentary to both publications)

Solitary fibrous tumor (SFT) is an uncommon mesenchymal neoplasm with fibroblastic differentiation. While originally thought to represent a mesothelial neoplasm primarily affecting the pleura, over the years the concept of SFT has undergone a significant evolution. Two main phenotypes of SFT are distinguished: the first is a classic "paucicellular SFT" with alternating cellular spindle cells and collagenized areas intermixed with branching thick-walled vessels. The second is the "cellular" or "HPC-like" SFT composed of more monotonous, highly cellular proliferation of ovoid to spindled cells with little intervening fibrous stroma and rather thin-walled vessels. However, the full morphological spectrum of SFT is extremely broad and also includes myxoid, lipomatous, angiofibroma-like/giant cell, and sarcomatous/dedifferentiated SFT (27). The concept of so-called dedifferentiation is a well-known phenomenon in soft tissue and bone tumors. The term dedifferentiated SFT was for the first time introduced by Mosquera et al. In their work, it was defined as an abrupt transition of conventional SFT into a poorly differentiated high-grade sarcoma (28). The dedifferentiated component most often shows epithelioid, spindled or pleomorphic morphology with occasional cases featuring heterologous chondro-osteosarcomatous or rhabdomyosarcomatous areas (29). Recently, a new and extremely rare phenomenon of transdifferentiation, which is well-known from embryology, was described in rare cases of SFT (30, 31). In contrast to dedifferentiation as defined above, transdifferentiation refers to the emergence of aberrant phenotypes (including expression of different immunohistochemical markers) distinct from the original neoplasm which might be associated with high-grade transformation/dedifferentiation (30, 31).

NAB2::STAT6 fusion gene leading to the overexpression of STAT6 protein was shown to be the main oncogenic molecular event in SFT and has led to the discovery of STAT6 immunohistochemical antibody which serves as a highly sensitive and specific marker for this tumor (32-34).

In our publication **ii.1.** we reported 3 new cases of SFT of the head and neck region in which transdifferentiation (n= 2) or dedifferentiation (n= 1) occurred. The reported tumors originated in the oral minor salivary glands, the base of the tongue, and sinonasal tract and closely resembled hyalinizing clear cell carcinoma of the salivary gland, adenocarcinoma not

otherwise specified and biphenotypic sinonasal sarcoma, respectively. All cases were positive for cytokeratins and variably expressed S100 protein but at the same time showed diffuse nuclear STAT6 positivity, and harbored *NAB2::STAT6* gene fusions consistent with the diagnosis of SFT. Both dedifferentiation and transdifferentiation represent a major diagnostic pitfall. It is important to be aware of this phenomenon when diagnosing SFT in order to avoid misdiagnosis.

The paper **ii.2.** studied the lipomatous variant of SFT, i.e. unusual form of SFT showing lipomatous admixture which might significantly alter the differential diagnosis (which might then include liposarcoma variants) of this tumor. A hybrid-capture-based NGS panel was employed to detect *NAB2::STAT6* gene fusions at the RNA level. In addition, the RNA expression levels of 507 genes were evaluated using this panel, and were compared with a control cohort of nonlipomatous SFTs. Five of 11 (45%) of lipomatous SFTs in this series harbored the uncommon *NAB2* exon 4-*STAT6* exon 4 gene fusion variant, which is observed in only 0.9% to 1.4% of nonlipomatous SFTs. Furthermore, lipomatous SFTs displayed significant differences in gene expression compared with their nonlipomatous counterparts, including up-regulation of the gene peroxisome proliferator activated receptor-γ. Peroxisome proliferator activated receptor-γ is a nuclear receptor regulating adipocyte differentiation, providing a possible explanation for the fat-forming component in lipomatous SFTs. The current study provided a possible molecular genetic basis for the distinct morphologic features of lipomatous SFTs.

iii. Nodular fasciitis, myositis ossificans and other USP6-rearranged lesions

1. Novel *EIF5A-USP6* Gene Fusion in Nodular Fasciitis Associated With Unusual Pathologic Features: A Report of a Case and Review of the Literature

2. Fibro-osseous Pseudotumor of digits and myositis Ossificans show consistent *COL1A1-USP6* rearrangement: a Clinicopathological and genetic study of 27 cases

(Joint commentary to both publications)

USP6-rearranged lesions represent a group of pseudosarcomatous proliferations which were initially thought to be non-neoplastic but were later shown to harbor various USP6 gene fusions. Besides similar molecular background, all these lesions have some overlapping clinical features such as the tendency for self-limited growth and low recurrence rate despite incomplete excision. Morphologically, they share the presence of plump (myo)fibroblasts, and variable bone production. Some of the most notable members of this group include nodular fasciitis, myositis ossificans and fibro-osseous pseudotumor of digits (35).

While consistently harboring *USP6* gene rearrangements, there are multiple possible 5' fusion partners in nodular fasciitis (35). In the publication **iii.1.** we reported yet another fusion partner, namely *EIF5A* which expanded the molecular genetic spectrum of this neoplasm. This particular case was also associated with some unusual clinical and morphological features such dermal location and the presence of atypical mitosis.

Myositis ossificans is a relatively rare tumor occurring in diverse soft tissue sites, most notably on the thigh or other extremity locations. In contrast, fibro-osseous pseudotumor of digits, as the name implies typically occurs on the fingers and toes. Both tumors were described as 2 separate entities but over the years, several papers pointed to very similar clinical and morphological features suggesting that both tumors represent only an anatomic variant of the same entity. Eventually, Flucke et al reported the presence of *USP6* gene rearrangements in both tumors. However, this study used FISH assay to detect the presence of USP6 gene rearrangement but did not attempt to characterize the fusion partner (36).

In our paper **iii.2.** we collected a large series of both myositis ossificans and fibro-osseous pseudotumor of digits and perform NGS study using Archer FusionPlex assay which is able to detect the whole fusion gene. As a result, we found that both tumors not only share *USP6* gene rearrangements but that also the fusion partner is identical and is represented by the *COL1A1* gene. Our finding put an end to speculations about the relationship of both tumors and confirmed they are identical tumors occurring at different locations.

iv. Myxoid fibroblastic tumor of the vocal cords with a TIMP3::ALK fusion

1. **Vocal cord inflammatory myofibroblastic tumor with mucoid deposits harboring *TIMP3–ALK* fusion: A potential diagnostic pitfall**
2. ***TIMP3::ALK* fusions characterize a distinctive myxoid fibroblastic tumor of the vocal cords: a report of 7 cases**

(Joint commentary to both publications)

In studies **iv.1.- 2.** we reported altogether 7 cases of an unusual variably myxoid tumor of the vocal cords which previously did not precisely fit into any known tumor category. While this tumor proved to be completely benign in our studies, due to the presence of certain atypical morphological features it might be confused for malignant lesions, potentially leading to significant overtreatment. Targeted RNA-sequencing revealed an identical *TIMP3::ALK* fusion with exon 1 of *TIMP3* gene being fused with exon 12 of ALK gene in all analyzable cases leading to positive ALK immunohistochemical staining in all cases. This finding point towards the possibility this tumor represents a myxoid variant of inflammatory myofibroblastic tumor, i.e. a long known tumor entity consisting of myofibroblastic proliferation with intermediate malignancy (rarely metastasizing). In our first paper on this topic (**iv.1.**) we even called this lesions as such. However, after studying additional cases of this tumor in study **iv.2.**, we have found that several features argue against this tumor being a variant of inflammatory myofibroblastic tumor. For this reason, we eventually decided to use a neutral name "Myxoid fibroblastic tumor of the vocal cords with a *TIMP3::ALK* fusion". Irrespective of the name used, it is a morphologically distinct and diagnostically challenging lesion that needs to be recognized by surgical pathologists in order to prevent overdiagnosis in this clinically very delicate area.

v. Others

1. *EWSR1-SMAD3*-rearranged fibroblastic tumor: An emerging entity in an increasingly more complex group of fibroblastic/myofibroblastic neoplasms

This study reported 4 cases of the shortly before described soft tissue tumor called *EWSR1-SMAD3* acral fibroblastic spindle cell tumor (37). This tumor has a very characteristic morphology and can be distinguished from its mimics by a consistent expression of ERG antibody and also by the presence of *EWSR1::SMAD3* fusion. To assess how specific is the ERG expression among the pertinent differential diagnostic entities, we also analyzed the staining characteristics of several such examples.

To find the particular tumors with *EWSR1::SMAD3* gene fusion, we have searched our soft tissue consultation files using a combination of pertinent keywords. Also, we reviewed all cases collected over the last 30 years and designated as "uncharacterized tumors of soft tissues". After review of more than 300 cases, we have found 3 tumors compatible with the diagnosis. Another case was recently received for a consultation at Cleveland Clinic, USA and was added to our study.

We scrutinized the tumors using light microscopy, IHC, FISH and NGS. Clinical information, including follow-up, were obtained, when possible.

All cases in our cohort occurred in women aged 5 to 68 years (mean: 36.5 years). Two were located on the hand, 1 on the foot and the last case arose on the calf. The tumor size ranged from 1 to 1.5 cm in the greatest dimension, with a mean size of 1.2 cm. Except for one recent case, follow-up was available, ranging from 7 to 18 years (mean, 11.7 years), with a recurrence noted in one case after 10 years. All tumors were subcutaneous and showed two main components. One consisted of bland, spindled cells with elongated nuclei which were round when observed on the cross-section. These cells mostly grew in relatively hypercellular, well-organized and intersecting fascicles. The second component was prominently hyalinized and paucicellular. Both components showed either a distinct zonation pattern or they were randomly intermingled with each other. In all 3 analyzable tumors, NGS showed *EWSR1::SMAD3* gene fusion in each case. By FISH, one tested case also revealed unbalanced rearrangement of the *EWSR1* gene. All 4 cases showed strong, diffuse nuclear expression of ERG, whereas none of the mimics stained with this antibody except for weak to moderate staining in calcifying aponeurotic fibromas (9/10 cases). Two tumors showed focal weak to moderate expression of SAT-B2.

The 4 cases presented in our study further broadened the clinicopathologic spectrum of tumors with *EWSR1::SMAD3* gene fusion. They also confirmed that they represent a novel entity for which we proposed the name *EWSR1::SMAD3*-rearranged fibroblastic Tumor. Our study also proved that in the context of fibroblastic/myofibroblastic tumors, ERG IHC is a relatively specific marker for these neoplasms. Based on the results of our paper and of the initial publication by Kao et al (37), *EWSR1::SMAD3*-positive fibroblastic Tumor was included as an emerging entity into the 2020 WHO classification of bone and soft tissue tumors. Since our paper was the second one published on this topic and remains one of the

largest series on these tumors to date, I was also invited to be a co-author of this chapter in the upcoming WHO classification of skin tumors.

2. Superficial CD34-Positive Fibroblastic Tumor and *PRDM10* - rearranged Soft Tissue tumor are overlapping entities: a comprehensive study of 20 cases

Superficial CD34-positive fibroblastic tumor (SCD34FT) and *PRDM10*-rearranged soft tissue tumor (PRDM10-STT) are recently described rare mesenchymal tumors (38, 39). Both lesions had been reported to share many clinicopathological features but their exact relationship remained unclear (39). Our study aimed to compare cases of SCD34FT and PRDM10-STT to explore their similarities and differences.

Ten lesions each of SCD34FT and PRDM10-STT were studied using immunohistochemistry, array-comparative genomic hybridization (aCGH), RNA sequencing and exome sequencing. Clinicopathological features were recorded and compared.

Overall, the only morphological features seen exclusively in PRDM10-STT were myxoid stromal changes (three of 10) and metaplastic bone (two of 10). All tumors diffusely expressed CD34, while pan-keratin and desmin were commonly seen focally. SynCAM3 was diffusely expressed in 12 cases (SCD34FT, n = 5; PRDM10-STT, n = 7), independently of fusion status. aCGH profiles were 'flat' (PRDM10-STT, n = 4; SCD34FT, n = 2) and exome sequencing showed no recurrent pathogenic mutations (PRDM10-STT, n = 2; SCD34FT, n = 4). This study expanded the knowledge on PRDM10-STT and SCD34FT and provided additional evidence for considering them as overlapping entities, which eventually became the prevalent view on these tumors.

3. Sclerosing epithelioid fibrosarcoma of bone: morphological, immunophenotypical, and molecular findings of 9 cases

Sclerosing epithelioid fibrosarcoma (SEF) is rare mesenchymal tumor primarily occurring in soft tissues. However, yet rarer cases may arise primarily in bone where they, due to their rarity and quite variable morphology, may be mistaken for other neoplasms. Since only 2 dozen of primary skeletal SEF had been reported previously (40), we collected 9 additional cases of this sarcoma and performed clinicopathological and molecular analysis with a special emphasis on immunohistochemical expression of SATB2, a commonly used marker for osteogenic differentiation.

Based on our study, SEF of bone proved to be a relatively indolent sarcoma of adults commonly located in the flat/irregular bones which has a conspicuous tendency for bone metastasis. It has characteristic radiographic features of a predominantly lytic expansile lesion with a sclerotic rim. Due to overlapping histological features, SEF is often misdiagnosed as osteosarcoma or as a chondroid tumor. Focal or patchy SATB2 expression which was seen in approximately half of SEF represent an important pitfall when differentiating this tumor from osteosarcoma. Two distinct morphological patterns referred to as classical SEF and hybrid SEF-low-grade fibromyxoid sarcoma seem to correlate with molecular features. While most examples of skeletal SEF exhibit *EWSR1* rearrangements, particularly the hybrid forms may rarely harbor *FUS* gene fusions.

4. Superficial acral fibromyxoma: clinicopathological, immunohistochemical, and molecular study of 11 cases highlighting frequent Rb1 loss/deletions

This study focused on a benign fibroblastic tumor called superficial acral fibromyxoma (SAF). Histologically, SAF have a fairly non-specific morphology and often elude confident diagnosis Moreover, the IHC profile of SAF is also unspecific, usually expressing only CD34 (41).

In a cohort of 11 cases, we studied the Retinoblastoma-1 (RB-1) protein expression as well as *RB1* gene deletion by FISH. Except for one case, all 9 analyzable cases showed a complete loss of RB-1 immunoexpression. Moreover, all 7 tested cases had *RB1* gene deletion by FISH. These results highlight frequent *RB1* deficiency as possible driver molecular event in SAF and indicate relationship of SAF to the RB1-deleted tumor family. This group includes, besides spindle cell and pleomorphic lipoma as the prototypes (42), also mammary-type myofibroblastoma and cellular angiofibroma (43, 44). All these lesions are unified by consistent expression of CD34, RB1 loss by IHC, as a consequence of *RB1* gene deletions, and a benign clinical course with a low recurrence rate. In this study, we added SAF as another lesion to the family of RB1-deficient neoplasms. The loss of RB1 IHC staining and perhaps even more importantly, the *RB1* gene deletions detectable by FISH provide a very useful adjuvant methods especially in difficult to diagnose cases, as some SAF are occasionally confused for low-grade sarcomas.

5. Recurrent novel *HMGA2-NCOR2* fusions characterize a subset of keratin-positive giant cell-rich soft tissue tumors

Giant cell tumor of soft tissue (GCT-ST) is an extremely uncommon mesenchymal tumor consisting of dual population of cells. The first consists of uniform histiocytoid mononuclear cells lacking nuclear pleomorphism admixed with bland osteoclastic giant cells. Metaplastic bone formation is a frequent feature of these tumors - a peripheral rim (shell) of woven bone is seen in almost half of the cases. GCT-ST originates predominantly in the superficial soft tissues of the extremities. Adults in their fifth decade are predominantly affected with a very wide age range (5–89 years) without sex predilection (45). At one point, they were thought to represent the soft tissue counterparts of GCT of bone but are now known to lack the *H3F3* mutations characteristic of osseous GCT. Immunohistochemically, in most cases, the neoplastic mononuclear cells of GCT-ST were reported as being negative with cytokeratins (45).

In this study, we presented 6 distinctive giant cell-rich soft tissue neoplasms that expressed cytokeratins and carried a recurrent *HMGA2::NCOR2* gene fusion. All GCT-ST that were negative for cytokeratins were also negative for this fusion. The *HMGA2::NCOR2*-positive cohort showed a distinct female predilection – five were females and one male aged 14–60 years (median, 29). All cases were indolent on follow-up. The tumors originated in superficial soft tissues of the upper extremity (2), lower extremity (2), head/neck (1), and trunk (1). Morphologically, the main difference between *HMGA2::NCOR2*-positive and negative subgroup was the absence of bone production in the former, whereas 4/9 cases of the latter exhibited areas with osteoid production. Immunohistochemically, SATB2 expression (3/4) was frequent in keratin negative subgroup but were lacking in keratin-positive tumors.

The distinctive immunophenotype and genotype of these tumors strongly suggest that they represent a discrete entity, differing from conventional, cytokeratin negative GCT-ST and other osteoclast-rich morphologic mimics. Their natural history appears favorable, although a study of additional cases and longer follow-up are warranted.

6. *ALK* Gene Fusions in Epithelioid Fibrous Histiocytoma: A Study of 14 Cases, With New Histopathological Findings

Fibrous histiocytoma is a benign tumor occurring in the dermis, superficial subcutis or rarely, in deep soft tissues over a wide anatomic range. When this tumor consists of more than 50% of epithelioid cells it is referred to as epithelioid fibrous histiocytoma (EFH). In 88% of cases, this subtype shows immunoreactivity with ALK antibody and even most of the immunonegative cases show *ALK* gene rearrangements (46). Another study found that a subset of these *ALK* rearranged lesions harbor *VCL::ALK* and *SQSTM1::ALK* gene fusions (47). However, the majority of *ALK* fusion partners remained unknown. This led us to analyze all EFH stored in our files to further characterize the molecular underpinnings and also, to look for novel, yet unreported morphological features of these tumors.

After morphological review, 14 well characterized examples of EFH were included in the study. All ALK immunonegative cases were excluded. Then, we further morphologically scrutinized the cases and performed *ALK* FISH to confirm or exclude the gene break. After the *ALK* gene break was confirmed by FISH, the cases were submitted for NGS-based panel analysis of 195 targets in 40 genes [The Comprehensive Thyroid Lung kit (ArcherDX Inc, Boulder, CO)].

We found several novel morphological features of EFH including occasional presence of dominant spindle cell population (as opposed to predominantly epithelioid cells) which expanded the possible differential diagnosis of this tumor. *ALK* genes fusions were found in all but 3 cases and included *SQSTM1::ALK* (3), *VCL::ALK* (3), *TMP3::ALK* (2), *PRKAR2A::ALK* (1), *MLPH::ALK* (1), and *EML4::ALK* (1). No correlation between histological features and type of *ALK* fusion was found.

Summarizing these results, ALK-positive EFH shows frequently ALK gene fusions that involve various protein-coding genes, implicated in a variety of biological processes. Rare variants of EFH rather consist of spindled “non-epithelioid” cells, thus occasioning a resemblance to PEComa or leiomyoma. Inclusion of such lesions into the spectrum of EFH must be validated by further observations.

c. PERIPHERAL NERVE SHEATH TUMORS

1. Multivacuolated mucin-filled cells: a unique cell characteristic of plexiform neurofibroma. A report of 11 cases

Neurofibroma is a benign peripheral nerve sheath tumor composed of a variable admixture of Schwann, perineurial-like, perineurial, mast and fibroblastic cells. Five neurofibroma subtypes are recognized, one of which is plexiform neurofibroma. Its recognition is important since as much as 85% of cases plexiform neurofibroma are associated with neurofibromatosis type 1 (48), and in addition, they are more prone to undergo a malignant transformation than the remaining four subtypes .

In our practice, we encountered several cases of plexiform neurofibroma, an extensively studied tumor type in the past, that exhibited a so-far unreported and highly distinctive type of cell. It typically featured a variably sized, multivacuolated cytoplasm divided by fine septa with a small polygonal nucleus that was dislodged to the periphery of the cells and was often compressed or slightly indented by the cytoplasmic mucous substance. Often the cells resembled a soccer ball or a jellyfish. Based on the appearance of these cells, we named them multivacuolated mucin-filled cells (MMFC). To collect as many cases as possible, more than 100 plexiform neurofibroma were reviewed which led to the identification of 11 such examples. Other (non-plexiform) neurofibroma subtypes, malignant neural tumors as well as some other entities were reviewed all of which lacked this type of cell.

With the Alcian blue stain, the vacuoles of MMFC showed positive staining, providing evidence that they contain mucus. In all nine cases tested, the MMFC stained positively for CD34, which selectively highlighted their presence, with some plexiform neurofibromas containing hundreds of these elements. The immunostaining was usually strong, and it outlined the cytoskeleton of the MMFC. Out of the 9 tested cases, in 6 lesions MMFC were also positive for GLUT-1, with the staining pattern similar to that of CD34. Two cases also showed expression of Claudin-1 antibody, again in a pattern similar to that of the CD34 antibody. As expected, S-100 protein reacted in all 9 tested cases with the Schwann cell population but was consistently negative in all MMFC, as were all the remaining antibodies. The common combined positivity of MMFC with CD34 and GLUT-1 and, in few cases, Claudin-1 antibodies may point towards their intermediate lineage between perineurial cells and fibroblasts. The awareness of this cell type in plexiform neurofibroma may be helpful especially in small biopsy specimens where its recognition may provide a clue for recognition. The correct diagnosis is clinically important since plexiform neurofibroma is a syndromic tumor. The same is true for some other differential diagnostic entities such as intramuscular myxomas. Moreover, other entities entering the differential diagnosis are malignant tumors, for example MIFS, myxofibrosarcoma or myxoid liposarcoma, further underscoring the importance of a correct diagnosis.

2. Whorling cellular perineurioma: A previously undescribed variant closely mimicking monophasic fibrous synovial sarcoma

Perineurioma is a benign peripheral nerve sheath tumor with several subtypes including intraneural, retiform (reticular) and sclerosing perineurioma. In our practice, we encountered a distinctive tumor that, based on morphology, immunohistochemistry and electron microscopy was shown to consist of perineurial cells and that closely mimicked monophasic fibrous synovial sarcoma (MSS), a highly malignant sarcoma. Eventually, we collected 4 examples of this perineurioma variant. We performed immunohistochemical analysis using perineurial markers EMA, Claudin 1 and Glut-1, along with other markers to exclude the possibility of MSS such as keratins and TLE-1. We also carried out FISH analysis using probes for *SYT/SSX* break to further exclude the possibility of MSS. Additionally, electron microscopy was used to confirm that the tumor has features of perineurial differentiation such as bipolar cytoplasmic processes with pinocytotic vesicles, tight-junctions and discontinuous external lamina.

The four cases of WCP presented in this study morphologically closely resembled the MSS. Three of the four tumors in our series were also located on the distal portion of extremities, with a mean size of 1.8 cm. The most essential similarity between our cases and MSS lies in the presence of a monotonous sheet-like growth of bland spindle-shaped cell with oval to round nuclei. Similar to WCP, a significant cellular pleomorphism and prominent mitotic activity can be absent in MSS as well as a prominent intervening stroma. However, in both tumors, foci of more prominent hyalinization may be encountered. Another potential pitfall is the immunohistochemical features of both neoplasms since the reactivity with the EMA antibody belongs to the typical characteristics of both MSS and perineurioma. Moreover, the expression of other antibodies frequently used to confirm the perineurial cell differentiation, namely Claudin-1 and GLUT-1, can be occasionally present in MSS as well (49).

Nevertheless, there are several histological as well as immunohistochemical differences between MSS and WCP which can be exploited in the differential diagnosis. One of the most helpful microscopic features is the whorling growth pattern which is not commonly seen in MSS. The focally discernible long, slender cytoplasmic processes typical for perineurial differentiation can also help in rendering the correct diagnosis. Although the cells constituting both tumors are bland, with no or little cellular pleomorphism, MSS almost always exhibits some mitotic figures, but these were found only after a prolonged search or were absent altogether in our cases of WCP. Conversely, the various features frequently encountered in MSS such as the vaguely epithelioid areas, hemangiopericytoma-like vascular pattern, calcifications, mast cells, hemorrhage or necrosis were not found in WCP. The IHC, as misleading as it may appear with respect to EMA, Claudin-1 and Glut-1 staining, can be a very useful adjunct as well. The expression of EMA antibody, albeit noncontributory by the positivity itself, proved useful in highlighting the whorling growth pattern of WCP. When used in a panel, at least focal cytokeratin expression is found in most MSS, while OSCAR and CK7 were completely absent in all studied cases of WCP as was another of the most frequently used markers of synovial sarcomas, the TLE-1. When necessary and available, FISH (and of course EM) will provide the definite answer.

Taking into account similar clinicopathologic features, it cannot be excluded that WCP represents a very cellular end of the morphological spectrum of sclerosing perineurioma with only a focal or completely absent areas of hyalinization, i.e. a feature typically found in sclerosing perineuriomas. Awareness of this PN variant is important in order to avoid its misinterpretation for a sarcoma, mainly for MSS.

d. TUMORS OF UNCERTAIN DIFFERENTIATION

i. KINASE FUSION-ASSOCIATED TUMORS

1. S100 and CD34 positive spindle cell tumor with prominent perivascular hyalinization and a novel *NCOA4-RET* fusion

2. Novel *BRAF* gene fusions and activating point mutations in spindle cell sarcomas with histologic overlap with infantile fibrosarcoma

(Joint commentary to both publications)

Soft tissue tumors with kinase gene alterations represent a large and heterogeneous group of mesenchymal neoplasms. Apart from traditionally recognized entities such as dermatofibrosarcoma protuberans or inflammatory myofibroblastic tumor, the recent widespread adoption of high-throughput molecular techniques such as RNA-sequencing has led to the emergence of a novel and not yet fully defined group of mesenchymal tumors with kinase gene alterations characterized by similar morphological, immunohistochemical and molecular genetic features. This group encompasses tumors that can exhibit several different but often co-occurring morphological patterns of which the most common are the lipofibromatosis-like neural tumor pattern and fibrosarcoma-like patterns with numerous other less common variants such as tumors with prominent perivascular and stromal hyalinization, and others (50). Many of these tumors exhibit immunohistochemical co-expression of CD34 and S100 protein and their molecular background is characterized by numerous different gene fusions and less often mutations in kinase genes such as *NTRK1/2/3, BRAF, RAF1, RET, ALK, ABL1* as well as *MET*. The list of these aberrations is broad and ever-expanding but in effect they all lead to the constitutive activation of the receptor tyrosine kinase (RTK)/RAS pathway, thus initiating tumorigenesis (51). While most tumors included in this group are benign, a significant minority (~10%) may metastasize and behave very aggressively. Thanks to the molecular genetic background of these tumors, targeted therapy with various more or less specific small molecule kinase inhibitors represents an effective way of treating these tumors(52). As a result, this chapter of soft tissue pathology has attracted a particular interest among oncologists and other clinicians.

In our paper **d.i.1.** we reported the first case of a mesenchymal tumor with *RET* gene fusion in the literature with other papers on this topic following soon thereafter (53). This case harbored *NCOA4::RET* gene fusion, co-expressed CD34 and S100 protein on immunohistochemical examination and exhibited a morphological pattern with prominent perivascular and stromal hyalinization.

Regarding the paper **d.i.2.**, we contributed an extremely rare case with *OSBP::BRAF* gene fusions into a study of mesenchymal tumors with *BRAF* gene alterations. A particularly important feature of this study was the fact that it reported not only several cases with *BRAF* gene fusions but also a couple of tumors with *BRAF* gene mutation, a feature reported in only a single study before (54).

ii. OTHERS

1. Phosphaturic Mesenchymal Tumors: Clinicopathologic, Immunohistochemical and Molecular Analysis of 22 Cases Expanding their Morphologic and Immunophenotypic Spectrum

Phosphaturic mesenchymal tumor (PMT) is a rare neoplasm of uncertain lineage. The hallmark of this tumor is the ability to induce osteomalacia as a result of inappropriate production of fibroblastic growth factor 23 (FGF23) by the neoplastic cells. FGF23 leads to increased loss of phosphate in the urine resulting in phosphaturic hypophosphatemia (55).

The diagnosis of PMT has traditionally been a difficult one to make, especially in cases when the TIO or phosphaturia was absent as there were almost no IHC or molecular markers to support the diagnosis. Morphologically, besides the already rare classical variant, there are numerous other, even rarer morphological subvariants that make this tumor prone to misclassification. Given the highly variable morphologic spectrum of these tumors and lack of defining phenotypic and/or genetic criteria, it remained controversial whether these tumors, particularly those lacking the classical PMT features, even belong to the same histogenetic group of neoplasms.

For this reason, it has been the aim of several research groups to find a useful IHC stain for the diagnosis of PMT. In last several years, various studies reported the utility of markers such as ERG, somatostatin receptor 2A (SSTR2A), CD56 or DOG1. Of note, recently, another group discovered a characteristic fusion in PMT, consisting of *FN1* gene that is fused either with *FGF1* (in 42% of cases) or FGFR1 (6%) (56, 57). However, the rearrangement in the other half of cases still remains obscure.

In this study, we analyzed a cohort of 22 PMT cases for the presence of special morphological patterns and the expression of multiple IHC markers including a novel (in the context of PMT) marker SAT-B2. Also, we carried out *FGFR1* FISH analysis to support the diagnosis of PMT. Eventually, we confirmed the uniform expression of SSTR2A, ERG, and CD56 in the vast majority of cases. We also described a novel consistent expression of SATB2 and confirmed the uniform reactivity for SSTR2A in the majority of in PMTs irrespective of the histologic variant. Furthermore, we described a distinctive ERG expression that is essentially weak compared with normal endothelial cells and slightly weaker or comparable with that seen in normal cartilage cells (58). Notably, expression of these immunomarkers is independent of the histologic pattern seen in PMT. Thus, taken together, it seems that in the majority of cases, PMTs are characterized by a distinctive combined immunophenotype showing positivity with SATB2, SSTR2A, CD56 and ERG and negativity with S100 protein and DOG1. Although all these markers are known to have limited general specificity and none is reliable in isolation to detect or confirm PMT, it seems promising that the combined expression of these markers can support the diagnosis of PMT, particularly in unusual looking cases and in cases without TIO. In our view, this immunophenotype is a strong argument for the notion that these tumors indeed represent a polymorphous neoplasm with many faces that is unified by prototypic immunophenotype of the neoplastic cells. Accordingly, the possibility that some variant lesions might have represented distinctive non-PMT lesions that happened to be incidentally associated with TIO seems unlikely.

e. OTHER BONE, SOFT TISSUES AND MESENCHYMAL TUMORS

i. UNDIFFERENTIATED ROUND CELL SARCOMAS

1. *EWSR1-PATZ1*-rearranged sarcoma: a report of nine cases of spindle and round cell neoplasms with predilection for thoracoabdominal soft tissues and frequent expression of neural and skeletal muscle markers

According to WHO classification, the group of undifferentiated round cell sarcomas recognizes 4 main tumor categories: a) Ewing sarcoma with *EWSR1-ETS* family fusions (mainly *EWSR1::FLI1* or *EWSR1::ERG*); b) *CIC*-rearranged sarcomas; c) *BCOR*-rearranged sarcomas and d) round cell sarcomas with *EWSR1*-non-*ETS* family fusions (59). The latter group include diverse extremely rare entities cause by different molecular events which were often reported only in the form of case reports or small case series. However, this group also includes two tumor subgroups whose clinicopathological features are more advanced stages of elucidation and which might be included as a stand-alone entities in the next WHO classification if additional data support this. These two subgroups are represented by sarcomas with *EWSR1::NFATC2* and *FUS::NFATC2* gene fusions as well as spindle and round cell sarcomas with *EWSR1::PATZ1* gene fusion. While multiple clinicopathological reports have been published recently on neoplasms with *NFATC2* rearrangements (59, 60), due to their extreme rarity, knowledge of clinical and, particularly, histopathological features of *EWSR1::PATZ1*-rearranged sarcomas (EPS) remains very limited, with only 19 cases reported, many of which lacked detailed clinical, pathological and/or molecular data (61).

In order to increase the collective understanding of these ultrarare neoplasms, we have undertaken a multicenter clinicopathological study which included cases from 8 different institutions from 3 different continents and led to the identification of additional 9 cases, i.e. the largest series reported to date.

Our findings confirmed that in contrast to other round cell sarcomas, the clinical features of these tumors are characteristic by their exclusive occurrence in soft tissues (no case affecting the skeleton has been reported) with a particular predilection for thoracoabdominal soft tissues. The uneventful outcome in some of our cases indicated that a subset of EPS might follow a much more indolent clinical course than previously appreciated.

Microscopically these tumors encompass a wide morphological spectrum albeit with recurrent microscopic features which were, for the first time, meticulously outlined in our paper. They also have a very unusual and characteristic immunophenotype distinguished mainly by the co-expression of neural (S100-protein, GFAP) and skeletal muscle (desmin, MyoD1, Pax-7) markers in most cases.

We believe that our paper combined with data from previous studies will represent solid evidence to include them as a separate entity in the upcoming WHO classification of soft tissue and bone tumors.

ii. SMOOTH/SKELETAL MUSCLE TUMORS

1. Inflammatory leiomyosarcoma shows frequent co-expression of smooth and skeletal muscle markers supporting a primitive myogenic phenotype: a report of 9 cases with a proposal for reclassification as low-grade inflammatory myogenic tumor

Inflammatory leiomyosarcoma (ILMS) is an extremely uncommon tumor first described in 1995 (62). The designation leiomyosarcoma was chosen due to a partially similar morphological and immunohistochemical features (expression of desmin and smooth muscle actin) with conventional leiomyosarcoma, i.e. a smooth muscle neoplasm. However, gene expression analysis performed by c et al in a 2017 study revealed a high expression of genes which take part in the development of skeletal muscle, i.e. *MYOD1, MYOG* or *PAX7* (63). Nevertheless, this study did not attempt to verify these findings on a protein level using immunohistochemistry.

This led us to further study the clinicopathological features of these elusive tumors with a particular focus on the expression of skeletal muscle markers. Eventually, we have collected 9 cases and using immunohistochemistry, we confirmed the immunohistochemical expression of skeletal muscle markers such as desmin, MyoD1, myogenin and PAX-7 in the vast majority of cases which excluded the possibility these tumors represent leiomyosarcoma variant but rather pointed towards skeletal muscle differentiation. We have also confirmed these tumors have an indolent behavior in most cases (some of our cases had over 20 years of follow-up). Our findings also suggested that due to overlapping clinicopathological features, these tumors are identical to lesions previously published under the designation „histiocyte-rich rhabdomyoblastic tumor“ (64) and a recent paper by Cloutier et al eventually confirmed our assumption (65).

As a result, our study and the study by Arbajian triggered an increased interest and research of these tumors and eventually led to the proposed name change to inflammatory rhabdomyoblastic tumor. It is almost certain that the next WHO classification of soft tissue and bone tumors will create a novel category of “intermediate grade skeletal muscle tumors” in which this entity will be included.

iii. CALCIFYING PSEUDOTUMORS OF JOINTS

1. Tenosynovitis With Psammomatous Calcifications: A Distinctive Trauma Associated Subtype of Idiopathic Calcifying Tenosynovitis With a Predilection for the Distal extremities of Middle-Aged Women-A Report of 23 Cases

The term “idiopathic calcifying tenosynovitis”, also known as calcific periarthritis or calcific/calcifying tendinitis refers to a clinically and radiologically defined syndrome of pain and tendinous calcification, most often involving the supraspinatus tendon of shoulder joint (66). A distinctive subset of idiopathic calcifying tenosynovitis, termed “tenosynovitis with psammomatous calcifications” (TPC) has been reported and typically occurs in the distal extremities and shows characteristic morphology with prominent psammomatous calcified bodies (67, 68). Some cases of TPC have been reported as examples of “tumoral calcinosis of distal extremity type (69).” However, abnormalities in calcium and/or phosphate metabolism have not been noted, and it is thus clinically relevant to differentiate these lesions from tumoral calcinosis. Moreover, TPC may be confused with various neoplastic and non-neoplastic processes. As only 14 cases have been reported before our study, TPC remained poorly recognized by both pathologists and clinicians. In an effort to improve our collective understanding of this rare pseudoneoplasm, we reported the largest study of TPC to date.

We collected 23 cases of this peculiar lesion and recorded its clinical and morphological characteristics. We have confirmed this lesion has distinct predilection for fingers and toes as the vast majority of cases occurred therein. Except for one case, all cases occurred in females (in one additional case the gender was unknown), usually of young to middle age. None of the patients had calcium and/or phosphate metabolism. We also confirmed that TPC appears to be related to trauma and/or repetitive activity and is cured with simple excision Since the psammomatous calcifications that are characteristic of this lesion on microscopic examination are usually surrounded by a highly cellular histiocytic proliferation, it may be confused for various neoplasms, including malignant tumors. However, we showed that a simple immunohistochemical work-up will enable a confident diagnosis.

Overall, TPC should be rigorously distinguished from other forms of idiopathic calcifying tenosynovitis, which typically involve large, proximal joints, and show simply dystrophic calcification involving tendinous tissues, and from tumoral calcinosis, which also involves large joints and often is associated with calcium and/or phosphate abnormalities. TPC appears to be related to trauma and/or repetitive activity and is cured with simple excision.

iv. PERIVASCULAR TUMORS

1. Recurrent Somatic PDGFRB Mutations in Sporadic Infantile/Solitary Adult Myofibromas But Not in Angioleiomyomas and Myopericytomas

Infantile myofibroma (MF) represents an uncommon proliferative mesenchymal lesion of infancy and early childhood that shows myofibroblastic differentiation. The disease occurs in 3 main clinicopathologic settings: solitary MF, multicentric MF/myofibromatosis, and generalized myofibromatosis (70, 71). This study utilized the knowledge of the recently reported presence of activating germline *PDGFRB* mutations in familial infantile myofibroma (MF) (72, 73). Since the molecular pathogenesis of sporadic infantile and adult solitary MF remained unclear, we undertook this study.

Overall, we collected and analyzed 25 non-familial solitary MFs (of which 9 were infantile and 16 were adult MFs), in order to find out, whether somatic *PDGFRB* mutations might be responsible for the sporadic form of the disease as well. Given the presumed histogenetic link of MF to myopericytoma and angioleiomyoma, we additionally analyzed a control group of 6 myopericytomas and 9 angioleiomyomas for *PDGFRB* mutations. We also tested the utility of the *PDGFRB* antibody in the distinction between these three entities.

We detected *PDGFRB* mutations in 6/8 (75%) analyzable infantile and in 11/16 (69%) adult MFs but in none of the angioleiomyomas or myopericytomas. Additional sequencing of the germline confirmed the somatic nature of *PDGFRB* mutations. The staining pattern of the PDGFRB antibody did not differ significantly between any of the three groups and thus currently seems not useful in the diagnostic practice.

Further studies are needed to uncover the genetic background of the fraction of cases that tested negative in our series as well as that of angioleiomyoma. Interestingly, probably due to a more sensitive molecular genetic method used, another group recently reported the presence of *PDGFRB* mutations in myopericytoma/myopericytomatosis as well (74).

v. MESENCHYMAL LESIONS OF THE LUNG

1. Recurrent *YAP1-TFE3* Gene Fusions in Clear Cell Stromal Tumor of the Lung

Hemangioblastoma is a rare mesenchymal neoplasm mostly involving the central nervous system (cerebellum) and rarely other extraneural/peripheral sites. None of 65 peripheral cases (15 within soft tissue, 6 of peripheral nerve origin, 5 within the bone, 26 renal, and 13 within other organs) was pulmonary in origin (75).

In 2013, Falconieri et al 6 described 2 primary lung lesions that were morphologically overlapping with hemangioblastoma but lacking phenotypic features of that entity. They coined the term “hemangioblastoma-like clear cell stromal tumor of the lung” for this rare putative tumor entity. More recently, a series describing 5 additional tumors was published by Lindholm and Moran (76, 77). However, the molecular background of these tumors remained unknown. In this paper we described 4 new cases of this rare lesion, in which targeted RNA-based NGS was performed and showed a novel *YAP1::TFE3* gene fusion in 3/4 cases.

These observations further underline the distinctness of this tumor from conventional hemangioblastoma, with which they have likely been confused in the past. Our study adds to the expanding family of *TFE3*-related neoplasms and represents a novel contribution to the *YAP1-TFE3*–rearranged subgroup. The biology of these tumors cannot be predicted from our few cases, but they seem to possess a malignant potential at least in a subset of cases. Future molecular analysis of reported visceral hemangioblastomas from different organs should address the justified question, whether pulmonary-type clear cell stromal tumor does exist in other organs.

2. PART:

TUMORS OF THE LYMPHOID SYSTEM AND TUMORS OF THE SPLEEN

a. HISTIOCYTIC LESIONS OF VARIOUS LOCATIONS

i. Histiocytosis With Raisinoid Nuclei: A Unifying Concept for Lesions Reported Under Different Names as Nodular Mesothelial/Histiocytic Hyperplasia, Mesothelial/Monocytic Incidental Cardiac Excrescences, Intralymphatic Histiocytosis, and Others: A Report of 50 Cases

Nodular mesothelial/histiocytic hyperplasia, nodular histiocytic aggregates, mesothelial/monocytic incidental cardiac excrescences (MICE), reactive eosinophilic pleuritis, histioeosinophilic granuloma of the thymus, and intralymphatic histiocytosis are all names for benign reactive histiocytic proliferations occurring in different body sites. Several previous publications have established a link between some of these conditions. However, no publication has ever comprehensively addressed all of these lesions together in one study in an attempt to explain and discuss their striking analogy and to provide an unifying concept for all these lesions.

We collected 50 cases of these proliferations from various body locations. To prove that all lesions represent the same process, we first analyzed their morphological features which were basically identical. However, based on the exact location, the histiocytes may be admixed with various other types of cells or tissues, and that was also the reason why most of them have not been linked to each other before. For example, lesions called nodular histiocytic hyperplasia occur on the serosal surfaces and therefore usually contain an additional admixture of mesothelial cells.

Secondly, we used a wide panel of histiocytic antibodies consisting of the following markers: CD68, CD163, CD4, Lysozyme, CD45, CD11c, CD64, CD14 to confirm that their frequency of expression in each of these lesions is the same and to find out which marker is the most sensitive. We also applied CD1a, Langerin and S100 protein to exclude the possibility of Langerhans cell histiocytosis, i.e. one of the main differential diagnoses which consistently yielded negative results.

Thirdly, besides analyzing the morphological and immunohistochemical features, we also performed an exhaustive literature review. We pointed out the similarities between these processes and found previous articles suggesting a potential link between some of the entities. Most of the evidence supports the concept that these proliferations occur as an unspecific reaction to an injury, that is, a trauma, inflammation, infiltratively growing malignancy, surgical procedure, etc. In most cases, they occur as an incidental finding during a diagnostic process for other purposes. Also, they are invariably benign and, with the exception of the skin, asymptomatic. These innocuous lesions can sometimes cause considerable differential diagnostic difficulties by resembling a metastatic carcinoma or Langerhans cell histiocytosis.

Eventually, we provided evidence that all of the lesions share the same morphologic, immunohistochemical, and pathogenetic properties, thus they all represent the same pathologic process and should be referred to as such. Based on their typical nuclear features we proposed for them a collective term “histiocytosis with raisinoid nuclei”.

b. MESENCHYMAL LESIONS OF THE SPLEEN

i. Littoral cell angioma of the spleen: a study of 25 cases with confirmation of frequent association with visceral malignancies

In this paper we have studies the largest series of rare splenic tumors called Littoral cell angioma (LCA). This tumor has two fairly unique features. Firstly, it seems to be associated with visceral malignancies as there are numerous literature reports regarding this association (78). Secondly, the cell of origin, i.e. the littoral cell, has a unique immunophenotype, expression both endothelial and histiocytic markers (79). Our study focused on both of these aspects as particularly the former one has not been sufficiently clear from the available literature.

Regarding the first feature, we have collected a detailed follow-up information for 11 of our cases. Additionally, an extensive literature review was undertaken to summarize all the known tumor associations published in the literature. To further analyze the immunophenotype, we applied an extensive panel of endothelial and histiocytic markers including LYVE-1, factor VIII, FLI-1, vascular endothelial growth factor receptor 2, VEGFR-3, claudin-5, ERG, LMO2, CD31, CD163, lysozyme, WT-1, CD4, D2-40, CD8, and factor XIIIa, many of which have not been previously tested in these tumors. Although a wide spectrum of these markers (except for the latter four) were expressed in the LCA, many of them also variably reacted with the surrounding splenic parenchyma. Eventually, the three antibodies we found most useful in the diagnosis of LCA were Claudin-5, Vascular endothelial growth factor receptor 2 and CD11c which were expressed exclusively in the LCA areas and not in the splenic tissue.

The result of this study is the expanded spectrum of immunohistochemical features of LCA, which may facilitate the diagnosis, especially in ambiguous cases. For practical purposes, we recommended the combined use of the vascular markers vascular endothelial growth factor receptor 2, claudin-5, and the more widely available factor VIII, together with the histiocytic markers CD163, CD4, and CD11c. Such a combination of vascular and histiocytic markers is highly specific for LCA. To add even more specificity, the absence of staining of WT-1 in LCA, which is very rare among splenic vascular tumors, can be also exploited. Finally, we confirmed frequent associations of LCA with various visceral malignancies which was present in more than 60% of cases, and showed that the associated malignancies are not always epithelial, as suggested previously (78), but comprise a whole spectrum of neoplastic diseases.

Since we published by far the largest study of LCA to date, I was invited to be the responsible author for this chapter in the upcoming WHO classification of hematolymphoid tumors.

c. REVIEW ON CHANGES IN THE UPCOMING 5TH EDITION OF WHO CLASSIFICATION OF HEMATOLYMPHOID TUMORS

i. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms

Thanks to my participation on the preparation of the upcoming WHO classification of hematolymphoid tumors, I also became a co-author of a large and very prestigious review article on changes made in this novel 5th edition as compared to the previous edition. Littoral cell angioma of the spleen represents one of the few novel entities included in this edition.

d. FOLLICULAR DENDRITIC CELL TUMORS

i. Follicular dendritic cell sarcoma: clinicopathologic study of 15 cases with emphasis on novel expression of MDM2, somatostatin receptor 2A, and PD-L1

Follicular dendritic cell sarcoma (FDCS) is a rare neoplasm whose cells show phenotypic features of normal follicular dendritic cells (FDC). Only few larger series of this entity have been published since the description. Being initially considered a low-grade malignancy, later series suggested a higher rate of aggressiveness and recommended considering FDCS a neoplasm of intermediate grade. Approximately half of the cases recurred locally after initial excision, whereas metastasis and death from the tumor were reported in half of cases (80, 81). Diagnosis of FDCS mainly relies on characteristic histologic appearance supplemented by IHC and, if necessary, electron microscopy. Due to rarity and hence limited familiarity with the histologic features of FDCS, this entity is known for being frequently misdiagnosed as other neoplasms of epithelial, mesenchymal, meningeal, or hematolymphoid origin. In addition to the classical FDC markers such as CD21, CD23, CD35, and CNA.42, the list of markers found to be frequently expressed in FDCS is ever increasing.

In this study, we reviewed clinicopathologic features of 15 FDCS and stained them for novel markers in addition to the survey of conventional FDC markers. Based on observations (by the first author) of somatostatin receptor type 2A (SSTR2A) expression in native lymphoid follicles surrounding a metastatic neuroendocrine tumor and of an MDM2-positive FDCS submitted for second opinion as dedifferentiated liposarcoma, we assumed that these 2 markers might be expressed in FDCS.

Altogether we collected 15 cases. Patients were 7 men and 7 women (1 unspecified), with a mean age of 47 years (20-75 years). The tumor site was lymph nodes (6) or spleen (2), both (1) and extranodal sites of head and neck (4) or abdominal cavity (2). Treatment was variable combinations of surgery and aggressive chemotherapy/radiotherapy. Four of 8 patients with follow-up died of disease within 1 to 10 years. To confirm the diagnosis, we performed a wide panel of antibodies, whose expression have been reported in FDCS. Additionally, to further analyze the cases, we stained them for newly reported or novel markers and performed *MDM2* FISH to assess the presence of this gene's amplification.

Eventually, all tumors expressed at least 1 FDC marker. Most importantly, five of 14 cases (36%) stained strongly for SSTR2A with a distinctive membranous pattern. Seven (54%) of 13 assessable cases showed moderate to strong membranous staining for PD-L1 in greater than 5% of the neoplastic cells. The Rb1 antigen was lost in 4 (28%) of 14 cases. MDM2 stained less than 5% to 20% of the tumor cells in 5 (36%) of 14 cases; 2 of them showed amplification by FISH.

This study adds to the existing few clinicopathologic series on FDCS and represents the first study to show *MDM2* amplification in this entity. Our results regarding frequent SSTR2A expression in FDCS are novel and might be of potential diagnostic and therapeutic relevance. Expression of SSTR2A in any neoplasm has the potential relevance of being of value as a biomarker used in imaging examination to detect tumors and their metastases (as a tracer for scintigraphy), but might also be of value for specific treatment such as radioactive

peptide receptor therapy as in neuroendocrine neoplasms. FDCS occurring within the retroperitoneum and/or the abdominal cavity may closely mimic dedifferentiated liposarcoma, particularly if MDM2 positive and/or amplified and should thus be carefully assessed for expression of FDC markers.

3. PART:

HEAD AND NECK TUMORS

a. SECRETORY CARCINOMA OF SALIVARY GLANDS AND NASAL CAVITY

i. Molecular Profiling of Mammary Analog Secretory Carcinoma Revealed a Subset of Tumors Harboring a Novel *ETV6-RET* Translocation: Report of 10 Cases

ii. Expanding the Molecular Spectrum of Secretory Carcinoma of Salivary Glands With a Novel *VIM-RET* Fusion

iii. A New Hitherto Unreported Histopathologic Manifestation of Mammary Analogue Secretory Carcinoma: "Masked MASC" Associated With Low-grade Mucinous Adenocarcinoma and Low-grade In Situ Carcinoma Components

iv. Mammary Analog Secretory Carcinoma of the Nasal Cavity: Characterization of 2 Cases and Their Distinction From Other Low-grade Sinonasal Adenocarcinomas

(Joint commentary to all 4 publications)

Mammary analogue secretory carcinoma (MASC), more recently called secretory carcinoma is a low-grade salivary gland carcinoma originally described by my colleagues (82). It usually harbors the canonical *ETV6::NTRK3* gene fusion but in a small subset of these tumors, the *ETV6* had an unknown fusion partner, as the *NTRK3* gene rearrangements were not found. Hence, this fraction was provisionally labeled as *ETV6*::X MASCs (83). In the study **3.a.i.**, we analyzed altogether 10 such *ETV6*::X cases using NGS methods with the hope of identifying the partner genes. Eventually, all cases were confirmed to harbor the *ETV6::RET* gene fusion. This finding is critically important since some cases of MASC may behave very aggressively and such patients profit from already available targeted therapies with small molecule NTRK inhibitors. However, these NTRK inhibitors, as the name implies, are effective only in cases with *NTRK3* fusions. In contrast, cases with *ETV6::RET* fusions respond well to RET-inhibitors (84). Therefore, the findings of this study are of utmost clinical importance with the potential to save or prolong patient lives in a very close future.

In the paper **3.a.ii.** we have collected further 49 cases of MASC and on this large cohort performed RNA-seq. Eventually, 40 cases (82%) presented the classical *ETV6::NTRK3* fusion while 9 cases (18%) revealed an alternate fusion. Of the 9 cases negative for the *ETV6::NTRK3* fusion, 8 cases presented with *ETV6::RET* fusion. Importantly, in the one remaining case, RNA-seq analysis identified a novel *VIM::RET* fusion transcript. In addition, the analysis indicated that one recurrent high-grade case in the submandibular gland was positive for both *ETV6::NTRK3* and *MYB::SMR3B* fusion transcripts. The expanded molecular spectrum provided a novel insight into SC oncogenesis and carries important implications for molecular diagnostics, as this is the first SC-associated translocation with a non-*ETV6* 5' fusion partner (some laboratories are using *ETV6* FISH as a highly sensitive method to confirm the diagnosis of MASC).

The case report **3.a.iii.** presented a unique manifestation of MASC characterized by a tumor showing a minor conventional MASC component (20%) admixed with an entirely different mucinous adenocarcinoma component comprising 80% of the lesion. This part consisted of morphologically nondescript low-grade intraductal carcinoma (in situ) component. Using FISH, a break in the *ETV6* gene was documented in the mucinous adenocarcinomatous, the conventional MASC, as well as in the intraductal (in situ) components. RT-PCR failed to reveal an *ETV6::NTRK3* fusion. The entire conventional MASC and only rare mucinous adenocarcinoma tumor cells were mammaglobin positive, whereas the low-grade intraductal carcinoma (in-situ) component was negative. S-100 protein stained only the MASC component (both antibodies represent very useful markers for the diagnosis of MASC). After several years when we adopted NGS sequencing techniques at our department we sequenced the case which revealed *ETV6::RET* gene fusion. This was published as a letter to the editor (85).

Most MASCs are localized to the parotid gland and intraoral minor salivary glands. Moreover, *ETV6*-rearranged carcinomas with secretory features have been reported recently in the thyroid (with and without a history of radiation exposure) (86), skin (87), and in very rare instances in the sinonasal tract (88). In the paper **3.a.iv.**, we described 2 cases of primary MASC in the sinonasal tract and provided a detailed clinical and histopathologic characterization of their morphology, immunohistochemical profile, and genetic background and highlighted features allowing its separation from its recently described molecular mimicker, *ETV6*-rearranged low-grade sinonasal adenocarcinoma (88).

b. OTHER TUMORS

i. Alterations in key signaling pathways in sinonasal tract melanoma. A molecular genetics and immunohistochemical study of 90 cases and comprehensive review of the literature

Sinonasal tract mucosal melanoma (SNTMM) consist of tumors developing in the nasal cavity, paranasal sinuses, and nasopharynx. SNTMM is an aggressive tumor which in the United States, it accounts for <1% of all melanomas (89). SNTMM is often diagnosed at an advanced stage, with surgery as the first-line treatment, but only 25–30% of patients survive more than five-years. As a result, adjuvant therapy, including postoperative radiation, chemotherapy and targeted therapy are often considered (90). As more options become available for the latter, the need to identify genetic markers has become increasingly important. Recently developed cutaneous melanoma molecular classification specified four subtypes: *BRAF-, RAS-, NF1*-mutants, and Triple-WT tumors (91). Oncogenic gene fusion involving *ALK, BRAF, MET, NTRK* and *ROS-1* have been detected predominantly in younger patients in a subset of cutaneous melanomas including Spitzoid and acral tumors (92). However, the mutation profile of SNTMM remained incompletely characterized. The aim of this study was to elucidate the molecular characteristics of SNTMM.

A large series consisting of 90 well-characterized cases of which about 1/4 was contributed from our department was evaluated using targeted NGS, Sanger sequencing, FISH, and IHC. This analysis showed that SNTMM were commonly driven by *RAS* (38/90, 42%), especially *NRAS* (n = 36) mutations and only rarely (4/90, 4%) displayed *BRAF* pathogenic variants in stark contrast to cutaneous examples. *BRAF/RAS* mutants were more frequent among paranasal sinuses (10/14, 71%) than nasal (26/64, 41%) tumors. *BRAF/RAS* wild type tumors occasionally harbored alterations of the key components and regulators of Ras-MAPK signaling pathway: *NF1* mutations (1/17, 6%) or *NF1* locus deletions (1/25, 4%), *SPRED1* (3/25, 12%), *PIK3CA* (3/50, 6%), *PTEN* (4/50, 8%) and *mTOR* (1/50, 2%) mutations. These mutations often occurred in a mutually exclusive manner. In several tumors some of which were *NRAS* mutants, *TP53* was deleted (6/48, 13%) and/or mutated (5/90, 6%). Variable nuclear accumulation of *TP53*, mirrored by elevated nuclear MDM2 expression was seen in >50% of cases. Furthermore, sinonasal melanomas (n = 7) including *RAS/BRAF*-wild type tumors (n = 5) harbored alterations of the key components and regulators of canonical WNT-pathway: *APC* (4/90, 4%), *CTNNB1* (3/90, 3%) and *AMER1* (1/90, 1%). Both, *TERT* promoter mutations (5/53, 9%) and fusions (2/40, 5%) were identified. The latter occurred in *BRAF/RAS*-wild type tumors. No oncogenic fusion gene transcripts previously reported in cutaneous melanomas were detected. Eight tumors including 7 *BRAF/RAS*-wild type cases expressed *ADCK4::NUMBL* cis-fusion transcripts.

In summary, this study documented mutational activation of *NRAS* and other key components and regulators of Ras-MAPK signaling pathway such as *SPRED1* in a majority of sinonasal melanomas. In contrast to cutaneous melanomas, *BRAF* mutation appear to be rare in SNTMM precluding a targeted therapy with BRAF-inhibitors.

ii. Immunohistochemical and genetic analysis of respiratory epithelial Adenomatoid Hamartomas and Seromucinous Hamartomas: are they precursor lesions to Sinonasal low-grade Tubulopapillary adenocarcinomas?

Respiratory epithelial adenomatoid hamartoma (REAH) and seromucinous hamartoma (SH) are rare lesions arising in the sinonasal tract. Although both lesions are still considered different entities, cases presenting histologic features of both REAH and SH have been reported, suggesting they both might be within a spectrum of the same lesion (93). Morphologically, REAH is mainly composed of invaginated slit-like spaces lined by respiratory epithelium, which may in some areas detach from the surface and creat small glandular structures. Cases of SH additionally contain small to large seromucinous acini and ducts.

Sinonasal adenocarcinomas are classified as either salivary or non-salivary adenocarcinomas. Non-salivary type adenocarcinomas encompass a wide spectrum of intestinal-type adenocarcinoma and non-intestinal type adenocarcinoma, of both low-grade and high-grade morphologies (94). Low-grade tubulopapillary adenocarcinoma (LGTA) has been recognized as a distinctive variant of low-grade non-intestinal type sinonasal adenocarcinoma. It is, besides others, characterized by morphologic features that resemble the serous glandular component of SH (95).

In recent studies on REAH and SH, several authors demonstrated molecular alterations more in keeping with a neoplastic than hamartomatous process (96). Other report described possible association of REAH with non-intestinal sinonasal adenocarcinomas in 6/29 cases (97). Therefore, there was a growing body of evidence that rather than hamartomas, REAH/SH may represent a benign neoplasm and carry a potential to become a precursor lesion of LGTA. To support this hypothesis, we analysed 10 cases of REAH/SH and 9 cases LGTA cases immunohistochemically and by molecular genetic methods and compared resulting features of both groups.

All cases of REAH/SH and LGTA were analysed immunohistochemically with a cocktail of mucin antigens, and with epithelial, and myoepithelial markers. NGS assay was performed in 10 cases of REAH/SH and the *EGFR::ZNF267* gene fusion was detected in one of them. Two female REAH/SH cases were assessed for the presence of clonality. Using the HUMARA assay, one case was proved to be clonal.

Eventually, this study demonstrated that REAH and SH share many morphological and immunohistochemical features, thus forming a continuous spectrum. The serous component of REAH/SH had identical immunoprofile as LGTA. Moreover, four cases of LGTA showed overlapping morphological features with REAH/SH. This was also the first study which demonstrated monoclonality and a gene fusion in one case of SH each, further supporting the notion that it is a neoplastic process. Although REAH/SH and LGTA are separate entities, our findings pointed towards the possibility that SH (or the serous component of REAH) might be the precursor lesion of LGTA.

iii. Papillary thyroid carcinoma with prominent myofibroblastic stromal component: clinicopathologic, immunohistochemical and next-generation sequencing study of seven cases

Papillary thyroid carcinoma (PTC) is a common malignant neoplasm of the thyroid frequently associated with BRAF mutations (98) which may present with various morphologic appearances one of which is a papillary thyroid carcinoma with desmoid-type fibromatosis or nodular fasciitis-like stroma. These are characterized by conventional areas of PTC admixed with a prominent myofibroblastic component that is present in varying proportions and resembles either desmoid-type fibromatosis or nodular fasciitis, i.e. lesions normally encountered in soft tissues. The molecular background of former tumor is characterized by frequent *CTNNB1* (β-catenin) mutations (99), while the latter often harbors *USP6* gene fusions (100). This subtype of PTC was not well defined and there were only ~30 cases reported before our publication. Previously, additional reports and a few small series have demonstrated that many but not all of these lesions are associated with *CTNNB1* mutations (101-104). Rebecchini et al. have suggested that these lesions be classified under the term PTC with desmoid-type fibromatosis for cases harboring *CTNNB1* mutations (104). However, due to the rarity of these tumors, few cases have been examined with current sequencing techniques to corroborate the extent of *CTNNB1* mutations. Only a single case report had existed with available NGS data in this tumor (103).

In this study, we described the clinicopathological characteristics of seven cases of PTC with desmoid type-fibromatosis/fasciitis-like stroma in which we examined immunohistochemical expression and molecular features by NGS in the epithelial and mesenchymal components to better characterize the underlying genetic abnormalities driving these tumors. Six out of 7 cases in our study had activating *CTNNB1* mutations consistent with the previously described cases of PTC-DTF. The case with fasciitis-like stroma was negative for beta-catenin by sequencing and immunostaining as well as negative for *USP6* gene rearrangement. Our findings indicate that papillary thyroid carcinoma with prominent myofibroblastic stroma may represent more than one category of lesions.

iv. Angioleiomyoma of the Sinonasal Tract: Analysis of 16 Cases and Review of the Literature

In this manuscript we analyzed the clinicopathological features of sinonasal angioleiomyoma. Angioleiomyoma is a rare mesenchymal tumor of skin which even more rarely occurs in this sinonasal location. Altogether we analyzed 16 cases from our files using light microscopy and immunohistochemistry which represented the largest series to date. We also reviewed 38 previously reported cases of submucosal sinonasal angioleiomyomas emphasizing their benign nature, frequent presence of fatty component, similarity to cutaneous angioleiomyoma and distinctness from renal-type angiomyolipoma.

This study increased the awareness of the histological spectrum of sinonasal angioleiomyoma which is necessary to distinguish these tumors from other potentially recurring or locally aggressive neoplasms.

v. Spectrum of lesions derived from branchial arches occurring in the thyroid: from solid cell nests to tumors

The purpose of this manuscript was to review and report the morphological spectrum of thyroid lesions derived from branchial arches and related structures, which is in the thyroid mainly the case of the ultimobranchial body. When inflamed, these structures lead to formation of cystic spaces that are lined by squamous or glandular epithelium and are surrounded by inflammatory infiltrate which focally forms germinal centers.

To investigate the spectrum of such thyroid lesions, the consultation files were reviewed for thyroid samples containing pathological structures regarded to arise from the ultimobranchial body. A panel of immunohistochemical markers was used to confirm the diagnosis. The specific subtype of the ultimobranchial body-derived lesion was then determined based on histological examination of H&E-stained slides. Twenty-one cases of ultimobranchial body-derived lesions were retrieved from the consultation files. Lesions derived from the ultimobranchial body were classified as follows: (hyperplastic) solid cell nests (nine cases), solid cell nests with focal cystic change (five cases), cystic solid cell nests (two cases), branchial cleft-like cyst (four cases), and finally a peculiar case of Warthin tumor-like lesion (already reported previously by us in a separate case report (105).

We suggested that the common denominator of the identified structures is that they all arise due to activation of inflammatory cells around the vestigial structures, which leads to cystic dilatation and proliferation of the epithelial component. The awareness of these lesions is important to avoid their misinterpretation for other potentially more aggressive lesions such as papillary thyroid (micro)carcinoma or squamous cell carcinoma metastasis.

4.PART:

TESTICULAR AND PENILE TUMORS

a. Solid pseudopapillary neoplasm (SPN) of the testis

i. Pancreatic analogue solid pseudopapillary neoplasm arising in the paratesticular location. The first case report

ii. Primary signet ring stromal tumor of the testis: a study of 13 cases indicating their phenotypic and genotypic analogy to pancreatic solid pseudopapillary neoplasm

iii. Solid pseudopapillary neoplasm (SPN) of the testis: Comprehensive mutational analysis of 6 testicular and 8 pancreatic SPNs

(Joint commentary to all 3 publications)

Couple years ago, we received for consultation a peculiar tumor that morphologically looked exactly like the solid pseudopapillary neoplasm of the pancreas (SPN-P). The only problem with this otherwise straightforward case was that this tumor was located in the testis/paratestis. We performed the IHC staining with classical SPN-P IHC markers and even these results were completely in concordance with the diagnosis of SPN-P. We also performed molecular genetic analysis which revealed the same oncogenic mutation in exon 3 of *CTNNB1* gene as is routinely found in SPN-P. This case of course triggered our curiosity. We looked for more such cases in our registry and indeed found more very similar tumors that also shared the same morphology, IHC and *CTNNB1* mutation. However, they were slightly morphologically different, so we decided to leave them for a separate study. The initial case was published only as a case report entitled "Pancreatic analogue solid pseudopapillary neoplasm arising in the paratesticular location. The first case report" (publication **5.a.i.**).

The remaining tumors from our registry as well as several others later received from our colleagues from various institutions were almost identical but featured a high amount of signet ring cells, and in this regard, they were identical to another study published by my colleagues years ago, entitled as "Primary signet-ring stromal tumor of the testis" (106). However, in many cases, these signet ring cells comingled with non-signet ring cells that were identical to the cellular population found in the case that was reported as "Pancreatic analogue solid pseudopapillary neoplasm arising in the paratesticular location". After a careful morphological review, a clear spectrum was found ranging from cases of pure signet ring morphology (scattered signet ring cells can, however, be present in SPN-P as well), to cases of mixed morphology comprising signet ring and non-signet ring component, the latter being identical to the cells of SPN-P. Altogether, we collected 13 such cases and published them in an article entitled as "Primary signet ring stromal tumor of the testis: a study of 13 cases indicating their phenotypic and genotypic analogy to pancreatic solid pseudopapillary neoplasm" (paper **5.a.ii.**).

The problem with this topic is that so called Sertoli cell tumors NOS, a long-established heterogeneous group of mostly benign testicular tumors may, in a subset of cases, have the same phenotype. It was also the problem of one of the reviewers of the latter paper. However, as mentioned, Sertoli cell tumors NOS, are a very heterogeneous group of tumors, with a very variable and uncharacteristic morphology, immunophenotype and molecular

genetic changes including *CTNNB1* mutation which is present in only about one half of cases. In fact, what we are attempting is to separate a morphologically, immunohistochemically and molecular genetically completely homogeneous group of tumors from a heterogeneous one. We also think that these "Sertoli cell tumors" with signet ring cell/SPN-P morphology, IHC staining with classical SPN-P IHC markers and *CTNNB1* mutation represent in fact analogues of solid pseudopapillary tumors of the pancreas occurring in the testis and not Sertoli cell tumors of any kind.

Eventually, we published a paper **5.a.iii.** in which we broadened the morphological spectrum of herein described tumors by adding 6 testicular tumors devoid of signet ring cells, thus being very similar/identical to the SPN-P. In order to provide further evidence for our concept we compared them morphologically, immunohistochemically and molecular genetically to 8 SPN-P. This latest paper was entitled "Solid pseudopapillary neoplasm (SPN) of the testis: Comprehensive mutational analysis of 6 testicular and 8 pancreatic SPNs."

b. Other testicular tumors

i. Novel insights into the mixed germ cell-sex cord stromal tumor of the testis: detection of chromosomal aneuploidy and further morphological evidence supporting the neoplastic nature of the germ cell component

Although ovarian mixed germ cell-sex cord stromal tumor (MGSCT) (without features of gonadoblastoma, i.e. another type of MGSCT) is generally accepted as an extraordinarily rare entity, the existence of a true testicular MGSCT is more controversial. The ovarian and testicular MGSCT differ in the morphologic appearance of both the germ cell and the sex cord component(107). Immunohistochemically, the sex cord component in both testicular and ovarian tumors show immunoreactivity with conventional sex cord markers but the immunophenotype of the germ cell component is different as well (107). The same discrepancy has been found in molecular genetic features. While the amplification of chromosome 12 has been observed in ovarian MGSCT, no such aberration was detected in testicular MGSCT (107).

These reported differences between testicular and ovarian MGSCT, together with the overall bland cytologic appearance of the germ cell component and lack of characteristic immunophenotype and molecular abnormalities in testicular MGSCT, have led some authorities to conclude that true MGSCT does not exist and that all reported examples likely represent sex cord stromal tumors with entrapped nonneoplastic germ cells (108). However, it is a widely accepted theory that genetic and epigenetic factors may induce clinicopathological differences between similar tumors of both ovarian and testicular origin (109, 110). Based on our experience with such tumors, we sought to study a series 8 cases of what we had regarded to be true testicular MGSCTs to determine their full histologic spectrum, immunophenotype, chromosomal aneuploidy and mutational status, clinical outcome, and relationship to spermatocytic tumor.

The herein presented series provided further evidence for the neoplastic nature of the germ cell component. The germ cells in 4/8 cases featured occasional atypical mitotic figures, which further supported earlier observations (107, 111). More importantly, in case 3 there was an invasion into the spermatic cord and hilar soft tissue and in case 4 into the tumor capsule. In each case, the invasive structures contained both the germ cell and sex cord stromal components, which we considered strong supportive evidence for the neoplastic nature of the germ cell component. In addition, the comprehensive chromosomal aneuploidy study revealed losses of chromosomes 21 and 22 and gains of 8, 9, 12, 13, and 20 in 4 cases.

Although the studied sample was relatively small, it provided the first unequivocal evidence that chromosomal abnormalities do occur in testicular MGSCT and further suggested certain level of relatedness to spermatocytic tumor. Based on all the above findings, we argued that although rare, true MGSCT of the testis do exist and should be accepted as a distinct entity.

ii. Protein expression of the transcription factors DMRT1, TCLF5, and OCT4 in selected germ cell neoplasms of the testis

In this article, we investigated the nuclear expression of DMRT1, TCFL5, and OCT4 protein (i.e. transcription factors expressed at different levels of germ cell maturation during spermatogenesis) in the germ cells of testicular MGSCT, spermatocytic tumor, and seminoma and compared the findings to those observed in the adult human testis during the reproductive years.

We noted a strong expression of DMRT1 together with the absence of TCLF5 in the germ cells of both testicular MGSCT and spermatocytic tumor which supported a premeiotic origin for both neoplasms. These low-grade neoplasms, however, differ histologically in that all the germ cell nuclei of testicular MGSCT resemble spermatogonia, whereas in spermatocytic tumor, the nuclei of the medium sized and large cells resemble those of primary spermatocytes. Although our morphological observations as well as other evidence did not support a close relationship between testicular MGSCT and spermatocytic tumor, we argued that molecular-genetic and ploidy studies of testicular MGSCT are required to confirm these findings (our study 5.b.i. which was conducted later on confirmed partially overlapping copy number aberrations between both tumors). By way of contrast, seminoma, a fully malignant germ cell tumor, strongly expressed OCT4 protein but lacked expression of either DMRT1 or TCLF5 which led us to the conclusion that it is completely unrelated to either testicular MGC-SCST or spermatocytic tumor.

c. Penile analogue of stratified mucin-producing intraepithelial lesion of the cervix

i. Penile Analogue of Stratified Mucin-Producing Intraepithelial Lesion of the Cervix: The First Described Case. A Diagnostic Pitfall

ii. Penile warty mucoepidermoid carcinoma with features of stratified mucin-producing intra-epithelial lesion and invasive stratified mucin-producing carcinoma

(**Joint commentary to both publications**)

Stratified mucin-producing intraepithelial lesion (SMILE) was originally described in the uterine cervix as a rare neoplastic intraepithelial process affecting the transformation zone and showing features of both cervical intraepithelial neoplasia and adenocarcinoma in situ. Histopathologically, the stratified epithelium contains mitotically active cells with atypical and hyperchromatic nuclei, involving the surface epithelium and/or the underlying endocervical glands. Simultaneously, in the absence of gland formation, mucin vacuoles are present throughout the full-thickness of the squamous epithelium (112). Two similar lesions were later described on the vulva, where collections of cells with intracytoplasmic mucin present at all epidermal layers occurred concomitantly with changes corresponding to classic vulvar intraepithelial neoplasia (113). These lesions, on both the cervix and the vulva, have been shown to be caused by high-risk Human Papillomavirus.

In the case report **5.c.i**, we presented a unique tumor affecting the penis which showed classic intraepithelial neoplasia admixed with goblet cells being present throughout the entire thickness of the epithelium which subsequently progressed into an invasive carcinoma featuring both squamous and glandular components. This was the first description of such a lesion occurring on the penis, which can be considered the penile analogue of cervical SMILE.

The lesion occurred in a 56-year-old HIV-positive man and evolved in three consecutive biopsies from only surface epithelium occupying numerous goblet cells in the first to variably sized solid nodules in the dermis composed of atypical squamous and/or basaloid cells intermixed with numerous goblet cells in the third biopsy. The initial biopsy was misinterpreted as extramammary Paget disease. The correct diagnosis was rendered retrospectively, after recognition of the existence of the vulvar lesion resembling cervical SMILE (113). After coming across the latter article, we stained the tumor with p16 antibody which yielded diffuse block type positivity in a full thickness of the epithelium, e.i. a staining pattern suggestive for the presence of a high-risk HPV infection. This prompted the HPV genotyping which disclosed the presence of human papillomavirus type 16 infection thereby confirming our hypothesis.

Invasive stratified mucin-producing carcinoma is another recently described cervical lesion (114), which represents an invasive variant of SMILE of the cervix which is its precursor. In the case report **5.c.ii**, we reported an unusual case of mixed variant of penile invasive squamous cell carcinomas (SCC) with features of warty SCC, usual SCC and features of SMILE/Invasive stratified mucin-producing carcinoma.

5. PART:

THE REST

a. Targeted next generation sequencing of MLH1-deficient, MLH1 promoter hypermethylated and BRAF/RAS-wild-type colorectal adenocarcinomas is effective in detecting tumors with actionable oncogenic gene fusions

Remarkable advances in the therapy of colorectal adenocarcinoma (CRC), i.e. one of the most common human malignancies, and improvements in patients' prognosis have been achieved in recent years by the introduction of targeted therapy, including agents targeting epidermal growth factor receptor (EGFR) pathway and angiogenesis. However, these is a large subset of CRC cases that is resistant to anti-EGFR therapy due to other aberrations in RAS-MAPK or PIK3KAKT-mTOR pathways, such as mutations in the *HRAS, MAP2K1, RAF1, PTPN11, PIK3CA*, or *PTEN* genes (115). Another subset of CRC which confers resistance to anti-EGFR inhibitors but that could be instead targeted by other currently available small molecule inhibitors is represented by CRC with kinase gene fusions involving *ALK, NTRK1/2/3, BRAF, RET, RAF1* and others. However, previous studies had shown that only 1.8% CRC cases have these potentially druggable receptor kinase fusions (116). Other studies reported fusions involving *NTRK, ALK, ROS1, RET*, or *BRAF* genes in 0.2% to 2.4% of CRC cases (117-119).

Since the frequency of gene fusions in CRC is so low, the screening for these alterations in general CRC patient population seems to be impractical and costly. To address this problem, several large-scale studies retrospectively showed that CRCs with gene fusions are highly enriched among groups of tumors characterized by the loss of expression of MLH1/PMS2 DNA mismatch repair proteins and hypermethylation of *MLH1* promoter, with or without the presence of microsatellite instability, while presenting *BRAF/KRAS* wild-type status (BRAFwt/KRASwt) (119-122). In this study, we used targeted NGS to explore the occurence of potentially targetable gene fusions *NTRK, ALK, ROS1, RET*, or *BRAF* in BRAFwt/KRASwt CRC cases that displayed IHC confirmed loss of expression of PMS2 and MLH1 proteins, and genetically proven *MLH1* gene promoter hypermethylation (MLH1d/MLH1ph).

From the initially identified group of 173 MLH1d CRC cases, 141 cases (81.5%) displayed MLH1ph. BRAFwt/RASwt genotype was confirmed in 23 of 141 (~16%) of MLH1d/MLH1ph cases. Targeted NGS of these 23 cases identified oncogenic gene fusions in nine patients (39.1%) Detected fusions involved *NTRK* (4 cases), *ALK* (2 cases), and *BRAF* genes (3 cases). As a secondary outcome of NGS testing, we identified PIK3K-AKT-mTOR pathway alterations in 2 CRC cases, which displayed *PIK3CA* mutation. Altogether, 11 of 23 (~48%) MLH1d/MLH1ph/BRAFwt/RASwt tumors showed genetic alterations that could induce resistance to anti-EGFR therapy.

Overall, this study confirmed that targeted NGS of MLH1d/MLH1ph and BRAFwt/RASwt CRCs could be a cost-effective strategy in detecting patients with potentially druggable oncogenic kinase fusions which might translate into a significantly improved outcome of these patients.

LIST OF ABBREVIATIONS

ALT – atypical lipomatous tumor

CRC – colorectal adenocarcinoma

DDL – dedifferentiated liposarcoma

EGFR - epidermal growth factor receptor

EPS - EWSR1::PATZ1-rearranged sarcomas

FDC - follicular dendritic cells

FDCS - follicular dendritic cell sarcoma

FGF23 - fibroblastic growth factor 23

FISH – fluorescence in-situ hybridization

GCT-ST - giant cell tumor of soft tissue

HPNST - hybrid peripheral nerve sheath tumors

IHC - immunohistochemistry

ILMS - inflammatory leiomyosarcoma

LCA - littoral cell angioma

LGTA - low-grade tubulopapillary adenocarcinoma

MASC - mammary analogue secretory carcinoma

MF - myofibroma

MGSCT - mixed germ cell-sex cord stromal tumor

MIFS – myxoinflammatory fibroblastic sarcoma

MSS - monophasic fibrous synovial sarcoma

NGS – next generation sequencing

PHAT – pleomorphic hyalinizing angiectatic tumor

PL – pleomorphic lipoma

PMT - phosphaturic mesenchymal tumor

PRDM10-STT - PRDM10-rearranged soft tissue tumor

PTC – papillary thyroid carcinoma

REAH - respiratory epithelial adenomatoid hamartoma

SH - seromucinous hamartoma

RNA-ISH - RNA in situ hybridization

RNA-seq – RNA-sequencing

SAF – superficial acral fibromyxoma

SCC - squamous cell carcinomas

SCD34FT – superficial CD34-positive fibroblastic tumor

SCL – spindle cell lipoma

SEF – sclerosing epithelioid fibrosarcoma

SFT – solitary fibrous tumor

SMILE - stratified mucin-producing intraepithelial lesion

SPN-P - solid pseudopapillary neoplasm of the pancreas

SNTMM - sinonasal tract mucosal melanoma

TIO - induced ostemalacia

TPC - tenosynovitis with psammomatous calcifications

WCP - whorling cellular perineurioma

WDL – well-differentiated liposarcoma

WHO – world health organization

REFERENCES

1. **French CA, Mentzel T, Kutzner H, Fletcher CD.** Intradermal spindle cell/pleomorphic lipoma: a distinct subset. *Am J Dermatopathol.* 2000;22:496-502.
2. **Shmookler BM, Enzinger FM.** Pleomorphic lipoma: a benign tumor simulating liposarcoma. A clinicopathologic analysis of 48 cases. *Cancer.* 1981;47:126-133.
3. **Azzopardi JG, Iocco J, Salm R.** Pleomorphic lipoma: a tumour simulating liposarcoma. *Histopathology.* 1983;7:511-523.
4. **Maggiani F, Debiec-Rychter M, Vanbockrijck M, Sciot R.** Cellular angiofibroma: another mesenchymal tumour with 13q14 involvement, suggesting a link with spindle cell lipoma and (extra)-mammary myofibroblastoma. *Histopathology.* 2007;51:410-412.
5. **Evans HL.** Anisometric Cell Lipoma: A Predominantly Subcutaneous Fatty Tumor With Notable Variation in Fat Cell Size But Not More Than Slight Nuclear Enlargement and Atypia. *AJSP: Reviews & Reports.* 2016;21:195-199.
6. **Agaimy A.** Anisometric cell lipoma: Insight from a case series and review of the literature on adipocytic neoplasms in survivors of retinoblastoma suggest a role for RB1 loss and possible relationship to fat-predominant ("fat-only") spindle cell lipoma. *Ann Diagn Pathol.* 2017;29:52-56.
7. **Kulkarni AS, Wojcik JB, Chougule A, et al.** MDM2 RNA In Situ Hybridization for the Diagnosis of Atypical Lipomatous Tumor: A Study Evaluating DNA, RNA, and Protein Expression. *Am J Surg Pathol.* 2019;43:446-454.
8. **Moll UM, Petrenko O.** The MDM2-p53 interaction. *Mol Cancer Res.* 2003;1:1001-1008.
9. **Mirkovic J, Fletcher CD.** Lipoblastoma-like tumor of the vulva: further characterization in 8 new cases. *Am J Surg Pathol.* 2015;39:1290-1295.
10. **Gambarotti M, Erdogan KE, Righi A, et al.** Lipoblastoma-like tumor of the spermatic cord: case report and review of the literature. *Virchows Arch.* 2021;478:1013-1017.
11. **Coindre JM, Mariani O, Chibon F, et al.** Most malignant fibrous histiocytomas developed in the retroperitoneum are dedifferentiated liposarcomas: a review of 25 cases initially diagnosed as malignant fibrous histiocytoma. *Mod Pathol.* 2003;16:256-262.
12. **Thway K, Jones RL, Noujaim J, et al.** Dedifferentiated Liposarcoma: Updates on Morphology, Genetics, and Therapeutic Strategies. *Adv Anat Pathol.* 2016;23:30-40.
13. **Agaimy A, Daum O, Markl B, et al.** SWI/SNF Complex-deficient Undifferentiated/Rhabdoid Carcinomas of the Gastrointestinal Tract: A Series of 13 Cases Highlighting Mutually Exclusive Loss of SMARCA4 and SMARCA2 and Frequent Co-inactivation of SMARCB1 and SMARCA2. *Am J Surg Pathol.* 2016;40:544-553.
14. **Hoffman A, Lazar AJ, Pollock RE, Lev D.** New frontiers in the treatment of liposarcoma, a therapeutically resistant malignant cohort. *Drug Resist Updat.* 2011;14:52-66.
15. **Meis-Kindblom JM, Kindblom LG.** Acral myxoinflammatory fibroblastic sarcoma: a low-grade tumor of the hands and feet. *Am J Surg Pathol.* 1998;22:911-924.
16. **Michal M.** Inflammatory myxoid tumor of the soft parts with bizarre giant cells. *Pathol Res Pract.* 1998;194:529-533.
17. **Montgomery EA, Devaney KO, Giordano TJ, Weiss SW.** Inflammatory myxohyaline tumor of distal extremities with virocyte or Reed-Sternberg-like cells: a distinctive lesion with features simulating inflammatory conditions, Hodgkin's disease, and various sarcomas. *Mod Pathol.* 1998;11:384-391.
18. **Michal M, Kazakov DV, Hadravsky L, et al.** High-grade myxoinflammatory fibroblastic sarcoma: a report of 23 cases. *Ann Diagn Pathol.* 2015;19:157-163.

19. **Kao YC, Ranucci V, Zhang L, et al.** Recurrent BRAF Gene Rearrangements in Myxoinflammatory Fibroblastic Sarcomas, but Not Hemosiderotic Fibrolipomatous Tumors. *Am J Surg Pathol.* 2017;41:1456-1465.
20. **Arbajian E, Hofvander J, Magnusson L, Mertens F.** Deep sequencing of myxoinflammatory fibroblastic sarcoma. *Genes Chromosomes Cancer.* 2020;59:309-317.
21. **Carter JM, Sukov WR, Montgomery E, et al.** TGFBR3 and MGEA5 rearrangements in pleomorphic hyalinizing angiectatic tumors and the spectrum of related neoplasms. *Am J Surg Pathol.* 2014;38:1182-1992.
22. **Antonescu CR, Zhang L, Nielsen GP, et al.** Consistent t(1;10) with rearrangements of TGFBR3 and MGEA5 in both myxoinflammatory fibroblastic sarcoma and hemosiderotic fibrolipomatous tumor. *Genes Chromosomes Cancer.* 2011;50:757-764.
23. **Hallor KH, Sciot R, Staaf J, et al.** Two genetic pathways, t(1;10) and amplification of 3p11-12, in myxoinflammatory fibroblastic sarcoma, haemosiderotic fibrolipomatous tumour, and morphologically similar lesions. *J Pathol.* 2009;217:716-727.
24. **Zreik RT, Carter JM, Sukov WR, 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.
25. **Helias-Rodzewicz Z, Perot G, Chibon F, et al.** YAP1 and VGLL3, encoding two cofactors of TEAD transcription factors, are amplified and overexpressed in a subset of soft tissue sarcomas. *Genes Chromosomes Cancer.* 2010;49:1161-1171.
26. **Kazakov DV, Pavlovsky M, Mukensnabl P, Michal M.** Pleomorphic hyalinizing angiectatic tumor with a sarcomatous component recurring as high-grade myxofibrosarcoma. *Pathol Int.* 2007;57:281-284.
27. **Huang SC, Huang HY.** Solitary fibrous tumor: An evolving and unifying entity with unsettled issues. *Histol Histopathol.* 2019;34:313-334.
28. **Mosquera JM, Fletcher CD.** Expanding the spectrum of malignant progression in solitary fibrous tumors: a study of 8 cases with a discrete anaplastic component--is this dedifferentiated SFT? *Am J Surg Pathol.* 2009;33:1314-1321.
29. **Olson NJ, Linos K.** Dedifferentiated Solitary Fibrous Tumor: A Concise Review. *Arch Pathol Lab Med.* 2018;142:761-766.
30. **Lu C, Alex D, Benayed R, Rosenblum M, Hameed M.** Solitary fibrous tumor with neuroendocrine and squamous dedifferentiation: a potential diagnostic pitfall. *Hum Pathol.* 2018;77:175-180.
31. **Tan NJH, Sun ISY, Low SW, et al.** A rapidly fatal intracranial anaplastic hemangiopericytoma with de-novo dedifferentiation: emphasis on diagnostic recognition, molecular confirmation and discussion on treatment dilemma. *Brain Tumor Pathol.* 2019;36:20-26.
32. **Chmielecki J, Crago AM, Rosenberg M, et al.** Whole-exome sequencing identifies a recurrent NAB2-STAT6 fusion in solitary fibrous tumors. *Nat Genet.* 2013;45:131-132.
33. **Robinson DR, Wu YM, Kalyana-Sundaram S, et al.** Identification of recurrent NAB2-STAT6 gene fusions in solitary fibrous tumor by integrative sequencing. *Nat Genet.* 2013;45:180-185.
34. **Doyle LA, Vivero M, Fletcher CD, Mertens F, Hornick JL.** Nuclear expression of STAT6 distinguishes solitary fibrous tumor from histologic mimics. *Mod Pathol.* 2014;27:390-395.
35. **Hiemcke-Jiwa LS, van Gorp JM, Fisher C, et al.** USP6-Associated Neoplasms: A Rapidly Expanding Family of Lesions. *Int J Surg Pathol.* 2020;28:816-825.
36. **Flucke U, Shepard SJ, Bekers EM, et al.** Fibro-osseous pseudotumor of digits - Expanding the spectrum of clonal transient neoplasms harboring USP6 rearrangement. *Ann Diagn Pathol.* 2018;35:53-55.
37. **Kao YC, Flucke U, Eijkelenboom A, et al.** Novel EWSR1-SMAD3 Gene Fusions in a Group of Acral Fibroblastic Spindle Cell Neoplasms. *Am J Surg Pathol.* 2018;42:522-528.

38. **Carter JM, Weiss SW, Linos K, DiCaudo DJ, Folpe AL.** Superficial CD34-positive fibroblastic tumor: report of 18 cases of a distinctive low-grade mesenchymal neoplasm of intermediate (borderline) malignancy. *Mod Pathol.* 2014;27:294-302.
39. **Puls F, Pillay N, Fagman H, et al.** PRDM10-rearranged Soft Tissue Tumor: A Clinicopathologic Study of 9 Cases. *Am J Surg Pathol.* 2019;43:504-513.
40. **Wojcik JB, Bellizzi AM, Dal Cin P, et al.** Primary sclerosing epithelioid fibrosarcoma of bone: analysis of a series. *Am J Surg Pathol.* 2014;38:1538-1544.
41. **Fetsch JF, Laskin WB, Miettinen M.** Superficial acral fibromyxoma: a clinicopathologic and immunohistochemical analysis of 37 cases of a distinctive soft tissue tumor with a predilection for the fingers and toes. *Hum Pathol.* 2001;32:704-714.
42. **Chen BJ, Marino-Enriquez A, Fletcher CD, Hornick JL.** Loss of retinoblastoma protein expression in spindle cell/pleomorphic lipomas and cytogenetically related tumors: an immunohistochemical study with diagnostic implications. *Am J Surg Pathol.* 2012;36:1119-1128.
43. **Howitt BE, Fletcher CD.** Mammary-type Myofibroblastoma: Clinicopathologic Characterization in a Series of 143 Cases. *Am J Surg Pathol.* 2016;40:361-367.
44. **Iwasa Y, Fletcher CD.** Cellular angiofibroma: clinicopathologic and immunohistochemical analysis of 51 cases. *Am J Surg Pathol.* 2004;28:1426-1435.
45. **Oliveira AM, Dei Tos AP, Fletcher CD, Nascimento AG.** Primary giant cell tumor of soft tissues: a study of 22 cases. *Am J Surg Pathol.* 2000;24:248-256.
46. **Doyle LA, Marino-Enriquez A, Fletcher CD, Hornick JL.** ALK rearrangement and overexpression in epithelioid fibrous histiocytoma. *Mod Pathol.* 2015;28:904-912.
47. **Jedrych J, Nikiforova M, Kennedy TF, Ho J.** Epithelioid cell histiocytoma of the skin with clonal ALK gene rearrangement resulting in VCL-ALK and SQSTM1-ALK gene fusions. *Br J Dermatol.* 2015;172:1427-1429.
48. **McCarron KF, Goldblum JR.** Plexiform neurofibroma with and without associated malignant peripheral nerve sheath tumor: a clinicopathologic and immunohistochemical analysis of 54 cases. *Mod Pathol.* 1998;11:612-617.
49. **Billings SD, Walsh SV, Fisher C, et al.** Aberrant expression of tight junction-related proteins ZO-1, claudin-1 and occludin in synovial sarcoma: an immunohistochemical study with ultrastructural correlation. *Mod Pathol.* 2004;17:141-149.
50. **Kao YC, Suurmeijer AJH, Argani P, et al.** Soft tissue tumors characterized by a wide spectrum of kinase fusions share a lipofibromatosis-like neural tumor pattern. *Genes Chromosomes Cancer.* 2020;59:575-583.
51. **Davis JL, Al-Ibraheemi A, Rudzinski ER, Surrey LF.** Mesenchymal neoplasms with NTRK and other kinase gene alterations. *Histopathology.* 2022;80:4-18.
52. **Baranov E, Winsnes K, O'Brien M, et al.** Histologic characterization of paediatric mesenchymal neoplasms treated with kinase-targeted therapy. *Histopathology.* 2022;81:215-227.
53. **Antonescu CR, Dickson BC, Swanson D, et al.** Spindle Cell Tumors With RET Gene Fusions Exhibit a Morphologic Spectrum Akin to Tumors With NTRK Gene Fusions. *Am J Surg Pathol.* 2019;43:1384-1391.
54. **Wegert J, Vokuhl C, Collord G, et al.** Recurrent intragenic rearrangements of EGFR and BRAF in soft tissue tumors of infants. *Nat Commun.* 2018;9:2378.
55. **Folpe AL.** Phosphaturic mesenchymal tumors: A review and update. *Semin Diagn Pathol.* 2019;36:260-268.
56. **Lee JC, Jeng YM, Su SY, et al.** Identification of a novel FN1-FGFR1 genetic fusion as a frequent event in phosphaturic mesenchymal tumour. *J Pathol.* 2015;235:539-545.
57. **Lee JC, Su SY, Changou CA, et al.** Characterization of FN1-FGFR1 and novel FN1-FGF1 fusion genes in a large series of phosphaturic mesenchymal tumors. *Mod Pathol.* 2016;29:1335-1346.
58. **Shon W, Folpe AL, Fritchie KJ.** ERG expression in chondrogenic bone and soft tissue tumours. *J Clin Pathol.* 2015;68:125-129.
59. **Sbaraglia M, Righi A, Gambarotti M, Dei Tos AP.** Ewing sarcoma and Ewing-like tumors. *Virchows Arch.* 2020;476:109-119.

60. **Perret R, Escuriol J, Velasco V, et al.** NFATc2-rearranged sarcomas: clinicopathologic, molecular, and cytogenetic study of 7 cases with evidence of AGGRECAN as a novel diagnostic marker. *Mod Pathol.* 2020;33:1930-1944.
61. **Bridge JA, Sumegi J, Druta M, et al.** Clinical, pathological, and genomic features of EWSR1-PATZ1 fusion sarcoma. *Mod Pathol.* 2019;32:1593-1604.
62. **Merchant W, Calonje E, Fletcher CD.** Inflammatory leiomyosarcoma: a morphological subgroup within the heterogeneous family of so-called inflammatory malignant fibrous histiocytoma. *Histopathology.* 1995;27:525-532.
63. **Arbajian E, Koster J, Vult von Steyern F, Mertens F.** Inflammatory leiomyosarcoma is a distinct tumor characterized by near-haploidization, few somatic mutations, and a primitive myogenic gene expression signature. *Mod Pathol.* 2018;31:93-100.
64. **Martinez AP, Fritchie KJ, Weiss SW, et al.** Histiocyte-rich rhabdomyoblastic tumor: rhabdomyosarcoma, rhabdomyoma, or rhabdomyoblastic tumor of uncertain malignant potential? A histologically distinctive rhabdomyoblastic tumor in search of a place in the classification of skeletal muscle neoplasms. *Mod Pathol.* 2019;32:446-457.
65. **Cloutier JM, Charville GW, Mertens F, et al.** "Inflammatory Leiomyosarcoma" and "Histiocyte-rich Rhabdomyoblastic Tumor": a clinicopathological, immunohistochemical and genetic study of 13 cases, with a proposal for reclassification as "Inflammatory Rhabdomyoblastic Tumor". *Mod Pathol.* 2021;34:758-769.
66. **Siegal DS, Wu JS, Newman JS, Del Cura JL, Hochman MG.** Calcific tendinitis: a pictorial review. *Can Assoc Radiol J.* 2009;60:263-272.
67. **Gravanis MB, Gaffney EF.** Idiopathic calcifying tenosynovitis. Histopathologic features and possible pathogenesis. *Am J Surg Pathol.* 1983;7:357-361.
68. **Shon W, Folpe AL.** Tenosynovitis with psammomatous calcification: a poorly recognized pseudotumor related to repetitive tendinous injury. *Am J Surg Pathol.* 2010;34:892-895.
69. **Laskin WB, Miettinen M, Fetsch JF.** Calcareous lesions of the distal extremities resembling tumoral calcinosis (tumoral calcinosislike lesions): clinicopathologic study of 43 cases emphasizing a pathogenesis-based approach to classification. *Am J Surg Pathol.* 2007;31:15-25.
70. **Chung EB, Enzinger FM.** Infantile myofibromatosis. *Cancer.* 1981;48:1807-1818.
71. **Oudijk L, den Bakker MA, Hop WC, et al.** Solitary, multifocal and generalized myofibromas: clinicopathological and immunohistochemical features of 114 cases. *Histopathology.* 2012;60:E1-11.
72. **Martignetti JA, Tian L, Li D, et al.** Mutations in PDGFRB cause autosomal-dominant infantile myofibromatosis. *Am J Hum Genet.* 2013;92:1001-1007.
73. **Cheung YH, Gayden T, Campeau PM, et al.** A recurrent PDGFRB mutation causes familial infantile myofibromatosis. *Am J Hum Genet.* 2013;92:996-1000.
74. **Hung YP, Fletcher CDM.** Myopericytomatosis: Clinicopathologic Analysis of 11 Cases With Molecular Identification of Recurrent PDGFRB Alterations in Myopericytomatosis and Myopericytoma. *Am J Surg Pathol.* 2017;41:1034-1044.
75. **Bisceglia M, Muscarella LA, Galliani CA, et al.** Extraneuraxial Hemangioblastoma: Clinicopathologic Features and Review of the Literature. *Adv Anat Pathol.* 2018;25:197-215.
76. **Falconieri G, Mirra M, Michal M, Suster S.** Hemangioblastoma-like clear cell stromal tumor of the lung. *Adv Anat Pathol.* 2013;20:130-135.
77. **Lindholm KE, Moran CA.** Hemangioblastoma-like Clear Cell Stromal Tumor of the Lung: A Clinicopathologic and Immunohistochemical Study of 5 Cases. *Am J Surg Pathol.* 2020;44:771-775.
78. **Bisceglia M, Sickel JZ, Giangaspero F, et al.** Littoral cell angioma of the spleen: an additional report of four cases with emphasis on the association with visceral organ cancers. *Tumori.* 1998;84:595-599.
79. **O'Malley DP, Kim YS, Weiss LM.** Distinctive immunohistochemical staining in littoral cell angioma using ERG and WT-1. *Ann Diagn Pathol.* 2015;19:143-145.
80. **Chan JK, Fletcher CD, Nayler SJ, Cooper K.** Follicular dendritic cell sarcoma. Clinicopathologic analysis of 17 cases suggesting a malignant potential higher than currently recognized. *Cancer.* 1997;79:294-313.

81. **Soriano AO, Thompson MA, Admirand JH, et al.** Follicular dendritic cell sarcoma: a report of 14 cases and a review of the literature. *Am J Hematol.* 2007;82:725-728.
82. **Skalova A, Vanecek T, Sima R, et al.** Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3 fusion gene: a hitherto undescribed salivary gland tumor entity. *Am J Surg Pathol.* 2010;34:599-608.
83. **Skalova A, Vanecek T, Simpson RH, et al.** Mammary Analogue Secretory Carcinoma of Salivary Glands: Molecular Analysis of 25 ETV6 Gene Rearranged Tumors With Lack of Detection of Classical ETV6-NTRK3 Fusion Transcript by Standard RT-PCR: Report of 4 Cases Harboring ETV6-X Gene Fusion. *Am J Surg Pathol.* 2016;40:3-13.
84. **Su YJ, Lee YH, Jin YT, Hsieh MS.** Using pan-TRK and RET Immunohistochemistry for the Detection of Fusion Types of Salivary Gland Secretory Carcinoma. *Appl Immunohistochem Mol Morphol.* 2022;30:264-272.
85. **Petersson F, Michal M, Ptakova N, Skalova A, Michal M.** Salivary Gland Mucinous Adenocarcinoma With Minor (Mammary Analogue) Secretory and Low-Grade In Situ Carcinoma Components Sharing the Same ETV6-RET Translocation and With No Other Molecular Genetic Aberrations Detected on NGS Analysis. *Appl Immunohistochem Mol Morphol.* 2020;28:e53.
86. **Dogan S, Wang L, Ptashkin RN, et al.** Mammary analog secretory carcinoma of the thyroid gland: A primary thyroid adenocarcinoma harboring ETV6-NTRK3 fusion. *Mod Pathol.* 2016;29:985-995.
87. **Bishop JA, Taube JM, Su A, et al.** Secretory Carcinoma of the Skin Harboring ETV6 Gene Fusions: A Cutaneous Analogue to Secretory Carcinomas of the Breast and Salivary Glands. *Am J Surg Pathol.* 2017;41:62-66.
88. **Andreasen S, Skalova A, Agaimy A, et al.** ETV6 Gene Rearrangements Characterize a Morphologically Distinct Subset of Sinonasal Low-grade Non-intestinal-type Adenocarcinoma: A Novel Translocation-associated Carcinoma Restricted to the Sinonasal Tract. *Am J Surg Pathol.* 2017;41:1552-1560.
89. **Marcus DM, Marcus RP, Prabhu RS, et al.** Rising incidence of mucosal melanoma of the head and neck in the United States. *J Skin Cancer.* 2012;2012:231693.
90. **Amit M, Na'ara S, Hanna EY.** Contemporary Treatment Approaches to Sinonasal Mucosal Melanoma. *Curr Oncol Rep.* 2018;20:10.
91. **Cancer Genome Atlas N.** Genomic Classification of Cutaneous Melanoma. *Cell.* 2015;161:1681-1696.
92. **Wiesner T, He J, Yelensky R, et al.** Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. *Nat Commun.* 2014;5:3116.
93. **Weinreb I, Gnepp DR, Laver NM, et al.** Seromucinous hamartomas: a clinicopathological study of a sinonasal glandular lesion lacking myoepithelial cells. *Histopathology.* 2009;54:205-213.
94. **Leivo I.** Sinonasal Adenocarcinoma: Update on Classification, Immunophenotype and Molecular Features. *Head Neck Pathol.* 2016;10:68-74.
95. **Weinreb I.** Low grade glandular lesions of the sinonasal tract: a focused review. *Head Neck Pathol.* 2010;4:77-83.
96. **Ozolek JA, Hunt JL.** Tumor suppressor gene alterations in respiratory epithelial adenomatoid hamartoma (REAH): comparison to sinonasal adenocarcinoma and inflamed sinonasal mucosa. *Am J Surg Pathol.* 2006;30:1576-1580.
97. **Jo VY, Mills SE, Cathro HP, Carlson DL, Stelow EB.** Low-grade sinonasal adenocarcinomas: the association with and distinction from respiratory epithelial adenomatoid hamartomas and other glandular lesions. *Am J Surg Pathol.* 2009;33:401-408.
98. **Zou M, Baitei EY, Alzahrani AS, et al.** Concomitant RAS, RET/PTC, or BRAF mutations in advanced stage of papillary thyroid carcinoma. *Thyroid.* 2014;24:1256-1266.
99. **Agaimy A, Haller F.** CTNNB1 (beta-Catenin)-altered Neoplasia: A Review Focusing on Soft Tissue Neoplasms and Parenchymal Lesions of Uncertain Histogenesis. *Adv Anat Pathol.* 2016;23:1-12.

100. **Oliveira AM, Chou MM.** USP6-induced neoplasms: the biologic spectrum of aneurysmal bone cyst and nodular fasciitis. *Hum Pathol.* 2014;45:1-11.
101. **Mardi K.** Papillary thyroid carcinoma with nodular fasciitis-like stroma: A rare variant with distinctive morphology. *Indian J Pathol Microbiol.* 2017;60:390-392.
102. **Zhou L, Shi L, Jiang Z, Xie L.** Papillary thyroid carcinoma with nodular fasciitis-like stroma and beta-catenin gene mutations: report of a recurrent case. *Int J Clin Exp Pathol.* 2018;11:2879-2883.
103. **Wong SBJ, Nga ME, Michal M, et al.** SOX11 expression in a case of papillary thyroid carcinoma with fibromatosis/fasciitis-like stroma containing BRAF c.1799_1801delTGA and CTNNB1 c.133T>C mutations. *Virchows Arch.* 2019;475:519-525.
104. **Rebecchini C, Nobile A, Piana S, et al.** Papillary thyroid carcinoma with nodular fasciitis-like stroma and beta-catenin mutations should be renamed papillary thyroid carcinoma with desmoid-type fibromatosis. *Mod Pathol.* 2017;30:236-245.
105. **Peckova K, Daum O, Michal M, Curcikova R, Michal M.** Selected Case From the Arkadi M. Rywlin International Pathology Slide Seminar: Benign Warthin Tumor of the Thyroid. *Adv Anat Pathol.* 2016;23:339-342.
106. **Michal M, Hes O, Kazakov DV.** Primary signet-ring stromal tumor of the testis. *Virchows Arch.* 2005;447:107-110.
107. **Michal M, Vanecek T, Sima R, et al.** Mixed germ cell sex cord-stromal tumors of the testis and ovary. Morphological, immunohistochemical, and molecular genetic study of seven cases. *Virchows Arch.* 2006;448:612-622.
108. **Ulbright TM, Srigley JR, Reuter VE, et al.** Sex cord-stromal tumors of the testis with entrapped germ cells: a lesion mimicking unclassified mixed germ cell sex cord-stromal tumors. *Am J Surg Pathol.* 2000;24:535-542.
109. **Conlon N, Schultheis AM, Piscuoglio S, et al.** A survey of DICER1 hotspot mutations in ovarian and testicular sex cord-stromal tumors. *Mod Pathol.* 2015;28:1603-1612.
110. **Roth LM, Lyu B, Cheng L.** Perspectives on testicular sex cord-stromal tumors and those composed of both germ cells and sex cord-stromal derivatives with a comparison to corresponding ovarian neoplasms. *Hum Pathol.* 2017;65:1-14.
111. **Michal M, Hes O, Mukensnabl P, Kazakov DV.** Mixed germ cell sex cord-stromal tumours of the testis. *Virchows Arch.* 2007;451:1095-1096.
112. **Boyle DP, McCluggage WG.** Stratified mucin-producing intraepithelial lesion (SMILE): report of a case series with associated pathological findings. *Histopathology.* 2015;66:658-663.
113. **McCluggage WG, Jamison J, Boyde A, Ganesan R.** Vulval intraepithelial neoplasia with mucinous differentiation: report of 2 cases of a hitherto undescribed phenomenon. *Am J Surg Pathol.* 2009;33:945-949.
114. **Lastra RR, Park KJ, Schoolmeester JK.** Invasive Stratified Mucin-producing Carcinoma and Stratified Mucin-producing Intraepithelial Lesion (SMILE): 15 Cases Presenting a Spectrum of Cervical Neoplasia With Description of a Distinctive Variant of Invasive Adenocarcinoma. *Am J Surg Pathol.* 2016;40:262-269.
115. **Costigan DC, Dong F.** The extended spectrum of RAS-MAPK pathway mutations in colorectal cancer. *Genes Chromosomes Cancer.* 2020;59:152-159.
116. **Kloosterman WP, Coebergh van den Braak RRJ, Pieterse M, et al.** A Systematic Analysis of Oncogenic Gene Fusions in Primary Colon Cancer. *Cancer Res.* 2017;77:3814-3822.
117. **Pagani F, Randon G, Guarini V, et al.** The Landscape of Actionable Gene Fusions in Colorectal Cancer. *Int J Mol Sci.* 2019;20.
118. **Gatalica Z, Xiu J, Swensen J, Vranic S.** Molecular characterization of cancers with NTRK gene fusions. *Mod Pathol.* 2019;32:147-153.
119. **Lasota J, Chlopek M, Lamoureux J, et al.** Colonic Adenocarcinomas Harboring NTRK Fusion Genes: A Clinicopathologic and Molecular Genetic Study of 16 Cases and Review of the Literature. *Am J Surg Pathol.* 2020;44:162-173.

120. **Chou A, Fraser T, Ahadi M, et al.** NTRK gene rearrangements are highly enriched in MLH1/PMS2 deficient, BRAF wild-type colorectal carcinomas-a study of 4569 cases. *Mod Pathol.* 2020;33:924-932.
121. **Cocco E, Benhamida J, Middha S, et al.** Colorectal Carcinomas Containing Hypermethylated MLH1 Promoter and Wild-Type BRAF/KRAS Are Enriched for Targetable Kinase Fusions. *Cancer Res.* 2019;79:1047-1053.
122. **Wang J, Yi Y, Xiao Y, et al.** Prevalence of recurrent oncogenic fusion in mismatch repair-deficient colorectal carcinoma with hypermethylated MLH1 and wild-type BRAF and KRAS. *Mod Pathol.* 2019;32:1053-1064.