

SUMMARY

The dissertation is the result of Antonina Kalmykova's doctoral research conducted at Charles University in Prague, at the Faculty of Medicine in Pilsen, from 2019 to 2025. The primary focus was on rare soft tissue and skin tumors. In her work she used various investigative methods, including morphology, immunohistochemistry, and molecular genetic techniques, with the aim of extending and/or improving existing classification and diagnostic approaches.

There are two parts in this dissertation.

The first part focused on rare skin tumors and included three articles.

In the first study, we focused on comprehensive clinical, morphological, and molecular characteristics of BAP1-inactivated melanocytic tumors (BIMT). During this research, we extended the morphological spectrum of BIMTs and identified novel BAP1 mutations. We stressed the necessity of further investigations to correctly define BIMTs and identify possible morphological or molecular prognostic factors.

In the second paper, we reported newly identified *MITF::CREM* fusion-associated tumor and summarized our findings together with two previously published cases. Our research extended the morphological characteristics of this rare entity. We stated that molecular confirmation is necessary for a definitive diagnosis and further studies are needed to determine the exact biological behavior.

The third paper is a review article focused on the main changes in the mesenchymal tumor chapter in the new 5th edition of WHO classification of skin tumors, primarily focusing on the new entities that were added [1]. These namely include *CRTC1::TRIM11*, *ACTIN::MITF*, and *MITF::CREM* rearranged tumors with melanocytic differentiation, *EWSR1::SMAD3*-rearranged fibroblastic tumors, superficial CD34-positive fibroblastic tumors, and *NTRK*-rearranged spindle cell neoplasms. Morphological features, immunohistochemical characteristics, molecular profile, and differential diagnosis were discussed in this article.

The second part focused on rare soft tissue tumors and included four articles.

The first article was focused on a rare soft tissue tumor with an indolent nature: inflammatory leiomyosarcoma (ILMS). We investigated the immunohistochemical profile and analyzed follow-up data to understand the long-term outcomes and, therefore, clinical behavior of this tumor. The molecular study results and immunohistochemical characteristics indicated that ILMS is not a subtype of leiomyosarcoma. All these findings for the first time suggested that ILMS originate from a more primitive myogenic lineage with smooth but mainly skeletal muscle differentiation. This has been confirmed in subsequent studies and eventually it has led to redefining of its current classification

from ILMS to a neoplasm with skeletal muscle immunophenotype in the upcoming WHO classification of soft tissue tumors.

In the second paper, we described a rare phenomenon of transformation of biphenotypic sinonasal sarcoma (BSNS) with *PAX3::MAML3* fusion into high-grade rhabdomyosarcoma (RMS). Therefore, we advocated for a careful clinical follow-up and thorough sampling of every BSNS case, as well as molecular profiling of sinonasal RMS of any type to prevent potential diagnostic errors.

In the third study, we investigated by then the largest case series of *EWSR1-PATZ1*-rearranged sarcomas (EPS), focusing on detailed clinicopathological analysis. We expanded the known morphological range and defined specific histological and immunohistochemical features of these tumors. Also, in some cases, we described more indolent clinical behavior than previously reported cases, suggesting that a subset of EPS may have a more favorable prognosis than previously thought, a finding confirmed in subsequent studies. Further investigation is needed to identify the potential morphological or molecular prognostic factors which can influence clinical outcomes.

In the fourth study, we focused on comprehensive clinicopathological, molecular, and methylation analysis of mesenchymal tumors with *NTRK* and other kinase gene aberrations, one of the largest series to date. A morphological spectrum defined by cellularity and degree of atypia was confirmed to correlate with chromosomal copy number changes as well as outcome. Several novel fusions were identified, including *PWWP2A::RET*, *NUMA1::RET*, *ITSN1::RAF1*, and *CAPZA2::MET* fusions. Additionally, the first cervical case with *BRAF* and *EGFR* mutations was discovered and published in this series. DNA sequencing revealed that secondary molecular alterations are rare in these mostly kinase fusion driven tumors. Most importantly, we have shown that the methylation profile of so called *NTRK*-rearranged spindle cell tumors largely overlaps with that of infantile fibrosarcoma, a currently separate entity in the WHO classification, thus supporting the notion that all these neoplasms are closely related and perhaps represent a morphological spectrum of one entity.