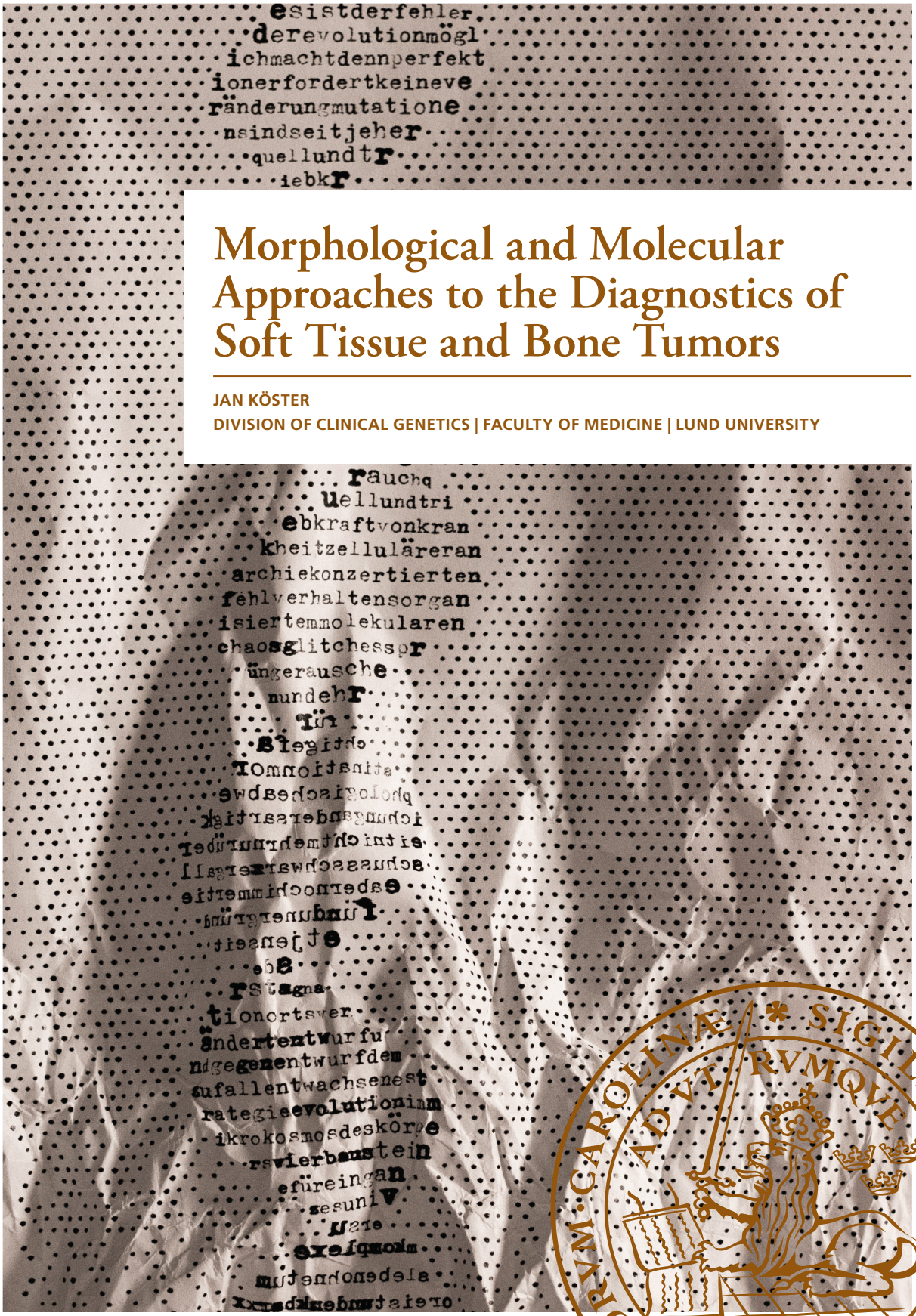
Morphological and Molecular Approaches to the Diagnostics of Soft Tissue and Bone Tumors

Köster, Jan

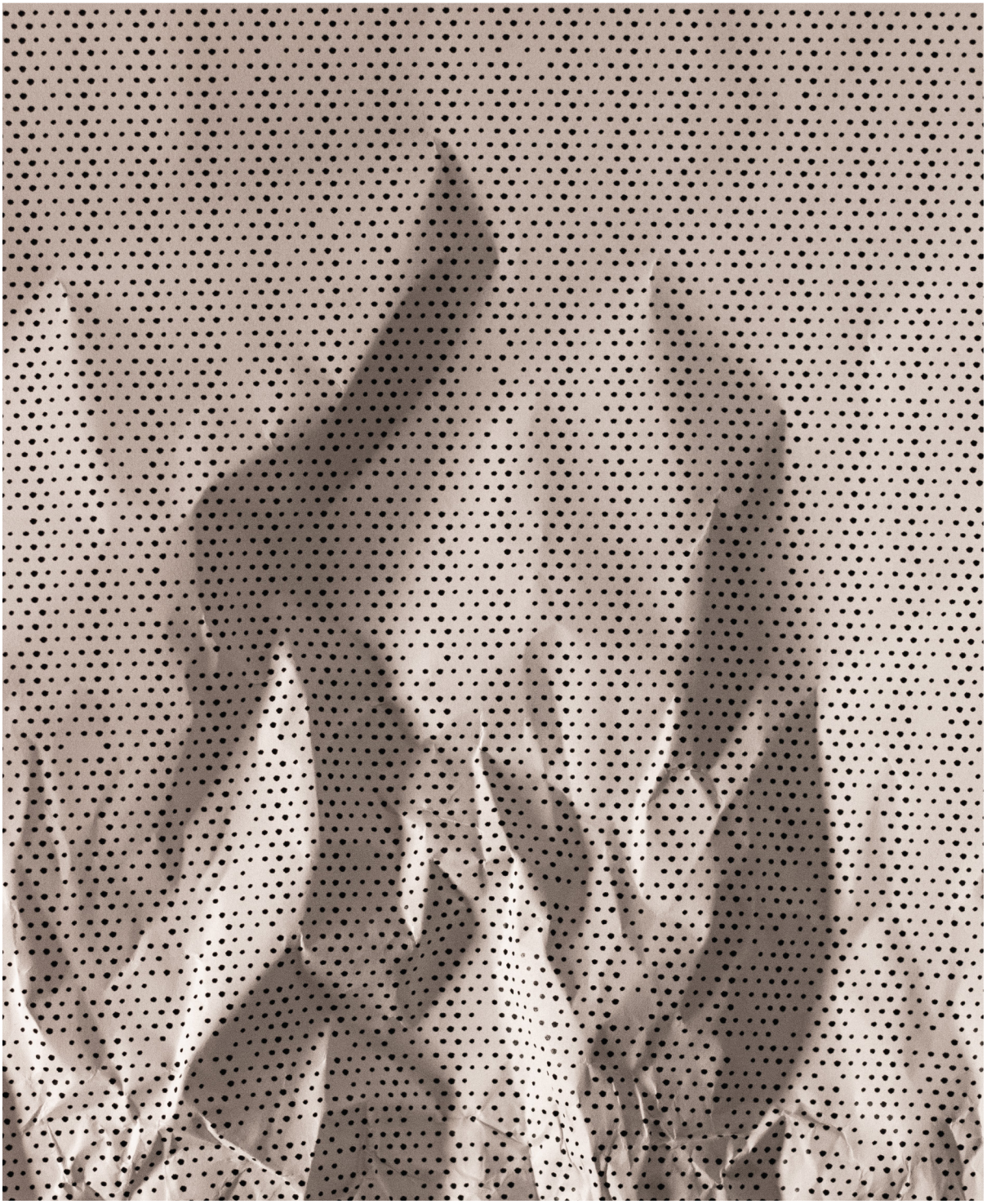
2024

Document Version:
Publisher's PDF, also known as Version of record



Morphological and Molecular Approaches to the Diagnostics of Soft Tissue and Bone Tumors

JAN KÖSTER
DIVISION OF CLINICAL GENETICS | FACULTY OF MEDICINE | LUND UNIVERSITY



LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Laboratory Medicine
Division of Clinical Genetics

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2024:137
ISBN 978-91-8021-635-7
ISSN 1652-8220

Morphological and Molecular Approaches to the Diagnostics of Soft Tissue and Bone Tumors

Jan Köster

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 29th of November 2024 at 09.00 in Föreläsningssalen, Department of Pathology

Faculty opponent
Professor Raf Sciot
Department of Pathology
KU Leuven/ Leuven University Hospitals, Leuven, Belgium

Organization: LUND UNIVERSITY, Faculty of Medicine, Department of Laboratory Medicine, Lund, Division of Clinical Genetics

Document name: Doctoral Dissertation **Date of issue:** 2024-11-29

Author: Jan Köster **Sponsoring organization:**

Title and subtitle: Morphological and Molecular Approaches to the Diagnostics of Soft Tissue and Bone Tumors

Abstract:

Soft tissue and bone tumors (STBT) constitute a large and both morphologically and genetically heterogeneous group of tumors. This thesis aimed at improving the diagnostics of STBT by both morphological and molecular approaches.

In **Article 1** we compared the diagnostic utility of fine needle aspiration cytology (FNAC) and core needle biopsies (CNB) in a large series of primary STBT. We found that FNAC is a useful tool to differentiate between benign and maligant lesions, but it is inferior to CNB in identifying the correct entity. In a second step we proposed and tested a standardised sytem for reporting soft tissue cytopathology. In **Article 2** we evaluated the diagnostic utility of FNAC from bone lesions, both primary and secondary manifestations of other diseases, and used the data to propose and test a system for reporting bone cytopathology. We found also here that FNAC is a suitable tool to differentiate between benign and malignant entities. Various entities can be reliably diagnosed by FNAC. The results of Articles 1 and 2 are a valuable contribution to the upcoming WHO Reporting System for Cytopathology.

In **Article 3** we evaluated the diagnostic utility of DNA copy number analysis of CNBs from STBT, using single nucleotide polymorphism (SNP) array analysis. We could show, that SNP array analysis is technically feasible on limited biopsies with low failure rate. The copy number profiles were compatible with the CNB diagnoses in the majority of cases and beyond that indicative for selected tumor entities. In addition, the copy number profiles were in most cases reprentative for the whole tumor. The focus of **Article 4** was a a specific tumor entity, the dermatofibrosarcoma protuberans family of tumors, mostly driven by a *COL1A1*::*PDGFB* gene fusion. A series of 42 tumor samples were investigated with various genetic methods with the aim to get a better understanding of the chromosomal origin of the driver mutation and secondary genomic alterations. We confirmed age-associated differences in the origin of the fusion gene and that the mutation most probably occurs after DNA replication. There are recurrent chromosomal aberrations, that however not differ among tumors of different grade.

Key words: soft tissue tumors, bone tumors, sarcoma, pathology, cytology, fine needle aspiration, core needle biopsy, reporting system, genomic array, copy number aberration, gene fusion, dermatifibrosarcoma protuberans

Classification system and/or index terms (if any) Supplementary bibliographical information

Language: English **ISSN and key title**: 1652-8220

ISBN: 978-91-8021-635-7

Recipient's notes **Number of pages**: 80

Price Security classification

Signature Date 2024-10-18

Morphological and Molecular Approaches to the Diagnostics of Soft Tissue and Bone Tumors

Jan Köster

LUND
UNIVERSITY

Faculty of Medicin

Department of Laboratory Medicine, Division of Clinical Genetics

ISBN 978-91-8021-635-7

ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University

Lund 2024

Wherever we are, what we hear is mostly noise. When we ignore it, it disturbs us. When we listen to it, we find it fascinating.

-John Cage-

Table of Contents

List of Articles 9

Abbreviations 10

Introduction 11

- Tumorigenesis 11
- Diagnostics of soft tissue and bone tumors 15
- The interventional cytopathologist 18
- Genetic aberrations in soft tissue and bone tumors 25

Aims 28

Materials and Methods 29

- Study cohorts 29
- Interventional procedures and morphological analyses 29
  - Fine needle aspiration and core needle biopsy sampling 29
  - Cytology specimens 30
  - Histology specimens 30
- Genetic analyses 31
  - Fluorescence in situ hybridization 31
  - Chromosome banding analysis 32
  - Single Nucleotide Polymorphism (SNP) array 33
  - Massive parallel sequencing 35

Results and Discussion 37

- Article 1 37
- Article 2 40
- Article 3 45
- Article 4 48

Context, Conclusion and Perspective 53

- Morphological and interventional aspects 53
- Molecular aspects 55

Other Publications 57
Populärvetenskaplig sammanfattning (Svenska) 58
Populärwissenschaftliche Zusammenfassung (Deutsch) 61
Acknowledgements 64
References 68

List of Articles

1. Köster J, Ghanei I, Domanski HA. Comparative cytological and histological assessment of 828 primary soft tissue and bone lesions, and proposal for a system for reporting soft tissue cytopathology. *Cytopathology* 2021;32:7-19.

2. Köster J, Bedeschi Rego de Mattos C, Domanski HA. Reporting bone cytopathology – A proposal based on a single tertiary center experience. *Under review (Cytopathology)*.

3. Köster J, Picinelli P, Arvidsson L, Vult von Steyern F, Bedeschi Rego de Mattos C, Almquist M, Nilsson J, Magnusson L, Mertens F. The diagnostic utility of DNA copy number analysis of core needle biopsies from soft tissue and bone tumors. *Lab Invest* 2022;102:838-845.

4. Köster J, Arbajian E, Viklund B, Isaksson B, Hofvander J, Haglund F, Bauer H, Magnusson L, Mandahl N, Mertens F. Genomic and Transcriptomic Features of Dermatofibrosarcoma Protuberans: Unusual Chromosomal Origin of the COL1A1-PDGFB Fusion Gene and Synergistic Effects of Amplified Regions in Tumor Development. *Cancer Genet* 2020;241:34-41.

Abbreviations

BT	Bone tumors
CB	Cell block
CNA	Copy number aberration
CNB	Core needle biopsy
DFSP	Dermatofibrosarcoma protuberans
DPFT	Dermatofibrosarcoma protuberans family of tumors
FISH	Fluorescence in situ hybridization
FNA	Fine needle aspiration
FNAC	Fine needle aspiration cytology
HE	Haematoxylin & Eosin stain
MPS	Massive parallel sequencing
RNA-seq	RNA sequencing
ROSE	Rapid on-site examination
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variants
STBT	Soft tissue and bone tumors
STT	Soft tissue tumors
WES	Whole exome sequencing
WGS	Whole genome sequencing
WHO	World Health Organization

Introduction

Tumorigenesis

The human body is a complex organism that is composed of a variety of specialized tissues, enabling us to perform different tasks like walking, tasting or making music. In order to develop a complex organism from a single fertilized egg, embryonal cells need to have the potential to proliferate and differentiate into a myriad of different cell types. The ability of cells to proliferate, differentiate as well as to concertedly die, if needed, are crucial mechanisms; not only during embryogenesis but also in adult life in order to maintain tissue homeostasis, for tissue renewal or regeneration, e.g. in case of tissue damage.

The genetic information of a human is organized in 23 pairs of chromosomes, located in the nucleus of a cell; 22 pairs of autosomes and one pair of sex chromosomes (X and Y). Broadly speaking, DNA is transcribed into RNA, which leaves the housing in the nucleus and is translated into proteins. Proteins come in a vast variety of flavours and display both the physical backbone of a cell and the final executing molecules, defining its structure and function. Although the vast majority of cells share the same genetic information, not the whole catalogue of genes is deployed at a given time, let alone in every cell type.

To replicate itself in a complex multi-step procedure called *cell cycle*, a cell must double its constituents, among those the genome, before a complete set of chromosomes can be distributed to the two daughter cells. DNA replication is a critical process as ideally the complete and correct genetic information should be transferred to the next generation of cells. Although having to pass several check points and underlying sophisticated control mechanisms, this process is not faultless; some genetic errors are always introduced into the genome of the daughter cells. Whereas errors in DNA replication stochastically occur

intrinsically, genetic errors can also be induced extrinsically, e.g. by irradiation, chemical carcinogens or viral infections.[1]

Tumorigenesis can be understood as the result of genetic errors. The common paradigm is, that mutations accumulate in a cell over time and drive the transformation from a normal cell into a tumor cell. Mutations that are regarded as the main causal factor for the development of a tumor are often labelled as *driver mutations*. Those mutations are not randomly distributed throughout the genome but instead accumulate in mainly three categories of genes: those that positively or negatively regulate cell growth and survival (*oncogenes* and *tumor suppressor genes*) and genes that are involved in the maintenance of the genomic stability (*caretaker genes*).

Tumors manage to escape normal tissue homeostasis and grow autonomically. To accomplish this, especially malignant tumor variants share a variety of molecular and cellular traits, illustrated by Hanahan and Weinberg as the *Hallmarks of Cancer*.[2–4] The list of initially six biological capabilities in 2010 has grown ever since, reflecting both the gain of knowledge but also complexity of tumorigenesis. Most, if not all, cells depend on external growth stimuli to proliferate. Especially oncogenes take advantage of this mechanism by permanently activating mitogenic pathways and thus decouple tumor cells from the dependency of growth signals from the tissue microenvironment (*self-sufficiency in growth signals)*. Furthermore, tumor cells gain *insensitivity to antigrowth signals*, mainly by altered functions of players in the cell cycle, e.g. the retinoblastoma protein. By *evading apoptosis*, neoplastic cells elude the attempt to get disassembled after triggering the apoptotic chain intrinsically or extrinsically, e.g. by immune cells. A key regulator of apoptosis is p53 and its gene, *TP53*, is mutated in about 50% of all cancers.[5] Non-neoplastic cells are normally bound to a limited number of cell divisions. Tumor cells, however, can achieve the ability of *limitless replicative potential* by telomere maintenance and by escaping cell senescence. Furthermore, neoplasms take advantage of *sustained angiogenesis* in order to ensure oxygen and nutrition supply. This factor, among others, illustrates that tumors are complex systems that not only consist of neoplastic cells but also a variety of other components, forming a complex microenvironment with diverse cell types that interact with each other (Figure 1). Tumor angiogenesis is commonly aberrant with hyperbranched, distorted vessels and abnormal blood flow. The phenomenon of tumor

microenvironments is of special interest for the pathologist as it affects the morphology of tumor tissue. *Tumor invasion and metastasis* are acquired capabilities, that are most often linked to a malignant behaviour of the neoplasm. These are by no means trivial processes as a tumor cell needs to leave its initial environment, migrate into a lymph or blood vessel and move to a different site of the body, leave the vessel again and lastly adapt to a new microenvironment and build a new cancer colony. The necessity to adapt to a different microenvironment might be a reason why certain tumors preferentially colonise specific distant anatomic sites. The first four mentioned hallmarks of cancer are regarded as prerequisites for tumor invasion and metastasis. The occurrence of metastases indicates a systemic disease, that is often associated with a poor outcome.[6] Two hallmarks of cancer were added in 2011. One of those is *reprogramming energy metabolism* and addresses altered ways of glucose breakdown in tumors. Secondly, *evading immune destruction* has been formulated regarding the ability of neoplasms to overcome the immune system as barrier to tumorigenesis. The to date last edition of *Hallmarks of Cancer* from 2022 adds four further capabilities. *Unlocking phenotypic plasticity* describes the ability of tumor cells to follow different paths of differentiation, among those dedifferentiation into a progenitor-like state, differentiation block (and thus stay in a progenitor-like state) or transdifferentiation into alternative cell lineages. There is furthermore evidence, that tumors benefit from epigenetic changes, both in the tumor cells themselves and in recruited cells of the tumor microenvironment (*non-mutational epigenetic reprogramming*). Especially in organs that display an interface between body and environment, the composition of the local microbiome can have both tumor-protective and -stimulating effects (p*olymorphic microbiomes*). Although initially thought to be a condition, that would protect from tumorigenesis, s*enescent cells* have been shown to be able to promote tumor development by interaction with the local microenvironment.

Most probably, this list is not exhausted and additional aspects will be added in the years to come. The relevance of the *Hallmarks of Cancer* is underlined by the fact that many of its aspects can be targeted in various therapeutic approaches.[7–9]

It is supposed to be unlikely that all those capabilities are achieved by a single mutation. Thus, it has been proposed, that tumorigenesis is paralleled by *genetic*

instability and thus a higher rate of mutations that accumulate in the genome. Although tumorigenesis is commonly understood as a long-termed multi-step procedure, there are, however, also mechanisms, that can cause significant genomic alterations within a single cell cycle, e.g. chromothripsis.[10] This term describes the fragmentation, shuffling and restitching of chromosomal segments of one or few chromosomes in a single event. Being a single-step event, the concept of, among others, chromothripsis does perhaps not challenge the classical view of tumorigenesis as multi-step procedure. But it might constitute a kind of fast-forward track in tumorigenesis by introducing structural aberrations and a higher degree of genomic instability. Genomic aberrations indicating chromothripsis have been identified in 49% of all cancers in adults.[11]

The majority of neoplasms occur spontaneously following acquired genetic errors, but there are also numerous known inherited genetic aberrations that predispose to the development of neoplasms, e.g. in the form of tumor syndromes.[12]

From a diagnostic point of view, it is noticeable, that each specialized tissue with unique tasks has a characteristic macro- and micromorphology, based on the global architecture, composition of cells and extracellular matrix components. Microscopically, each specialized cell type has a unique morphology, that is caused by its cell shape and both intra- and extracellular structures. The micromorphology of whatever tissue is mirrored by the composition of active genes in the given state of the cells. This implies also that a deviation from a cell's "normal" state, e.g. in case of inflammation or a neoplasm, is mirrored by an altered cell and tissue morphology. In other words, the interdependency of genomics, transcriptomics and proteomics are essential for cyto- and histopathological examination and diagnostic of diseases.

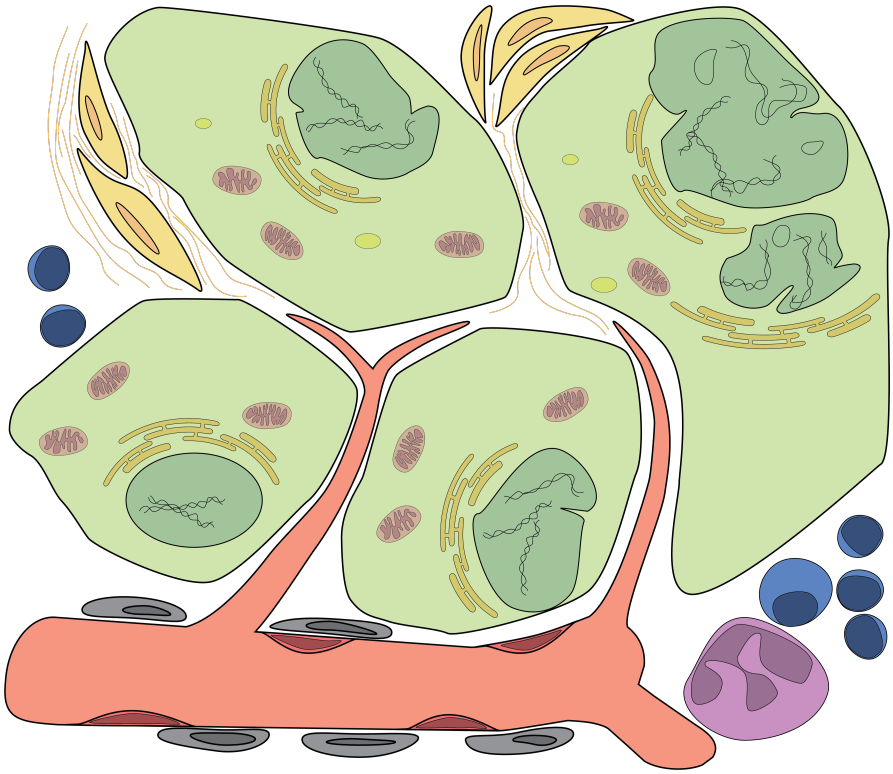


Figure 1. Tumor microenvironment
Tumor tissue consists not only of neoplastic cells (green) but also of a variety of non-neoplastic cells, exemplary depicted as fibroblasts with extracellular matrix (yellow), various inflammatory cells (blue and purple), vessels (red) and pericytes (grey) that interact with each other and form a complex microenvironment.

Diagnostics of soft tissue and bone tumors

Soft tissue and bone tumors (STBT) comprise large and heterogenous groups of neoplasms, that are thought to derive from cells of mesenchymal origin. The term mesenchymal tissue summarizes fatty and fibrous tissues, vessels, nerves but also bone and cartilage. Thus, mesenchymal tissues have mechanical tasks, ranging from the formation of skeleton and joints to supporting

parenchymatous organs with capsules and embedding glandular lobes. Vessels and nerves are responsible for the perfusion and innervation of tissues.

Benign mesenchymal tumor entities outnumber malignant tumor variants (sarcomas) by far. The true incidence of benign mesenchymal neoplasms is unknown as many do not cause any clinical symptoms and are found incidentally. According to the WHO, the annual incidence of soft tissue sarcomas (malignant mesenchymal tumors) is about 50 cases / 1 million population and thus account for less than 1% of all malignant neoplasms. Bone sarcomas have an annual incidence of 0.75 / 100 000 population.[13] In Sweden with a population of about 10 million, about 300 primary sarcomas are diagnosed annually, excluding sarcomas occurring in the abdominal cavity/retroperitoneum (https://statistik.incanet.se/OrthopedicSarcoma/). To maintain optimal care for patients with sarcomas in Sweden, the competences regarding diagnostics and treatment are concentrated to a few sarcoma centers, among those Stockholm, Gothenburg and Lund. National guidelines recommend that all deeply seated (under the level of muscle fasciae) and all large (>5 cm) superficial suspected mesenchymal tumors should be referred to a sarcoma center.

The diagnostic chain of suspected soft tissue and bone tumors is a multidisciplinary procedure. Clinical setting, radiology as well as microscopic examination are essential steps in the diagnostic chain. The recent WHO Classification of Soft Tissue and Bone Tumors counts more than 100 soft tissue and more than 50 bone tumor entities, some of those extremely rare.[13] Beyond that, non-neoplastic conditions that can mimic tumors, both clinically and morphologically. Many tumors share similar morphologic features, but a marked morphological heterogeneity within one tumor entity is not unusual (Figure 2). Thus, the pathological examination of STBT is often a diagnostic challenge, especially on limited, preoperative biopsies or cytological aspirates, as only a small fraction of the lesion can be analysed. Cyto- and histomorphological examinations are thus often aided by immunohistochemical stains to define the line of differentiation or specific cell types. Additionally, an increasing number of immune stains were introduced as surrogate markers for genetic aberrations.[14,15] However, immune profiles can be inconsistent and morphological and immunohistological approaches are

increasingly complemented by genetic analyses. Molecular characterization of STT has been shown to improve the diagnostic accuracy.[16]

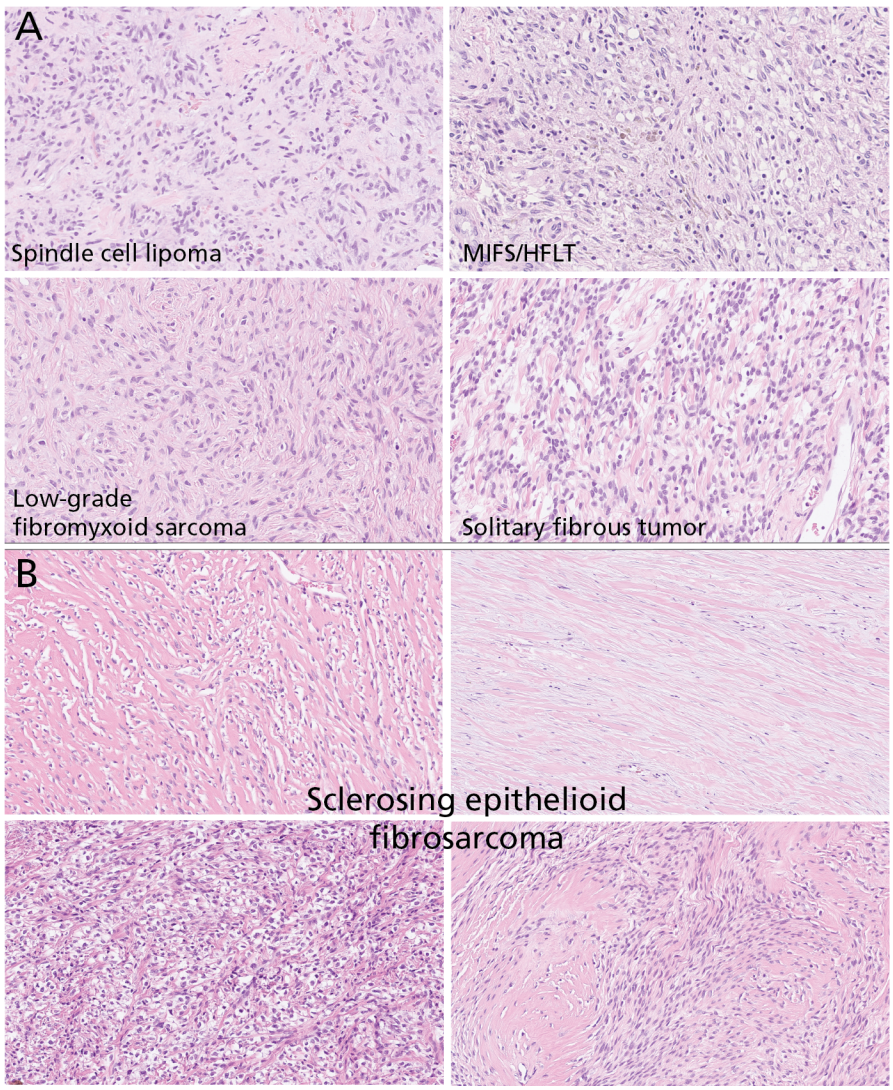


Figure 2. Morphological challenges among soft tissue tumors
(A) Exemplary depiction of four different soft tissue tumors that can show considerable morphological overlap, but vary in their biological behaviour. All tumors in these examples show often plump, mostly inconspicious spindled cells in a collagenous, partly myxoid background.

Spindle cell lipomas are benign. This myxoinflammatory fibroblastic sarcoma (MIFS)/Haemosiderotic fibrolipomatous tumor (HFLT) hybrid showed areas with only low-grade atypia. These tumors grow locally aggressive. Low-grade fibromyxoid sarcomas have a relatively high long-term risk for metastatic disease and disease-related death. Solitary fibrous tumors show a variable biological behaviour. (B) Different morphological features of one single tumor entity. The upper left picture shows the classic morphology of sclerosing epithelioid fibrosarcoma with small, partly epithelioid cells with pale cytoplasm, arranged in chords, surrounded by sclerotic stroma. However, those tumors can also show almost acellular components (upper right), nested growth of plump epitelioid cells (lower left) or components that resemble low-grade fibromyxoid sarcoma (lower right).

The interventional cytopathologist

Fine needle aspiration cytology (FNAC) from STBT played a major role in this thesis. Hence, this diagnostic tool deserves a historical round-up and closer look from a diagnostic perspective.

Cytopathological specimen types can generally be divided in two different groups: exfoliative cytology and fine needle aspiration cytology. Exfoliative cytology specimens (like pleural fluid, urine or brush samples from the bronchial tree or bile ducts) contain by nature mainly cells and material from the surface they released from, either spontaneously or by manipulation (washings, scraping, brushing). In contrast, FNAC specimens are obtained by a puncture procedure with a needle into the region of interest. It can contain everything that the sampled lesion consists of, liberated from the tissue and retrieved for diagnostics by aspiration/capillary action, together with the movement of the introduced needle. The diagnostic yield can contain single cells, cell groups, extracellular matrix components, fluids and sometimes even small tissue fragments, maintaining the original tissue architecture. The diagnostic challenge when interpreting FNAC is to reassemble those reshuffled tissue components into a coherent picture, not unlike small stones in a mosaic (Figure 3). Hence, knowledge of the histopathological morphology is no disadvantage when examining FNAC material.

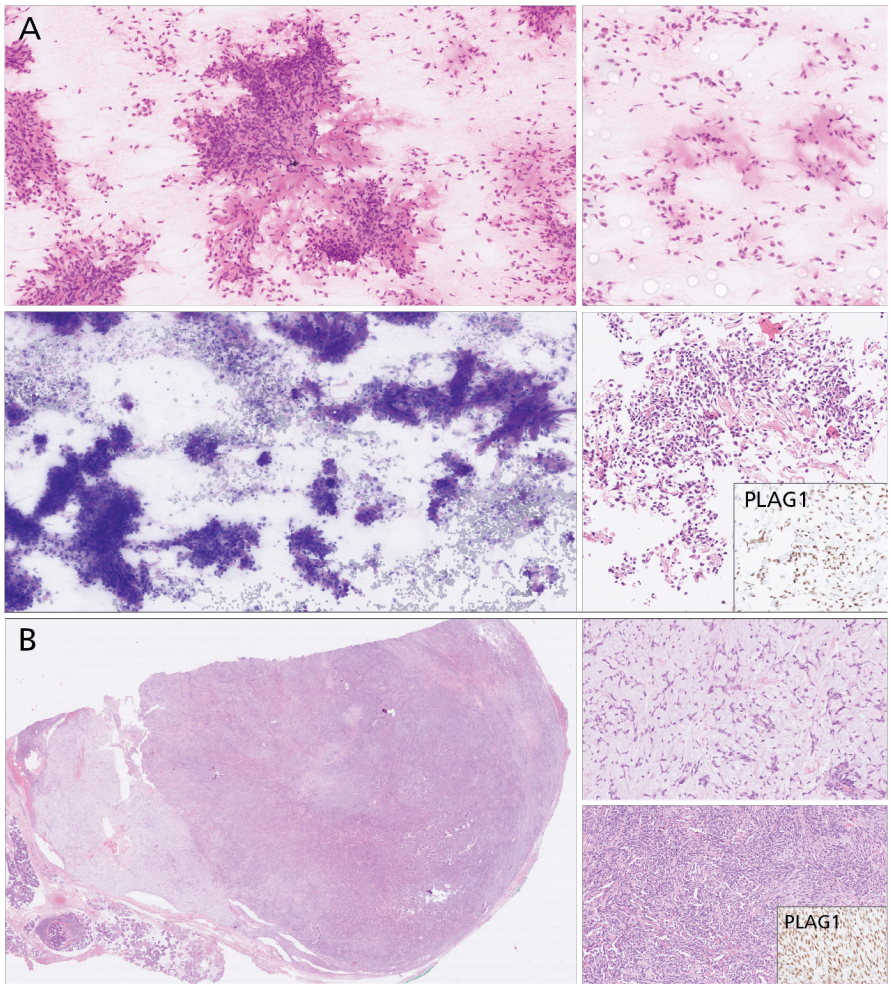


Figure 3. Fine needle aspriation cytology and its corresponding histopathology
(A) Fine needle aspiration cytology of a parotid mass showing a pleomorphic adenoma. The smear preparations with both Hematoxylin&Eosin stains (upper panel) and Giemsa stain (lower left) show a varying cellularity with both tissue fragments and dissociated cells. Those show partly spindled, partly plasmocytoid features without atypia.There are areas that are dominated by fibrillar extracellular matrix, that is better visualized by Giemsa staining. Cellblock preparation (lower right) with a similar appearance and a positive PLAG1 immunostain, that supports the diagnosis. (B) Corresponding surgical resection with whole tumor section (left) showing both a paucicellular, matrix-rich and a cell-rich, matrix-poor component. Those tumor components are highlighted in the right panel, with additional positive PLAG1 immunostain.

The very first reports of puncture procedures with hollow needles can be traced back to Arab medicine in the medieval period and were performed by the influential physician Abū al-Qāsim Khalaf ibn al-´Abbās Al-Zahrāwī (936-1013). He illustrated the therapeutic use of hollow needles of unknown dimension in thyroid tumors, that he called the "elephant of the throat".[17]

However, it was first in the late 19th and early 20th century, that material obtained by FNA was more widely used for diagnostic purposes. Light microscopes were already known since the Renaissance. But the ability to perform detailed microscopic analyses was linked to another development: the invention of appropriate staining techniques. Not the only, but undoubtedly one of the central figures in this context was the chemist and physician Paul Ehrlich (1854-1915). In the beginning of his career, he experimented with a variety of chemicals, especially dyes developed for the textile industry, and tested those on tissue and blood samples. He discovered that different cells and cell components interacted with chemicals in different ways and could thus not only be made visible but, more importantly, distinguishable.[18] As a side note, it should be mentioned, that the discovery of chemicals, selectively targeting specific structures, was further developed and refined in Ehrlich's research in various theories and concepts with huge impact on various medical disciplines, among those haematology, immunology and oncology ('chemotherapy').[18,19] Ehrlich was honoured for his work with the Nobel Prize in Physiology or Medicine in 1908.

In the first two decades of the 20th century there were numerous reports about the diagnostic use of material obtained by needle aspiration, however largely without the claim to evaluate the technique scientifically. The sampled lesions were initially mostly lymph nodes and cutaneous tumors, but later even abdominal organs like liver and spleen.[17]

Most authors agree that Hayes Martin and Edward Ellis from New York published in this context one of the most influential papers in 1930 on a series of 65 cases approached by FNA as diagnostic procedure.[20] By utilizing this method, the authors, a head & neck surgeon and a laboratory technician, aimed to meet the contraindications for "biopsy by surgical exposure", that were besides the general risks for surgery (infections and bleedings) the acquisition of diagnostic material from less assessable, deep-seated lesions and the risk for local dissemination of tumor tissue. They performed local anaesthesia, incised

the skin with a scalpel and performed the FNA with a comparably thick 18-gauge needle, attached to a 20-ml syringe. The authors describe the use of vacuum and the needle movement, that is needed to secure diagnostic material ("Aspiration with the needle at rest is not sufficient to draw tissue into the needle in most cases."). Although the paper perfectly describes the technical principles of FNA as it is used today, the preparation with local anaesthesia and incision of the skin resembles more the procedure that is nowadays applied for taking core needle biopsies. It was described that the obtained material regularly contained larger fragments of tissue, that were fixated in formalin and treated as biopsies.

One year after Martin and Ellis's first publication on FNA, the physician Ernst Mannheim published a comparable work, based on 43 patients with palpable tumors, approached by FNA.[21] The view of various authors, that it was the German Mannheim who should be honoured for being the first who used a thin needle for punctures that deserves the term *fine needle aspiration*, is entertaining.[17,22] Mannheim was aware of Martin and Ellis's work but described their work as "heroic" as they incised the skin and made use of a very thick needle ("einen sehr starken Troikart"). Furthermore, he expressed his worries, that the risk of a local spreading of the tumor with such a thick needle should not be underestimated. Curiously, when reading the original manuscript, written in German, one can notice that he describes the use of a needle with a 1 mm diameter in his own work. This is not much thinner than Martin's and Ellis's 18-gauge needle (corresponding to 1.2 mm). At least, Mannheim proved, that FNAC could be performed without local anaesthesia and skin incision.

Although early scientific evaluation of FNAC originates from America, the method remained here relatively unpopular in daily diagnostic routine. In Europe, however, and especially in Sweden, it was adopted already in the late 1940s and successively became an accepted diagnostic tool in the decades to come. Sixten Franzén introduced FNAC at the Karolinska University Hospital in Stockholm in the late 1940s.[23] Being haematologist in the first place, he was initially mainly interested in bone marrow smears but soon shifted his attention to solid tumors as well. Together with (among others) the pathologists Josef Zajicek and Torsten Löwhagen, he formed a scientific collaboration, that established itself as *The Karolinska Group*.[24] In the 1960s and 70s, this group

published several scientific works on FNAC with large study cohorts.[25–27] In many of the publications, the cytological findings were supported by histological follow-up which emphasized the diagnostic power of FNAC and silenced critics of the method of being much inferior to histological samples. The Karolinska Group also developed hardware devices, e.g. a needle guide for the sampling procedure of the prostate. Syringe holders that allowed one-handed FNA operation, were widely utilized at this time, as they are still used today (Figure 4). The Karolinska Group was internationally recognized not only due to their scientific output, but also due to teaching activities (e.g. in the USA), the first monograph textbooks on FNAC and the first international course in FNAC, that was held in Stockholm in 1970.[24,28] The popularity of FNAC in Scandinavia spread to different parts of the world, with varying resonance.

Figure 4. Palpation-guided fine needle aspiration.
Sampling of a subcutaneous mass on the shoulder. Syringe holder with attached 10-ml syringe and standard 23-gauge needle. *The photograph is used with permission of the patient.*

Simultaneously with the Karolinska Group, although less visible in the scientific world, the internist Nils Söderström introduced FNAC at the University Hospital in Lund.[23] In the early 1960s the pathologist Nils Stormby established the puncture service, driven by the department of pathology. Soon after, the pathologist Måns Åkerman joined. Together with the orthopaedic surgeon Anders Rydholm, he established FNAC as primary diagnostic tool for STBT in local sarcoma care in the early 1970s.[29–31] FNAC of STBT became the main scientific focus of Åkerman and his successor, my colleague and co-supervisor Henryk Domanski.[32–35] They also introduced the utilization of palpation-guided core needle biopsy sampling, simultaneous to FNAC in daily diagnostic routine, performed by the pathologist (Figure 5).[36]

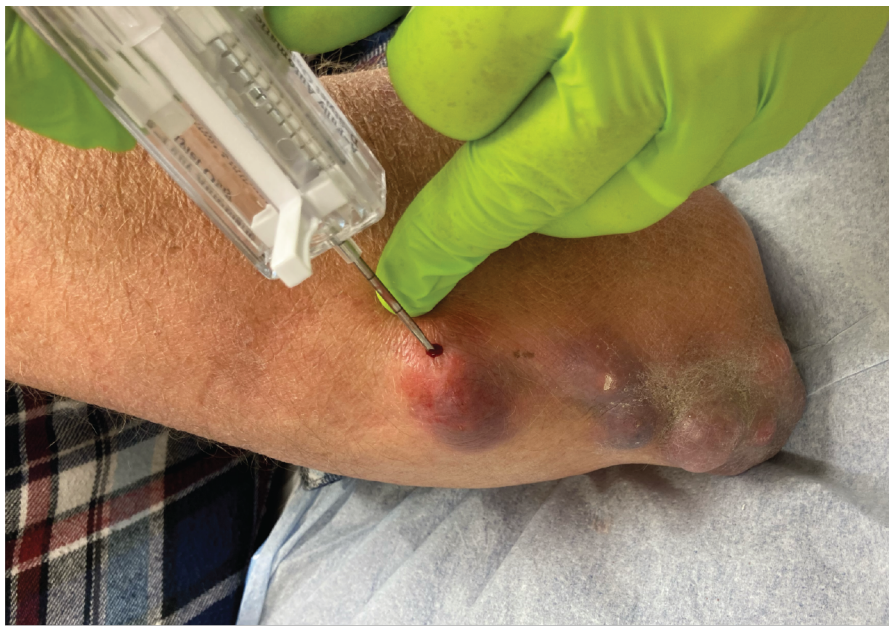


Figure 5. Palpation-guided core needle biopsy sampling
Sampling of a subcutaneous lesion close to the elbow with a 14-gauge needle. *The photograph is used with permission of the patient.*

Already Martin and Ellis reported the first cases of FNAC sampling from soft tissue and bone lesions in their publication from 1930, extended to a larger cohort some years later by Fred Stewart, who adopted the method proposed by

Martin and Ellis.[37] Although numerous reports and scientific studies regarding FNAC of STBT accumulated over the years, originating from different parts of the world, it still remains an unusual application in daily diagnostic routine until today.

One important development in the application of FNAC of palpable masses in Scandinavia was, that it rapidly moved into the hand of the pathologist and thus much closer to the patient. This new discipline would be established as *interventional cytopathology*.[23,38] Sampling by the cytopathologist and thus the diagnostician himself has several advantages. One obvious benefit is that the executing physician is in direct contact with the patient and can enquire anamnestic data and communicate during the examination. Furthermore, it is favourable also from a diagnostic perspective, to get a clinical impression of the sampled lesion; apart from the size also where it is exactly located, to what extent it is movable, the palpatory quality and potential reactions of the surrounding tissue. An additional and very essential advantage is the possibility of rapid on-site evaluation (ROSE) of the diagnostic yield. This means, that a cytological smear or imprint preparation is directly stained and examined under the microscope. ROSE is also regularly performed, when the cytopathologist is not executing the sampling her/himself, but is attending a sampling procedure, performed by, e.g. a radiologist or a surgeon. ROSE procedures are also possible on distance via an automated microscope with video link. At Skåne University Hospital in Lund, ROSE of endobronchial ultrasound-guided fine needle aspirations of mediastinal lymph nodes are routinely performed by cytotechnicians.[23] ROSE is not solely a possibility to verify the representativity of the diagnostic material but also to triage the diagnostic material for further analyses, that could be important for the diagnostic work-up. Important examples are flow cytometry in suspected lymphomas, material for cellblock preparations for metastatic disease or tissue for genetic analyses for the diagnostics of e.g. STBT. ROSE is integrated in our workflow both in our FNA clinic and in various external settings (Figure 6).

Figure 6. Trolley for external rapid on-site evaluation procedures

Genetic aberrations in soft tissue and bone tumors

The morphological variety among STBT is reflected by an extensive catalogue of genetic aberrations. Those range from small genetic variants over fusion genes to numerical and structural chromosomal aberrations of varying complexity.

Small genetic variants encompass mutations of single nucleotides (single nucleotide variants, SNV) to insertions/deletions (Indels) of up to 10.000 nucleotides. The impact of those mutations might be difficult to predict, as they occur in the genome of neoplasms as well as in constitutional DNA, both in coding and non-coding regions. SNVs and indels in coding genomic regions can lead to altered amino acid sequences or truncated proteins. In addition, Indels can be responsible for frameshifts. In contrast to other solid tumors like

carcinomas, small genetic variants as driver mutations are uncommon in sarcomas. One prominent exception are gastrointestinal stromal tumors (GIST). Those neoplasms are in the majority of cases driven by *KIT* and *PDGFRA* oncogene mutations, encoding for receptor tyrosine kinases.[39,40] Those mutations provide therapeutic targets, as many GISTs respond to the tyrosine kinase inhibitors. Furthermore, the mutation status in GIST has prognostic and predictive relevance. *KIT*-mutated tumors tend to be clinically more aggressive than neoplasms with *PDGFRA* mutations or wild-type GISTs.[41]

About one-third of STT harbour gene fusions as the result of intra- or interchromosomal rearrangement of genomic material.[42] The juxtapositioning of two otherwise unrelated genes or parts of genes may lead to chimeric proteins, altered gene expression or truncation. Some gene fusions are regarded as passenger mutations, less important for tumorigenesis and more a sign of genetic instability. Others constitute strong driver mutations, especially in tumors that otherwise lack genetic aberrations.[43] The majority of gene fusions among STT engage a transcription factor (e.g. *EWSR1*::*FLI1* in Ewing sarcoma or *NAB2*::*STAT6* in solitary fibrous tumors) or a gene coding for a tyrosine kinase (e.g. *ETV6*::*NTRK3* in infantile fibrosarcoma) as one of the fusion partners. STT with gene fusions involving tyrosine kinases may be, like other tumors with altered tyrosine kinase activity, susceptible to therapies with kinase inhibitors.[44] Recurrent translocations can be useful diagnostic tools. However, a certain fusion gene can be detected in various different tumors. One well known example is the above mentioned *ETV6*::*NTRK3* fusion gene, that has not only been identified in infantile fibrosarcomas, but among others also in acute myeloid leukemia, secretory carcinoma of various sites (among those breast and salivary glands) as well as in adenocarcinomas of the colon and thyroid.[42] This illustrates that identified mutations need to be interpreted in a clinical and histomorphological context, when used for diagnostic purposes.

Instead, or in addition to small genetic variants and gene fusions, soft tissue and bone tumors often display numerical or structural chromosomal aberrations. Numerical changes include altered ploidy levels with loss or gain of either the whole chromosome set or single chromosomes. The former is strongly associated with malignant tumor entities.[42] As numerical aberrations affect a vast number of genes, the pathogenic effect is difficult to estimate.

Generally though, the gene expression levels tend to follow the number of chromosomes.[45] In some neoplasms, recurrent numerical changes are unlikely to affect tumorigenesis, like trisomy 8 and/or 20 in desmoid fibromatoses. This tumor entity is driven by point mutations in the β-catenin gene *CTNNB1*.[46] In contrast, complete or partial loss of one chromosome 13 in spindle cell lipomas seems to be sufficient for tumorigenesis.[47]

Gene amplifications are structural aberrations, loosely defined as at least a three to fivefold copy number gain, that play an important role in tumorigenesis.[48] One example are amplicons on chromosome arm 12q, harboring *MDM2* and *CDK4*, in parosteal osteosarcomas, genetically undistinguishable from the extensively described genomic alterations in well-differentiated liposarcomas.[49,50]

Homozygous deletions are structural aberrations that can promote tumorigenesis, typically by inactivating tumor suppressor genes. As an example, multiple solid tumors were described with biallelic deletion of *SMARCB1* on chromosome arm 22q, among those several sarcomas like epithelioid sarcomas and extrarenal rhabdoid tumors.[51,52]

Aims

The general aim of the thesis was to improve the diagnostics of soft tissue and bone tumors with immediate impact on patient care. The different research projects followed two main approaches:

1. Improving *morphological analyses* by evaluating the diagnostic use of fine needle aspiration cytology for soft tissue and bone lesions and by developing standardised reporting systems (Articles 1 and 2)
2. Evaluation of *genetic approaches* that can support morphological analyses in the diagnostic process (Article 3).

   Article 4 aimed at obtaining a better understanding of the spectrum and origin of genetic aberrations in one specific group of sarcoma entities, the dermatofibrosarcoma protuberans family of tumors.

Materials and Methods

Study cohorts

The different studies included in this thesis are based on diagnostic cases of patients and patient material, referred the Department of Clinical Genetics, Pathology and Molecular Diagnostic within the section of Laboratory medicine Skåne in Lund (Article 1), respective different branches within this department, located in Lund, Malmö, Helsingborg and Kristianstad (Article 2). Articles 3 and 4 are based on tissue samples from patients referred to the sarcoma centers at Skåne University Hospital in Lund as well as to the Karolinska Hospital, Stockholm.

Data and samples in the different studies originated from varying time periods between 1985 and 2023 and were approved by the national ethical review board.

Interventional procedures and morphological analyses

Fine needle aspiration and core needle biopsy sampling

FNA and CNB sampling was either performed palpation-guided by cytopathologists in the FNA clinic or imaging-guided in the radiology departments. A minority of sampling procedures were performed by cytopathologists in the operation theatre together with orthopaedic surgeons with fluoroscopy-guidance.

For FNA, two to six puncture passes were executed without local anaesthesia, using 22-27-gauge needles (diameter 0.4-0.7 mm) on 10-ml disposable syringes on a syringe holder to enable one-handed FNA operation. CNB sampling required local anaesthesia and was carried out using 18-12-gauge needles (diameter 1-2 mm). Radiologists tended to incise the skin prior to the sampling procedure while pathologists did not.

Cytology specimens

Smear preparations were either air-dried or fixated in 96% ethanol. Air-dried preparations were stained with May-Grünwald Giemsa and ethanol fixated preparations were stained with Hematoxylin&Eosin (HE, Figure 3A). For ROSE, air-dried smear preparations were Diff-Quik stained, which belongs, like Giemsa stains, to the Romanowsky-type stains, with comparable morphology. Diff-Quik stains can be prepared in less than a minute.

The needles were often rinsed in various fluids for additional analyses or preparations. FNA material in CytoLyt solution was used for automated cell block preparations (CB, Figure 3A). CBs can be treated like formalin-fixated paraffin-embedded (FFPE) specimens using routine protocols. In case of a suspected lymphoma or plasma cell neoplasia, the needles were rinsed in a buffer that could be prompted to flow cytometry. Occasionally, FNA material was sent to the microbiology department for cultivation.

Histology specimens

CNB specimens, surgical biopsies and resection specimens were fixated in 4% buffered formaldehyde between 12-24 hours, depending on the specimen size. After the grossing, the tissue fragments were embedded in paraffine blocks that were sectioned and routinely stained with HE. Immunohistochemistry followed routing protocols with commercial antibodies.

FNAC was the main method in Articles 1 and 2. CNB sampling was also performed for both articles, however a detailed comparison of FNAC and CNB results was a purpose only in Article 1. CNB as main source material was used

in Article 3. Histological examinations in general were pivotal in all four articles for diagnostic purposes.

Genetic analyses

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) is a cytogenetic method that utilizes fluorescence-stain labelled DNA probes in order to visualize specific genetic targets. Those can be whole or parts of chromosomes or specific genes/loci. Both cytologic and histologic material in both fresh and formalin-fixed paraffin-embedded (FFPE) condition is assessable by this method. In diagnostic routine, FISH analysis is a well-established tool and frequently used to detect gene translocations and amplifications (Figure 7). A disadvantage, at least when using break-apart probes, is that only a rearrangement of the targeted gene is detected, but information about the fusion partner is not provided. If this information is of interest, reverse-transcriptase polymerase chain reaction (RT-PCR) or RNA-sequencing could be alternatively or additionally used.

In Articles 1 and 2, FISH analyses were used as part of the daily diagnostic routine on both cytological material and histological slides, using commercial probes against the *ALK*, *BCL2*, *EWSR1*, *FOXO1*, *FUS*, *HER2, MDM2*, *MYC*, *ROS1*, *SS18* and *USP6* loci. Metaphase FISH analyses were performed in Article 4 in order to detect abnormal chromosomal structures and *PDGFB* rearrangement.

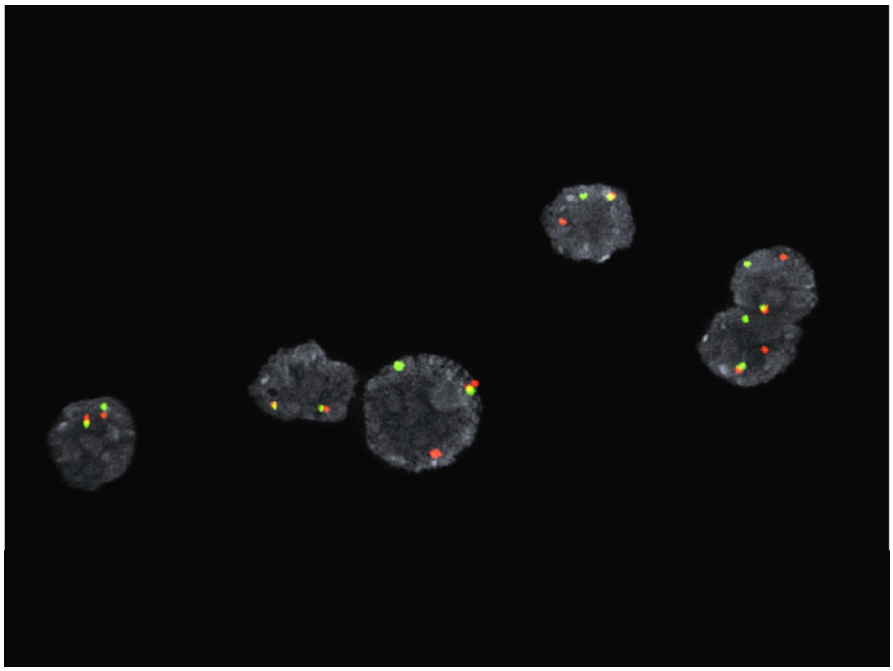


Figure 7. FISH analysis targeting the *EWSR1* locus
Interphase FISH analysis in a case of Ewing sarcoma, in the majority of cases driven by a *EWSR1*::*FLI1* gene fusion through a t(11;22). The *EWSR1* rearrangement is indicated by a split signal in the nuclei (separation of the red and green fluorescent signals)

Chromosome banding analysis

In the metaphase of the cell cycle, the chromosomes are maximally condensed and can be examined microscopically. After staining, a characteristic pattern of chromosome bands is visible. Those bands together with the size and shape of the chromosomes are characteristic for each individual chromosome and allow the identification of both numerical changes and structural aberrations with a relative low resolution of about 5-10 Mb (Figure 8). An advantage of chromosome banding analysis is the possibility to visualize abnormal chromosomal structures like ring chromosomes or double minutes, as well as to assess the ploidy level and inter-cellular variation. In addition, and in contrast to genomic arrays, balanced translocations can be detected.

Chromosome banding analysis was performed in Articles 1 and 2 in a small portion of cases as part of the daily diagnostic routine. The method was used in Article 4 to describe the spectrum of karyotypes in the dermatofibrosarcoma protuberans family of tumors.

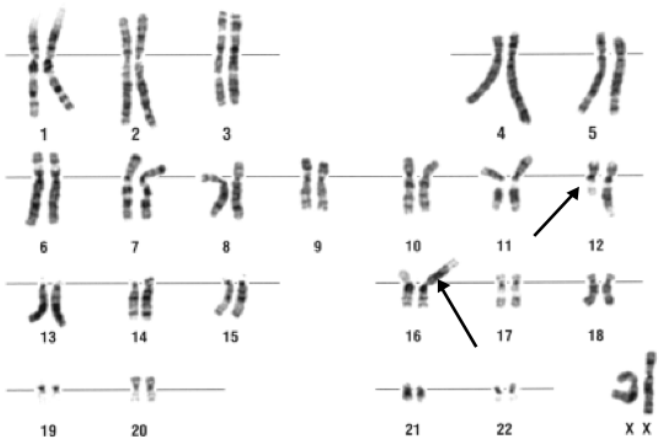


Figure 8. Karyotype of a myxoid liposarcoma with t(12;16)
Most myxoid liposarcomas are genomically characterized by a t(12;16), leading to a *FUS*::*DDIT3* fusion gene. The translocated chromosomal parts are indicated by the two arrows.

Single Nucleotide Polymorphism (SNP) array

SNP array analyses are used to detect genomic and allelic imbalances. SNPs are single nucleotide variations occurring in >1% of the population and are widely distributed throughout the genome. In modern array assays, more than 2 million probes can be targeted by specific DNA probes, covering the whole genome with a resolution of <100 kb. Fragmented DNA samples are allowed to bind to complementary probes, that are immobilized on a surface. The emitted fluorescence signal of each probe can be measured and corresponds to the amount of hybridized DNA. This signal is normalized against the average signal and log2 transformed, reflecting the copy number. A diploid count ideally equals 0. Deviations from the average copy number are interpreted as genomic gains and losses (Figure 9). Constitutional copy number variations can be excluded through comparison with the Database of Genomic Variants

(http://projects.tcag.ca/variation/). As the composition of SNPs show interindividual differences, even information about allelic distribution can be extracted. This allows the identification of loss of heterozygosity (LOH), including copy number neutral LOH, which, besides being diagnostically interesting, also can be used to estimate the ploidy level in the analysed sample.

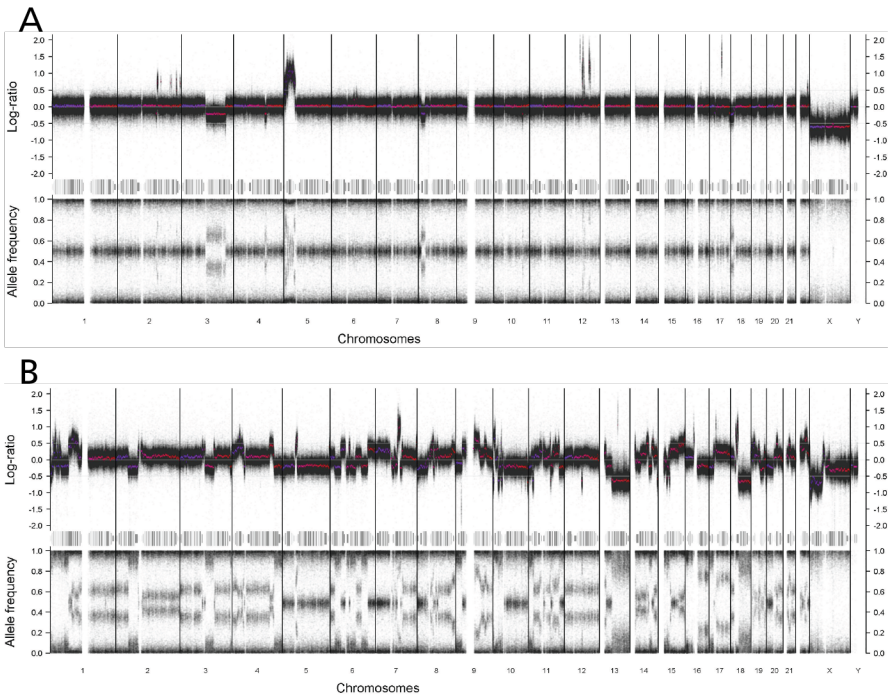


Figure 9. Single nucleotide polymorphism (SNP) array
Whole genome view of SNP array results from a dedifferentiated liposarcoma (A) and an undifferentiated pleomorphic sarcoma (B). Relative copy numbers are depicted in the upper graph and the allele frequency in the lower graph, respectively. (A) The dedifferentiated liposarcoma shows a dipoid stem line with amplified regions on chr 12 (including *MDM2* and *CDK4*). The depicted tumor shows additional copy number aberrations on several other chromosomes. (B) This undifferentiated pleomorphic sarcoma shows a non-diploid stem line and complex copy number shifts, involving the majority of the chromosomes.

One main drawback of SNP array analysis is that it fails to detect balanced chromosomal aberrations. However, in case of unbalanced translocations, copy

number shifts in or near the fusion partners can often be registered. The underlying cause of genomic imbalances cannot be defined by SNP array analysis. An additional disadvantage is, that the quality of the results is affected by contamination of non-neoplastic cells.

SNP array assays were used in all articles in this thesis. In Articles 1 and 2, the method was used as a diagnostic tool in selected cases in daily diagnostic routine. In Article 3 it was the main method, evaluated for its eligibility as diagnostic tool in STBT. In Article 4 the method was used to identify potential secondary genomic aberrations.

Massive parallel sequencing

The term *massive parallel sequencing* (MPS) or *next generation sequencing* covers several approaches, that make it possible to sequence multiple DNA or RNA fragments in a single analysis. An analysis can be restricted to selected regions of interest or cover the whole genome or transcriptome and, depending on the chosen method, can detect single nucleotide variants, indels, fusion genes as well as gene expression and copy number profiles. With sufficient reading depth, even rare, subclonal variants are traceable.

Targeted gene panels and RNA-sequencing (RNA-seq) are currently the methods that are most widely used as molecular-pathologic tools in diagnostic routine. Commercial gene panels of varying size (20-4,000 genes), designed to work with DNA and/or RNA, are frequently tailored to work with a particular group of neoplasms. The detection of small genetic variants, gene amplifications and fusions provide both diagnostic and predictive information. While predictive testing is nowadays a standard procedure for many solid tumors, e.g. lung carcinomas to identify targets for specific chemotherapy, it is still only rarely performed in sarcoma pathology, with the already mentioned GISTs as one of few exceptions.[53,54]

RNA-seq is commonly used to identify gene fusions. In diagnostic routine though, the use of RNA-seq is often restricted to challenging cases, that cannot be solved solely by morphologic approaches and FISH analysis. Provided that the analysis is run with sufficient reading depth and the RNA is of sufficient

quality, RNA-seq potentially detects all gene fusions in a sample. This includes unusual or even unknown variants, that are not covered by e.g. gene fusion panels. Besides detecting gene fusions, the information collected by RNA-seq can be used to generate gene expression profiles that could serve as signatures for particular tumors or groups of neoplasms. Such an approach is of particular clinical interest as it might not only provide diagnostic support but could also identify differentially expressed genes, that are of prognostic relevance.[55]

Other variants of MPS are whole exome sequencing (WES) and whole genome sequencing (WGS), the former targeting the coding part of the genome and the latter the entire genome. By WES, poorly explored small genetic variants, not covered by e.g. gene panels, can be analysed. Apart from exonic mutations, WGS detects also mutations in non-coding DNA, e.g. in promotor regions, and provides information about large-scale chromosomal aberrations. Both WES and WGS are still expensive methods that produce large data sets that require an extensive bioinformatical workup. To date, WES and WGS are performed only in selected cases for diagnostic purposes, e.g. childhood cancer. At least in Scandinavia WES/WGS are mainly used as scientific tools. However, this may quickly change, and especially WGS is utilized as a diagnostic tool on a larger scale in various countries, e.g. in Great Britain. This development has recently also reached Sweden and WGS as routine diagnostic tool is currently discussed also here.

In Articles 1 and 2, MPS-based gene panels, WGS and RNA-seq were performed in selected cases for predictive and diagnostic purposes. In Article 4, selected cases of dermatofibrosarcoma protuberans were analysed with gene panels, RNA-seq and WES.

Results and Discussion

Article 1

This project elucidated the morphologic part in the diagnostic chain of soft tissue tumors. We evaluated and compared the diagnostic utility of fine needle aspiration cytology (FNAC) and core needle biopsies (CNB) of 828 primary soft tissue and bone tumors from the archives of an 11-year period at the sarcoma center in Lund. Unlike many other sarcoma centers, FNAC is used in Lund as a primary diagnostic tool for soft tissue and bone tumors. The idea of comparing the diagnostic utility of FNAC and CNB is not new and has been examined by many groups, but our study includes, to our knowledge, one of the largest cohorts of primary soft tissue and bone lesions. The mean follow-up time was 57 months (range 0-171 months).

FNA cytology correctly identified malignant lesions in the majority of cases with a sensitivity and specificity of 87% and 89%, respectively. Although most authors report sensitivity and specificity values of around 90%, the variance across the different studies is very high, ranging from 25%-100%.[56–59] The reasons for this enormous variation are difficult to address retrospectively. Possible explanations are heterogeneous study populations, i.e. mixed mesenchymal and non-mesenchymal lesions including metastatic disease and lymphomas, varying use of ancillary techniques and probably varying expertise. The diagnostic accuracy, here defined as the possibility to define the correct histopathologic entity, was in our study 55% for all cases with surgical follow-up. This accounts for 88% of the cases, where a specific diagnosis rather than a descriptive diagnosis was provided by FNA cytology. Others reported diagnostic accuracies between 33%-93%.[60–62] Reasons for this high variation might include the same as described above.

The sensitivity and specificity of CNB analyses were 94% and 95%, respectively. Hence, CNB examination was slightly superior to FNA cytology in differentiating between benign and malignant lesions. Comparable results were reported in other studies with sensitivity/specificity values ranging between 72%-94%.[60,63] Moreover, CNB diagnoses are more accurate than FNA analyses when it comes to identifying the correct histopathologic entity; the accuracy in our hands was 66% of all CNB diagnoses with surgical follow-up, accounting for 90% accuracy for all CNB diagnoses, providing a specific histopathologic entity. Comparable analyses by others yield accuracy values between 45% and 92%.[60,63]

Although the global results show that CNB diagnoses were superior to FNA diagnoses in the calculated parameters, the former was not faultless. Both FNA and CNB analyses resulted in false positive (FP) and negative (FN) diagnoses, with 2% FN and 1% FP FNA diagnoses and 1% FP and 1% FN CNB diagnoses. Two main groups of neoplasms could be identified that caused most of the diagnostic errors. Firstly, spindle cell tumors with no or low-grade atypia can be challenging on both FNA and CNB biopsies, because of the shared morphologic features of both benign and malignant entities. The former included in our hands benign nerve sheath tumors, fibrous histiocytomas and myxomas; misdiagnosed malignant entities were malignant peripheral nerve sheath tumors, low-grade fibromyxoid sarcomas and dermatofibrosarcoma protuberans. The diagnostic difficulties among low-grade spindle cell tumors are known pitfalls that have been described before.[34,64–66] Secondly, another group that caused diagnostic errors were well-differentiated lipomatous tumors. The morphologic differentiation between lipoma and atypical lipomatous tumor is especially challenging or even impossible on FNA material when atypical spindle cells or lipoblasts are missing. Regressive changes/ histiocytic reactions can even cause FP diagnoses.[67,68] Genetic analyses (FISH and cytogenetics) were applied only in a fraction of cases (4% of both FNA and CNB specimens) and were thus not the main focus in this project. FISH analyses targeting *MDM2* have in our series not been performed on FNA material but could improve the identification of atypical lipomatous tumors.[69]

We used the data set to propose and test a reporting system for soft tissue FNA as a first attempt to standardize the communication between pathologists and other disciplines involved in the diagnostics and treatment of patients with

suspected soft tissue lesions. Comparable reporting systems have in the last decade been successfully established in various diagnostic fields, including thyroid and salivary glands.[70,71]

Our proposal implied six categories, including (I) non-diagnostic, (II) non-neoplastic, (III) atypia of unknown significance, (IVa) benign neoplasms, (IVb) neoplasms of unknown malignant potential, (V) suspicious for malignancy and (VI) malignant. To keep the material as homogeneous as possible, only soft tissue lesions were considered in this analysis. After distribution of the cases to the different categories, the fraction of truly malignant entities in each were calculated. The results are summarized in Table 1. As 42% of the cases in category I turned out to be malignant, resampling of non-diagnostic FNA yields should be encouraged. Both the non-neoplastic and benign neoplastic categories included none (II 0%) or only few malignant tumors (IVa 3%). The vast majority of the cases in the malignant categories were truly malignant (V 72%, VI 97%). According to our results, categories II, IVa, V and VI could, in conjunction with the clinical setting, be linked to treatment recommendations. The heterogeneity of soft tissue tumors in our proposal is mirrored by the intermediate categories III and IVb, with malignancy frequencies of 46% (III), respectively 27% (IVb). Although those categories are often not directly useful for the clinician, they help to keep the benign and malignant categories as well-defined as possible. The benefit of this approach has been proven in other reporting systems, dealing with a heterogeneous diagnostic field, e.g. salivary gland lesions.[71]

Table 1. Proposed reporting system for soft tissue cytopathology (ROM in grey shade)

	I. Non-diagnostic	II. Non-neoplastic	III. Atypia	IVa. Neoplasm benign	IVb. Neoplasm UMP	V. Suspicious for malignancy	VI. Malignant
n (of total)	24 (3%)	66 (9%)	11 (2%)	339 (46%)	70 (10%)	32 (4%)	190 (26%)
n (within category)							
Dx Benign	14 (58%)	66 (100%)	6 (54%)	329 (73%)	51 (73%)	9 (28%)	5 (3%)
Dx Malignant	10 (42%)	- (0%)	5 (46%)	9 (3%)	19 (27%)	23 (72%)	184 (97%)
Dx UMP	-	-	-	1 (0.3%)	-	-	1 (0.5%)

Abbreviations: ROM= risk of malignancy, UMP=tumor of unknown malignant potential, Dx=final histopathological diagnosis

In this article we focused only on soft tissue and bone cytopathology. Although this might be interesting in a scientific context, daily diagnostic routine is, of course, much more complex. Evaluating soft tissue or bone lesions from a clinical point of view obviously does not only include primary tumors but also manifestations of other diseases, like metastases or hematologic malignancies.

In conclusion, we show that FNA cytology is a suitable tool to detect malignant soft tissue or bone lesions, but it is inferior to CNB when it comes to identifying the correct histopathological entity. A standardised reporting system for soft tissue cytopathology could improve the clinical management of patients with soft tissue lesions.

Recently, the WHO has published the first two volumes of a new diagnostic series, the WHO Reporting System for Lung Cytopathology and Pancreatobiliary Cytopathology.[72,73] Soft tissue cytopathology will be part of this series, but is still unpublished.

Article 2

This article is thematically related to Article 1, in which a reporting system for soft tissue tumors was proposed. Article 2 aimed at the development of a reporting system for bone cytopathology. The retrospective study based on 721 cases from 717 patients with bone lesions, that were approached by cytopathology in a nine-year period between 2015-2023 in Region of Skåne. The cases were retrieved from the archives of the Department for Clinical Genetics, Pathology and Molecular Diagnostics. Contrary to Article I, not only primary bone lesions but also secondary involvement of the bone, like metastatic disease, was considered. Various ancillary analyses were applied on cytology material, including immunohistochemistry on CB preparations (24%), flow cytometry (20%) and genetic analyses (mainly FISH analyses, 2%). Histological follow-up was available in 87% of the cases. The mean follow-up time was 31 months (range 0-96 months).

The study included cases with a variety of different conditions, covering metastatic disease, primary bone tumors, bone engagement of hematopoietic tumors and non-neoplastic conditions. Metastatic disease constituted more

than 40% of the largest group (n=299), followed by various benign and malignant primary bone tumors (26%, n=188). Importantly, in about 20% of the cases (n=149), various non-neoplastic conditions were sampled. While writing this summary, the article is still under review, but it can be expected that this might be an issue for many reviewers. The problem is the question of representativity. How certain is it, that a diagnostic yield on cytology with solely normal bone and bone marrow components is representative for a lesion, discovered by imaging? In our opinion, those sampling results should not be haphazardly regarded as non-diagnostic but must be critically evaluated in conjunction with clinical and radiological data. In case of discrepant information, normal bone and bone marrow components should be regarded as sampling error and discarded as non-diagnostic.

Although and with that in mind, we reported 12 FN and two FP cases. On review, ten of the 12 FN diagnoses, mostly metastatic disease and hematopoietic tumors, were rendered due to non-diagnostic cytological material. This is obviously a recurring issue in diagnostic pathology and has in the context of bone cytopathology also been reported by others.[74,75] Notably, our approach did not lead to a significantly higher fraction of FN cytological diagnoses due to examination of non-diagnostic material, compared to Bommer and Saoud et al. (1.2% and 1.3% compared to 1.4% in our study).

Adequate FNAC material could be obtained in 86% of the cases. For every case it was recorded if the sampling procedures were accompanied by ROSE. We found that the application of ROSE led to a significantly approved sampling adequacy of bone FNAC, as also suggested by others.[74]

In order to be able to distribute cytological diagnoses into meaningful diagnostic categories, the diagnostic performance was first evaluated by sensitivity, specificity and accuracy analyses.

With a malignant diagnosis defined as positive, a benign diagnosis as negative rest result, cytopathology correctly identified the malignant potential of a bone lesion with a sensitivity of 89% and a specificity of 99%. For this analysis, cases with non-diagnostic cytology material (n=101) were excluded. We chose to treat equivocal diagnostic results, meaning that a benign or malignant diagnosis could not be established, as false diagnoses (n=56). This might appear as a harsh approach, which negatively affected the sensitivity in this study. But, in our

opinion, it better reflects reality than simply excluding those cases from the analysis, which, to us, would artificially enhance the results. However, excluding equivocal results is sometimes the approach that is chosen in other publications.[74] If also equivocal diagnostic results would be excluded, the diagnostic sensitivity would improve to 97% with unchanged specificity of 99%. In summary, our results show, that bone FNAC is a suitable diagnostic tool to differentiate between benign and malignant bone lesions. The diagnostic performance of bone FNAC has been evaluated in numerous publications, with reported sensitivities between 55-97% and specificities of 79-100%.[74,76,77] Possible reasons for the large range in sensitivity and specificity analysis results in soft tissue and bone cytopathology have already been discussed in Article 1.

Bone tumors are not as numerous and heterogeneous as soft tissue tumors but still represent a large group of different neoplasms.[13] The overall diagnostic accuracy in our hands was 65% but it varied tremendously between different tumor types. In the literature the overall diagnostic accuracies range between 54-95%.[74,76–79] The best diagnostic accuracy in our hands was achieved for conventional chordomas (100%), Ewing sarcomas (92%) and conventional osteosarcomas (88%) and comparable results were achieved by others.[80,81]

The diagnostic accuracy for chondrosarcomas was in our hands 67% and surprisingly homogeneous among tumors of different grades. Diagnostic accuracies up to 86% have been reported.[81] Especially the evaluation of low-grade chondroid tumors is often a diagnostic challenge, even on biopsy material. Morphological features are shared between various benign entities (enchondromas, parosteal chondromas, osteochondromas) and malignant ones, especially low-grade chondrosarcomas. A diagnosis should only be established in agreement with the clinical and radiological findings. We achieved a diagnostic accuracy for enchondromas/parosteal chondromas and osteochondromas in 30% and 40%, respectively.

Osteoclast giant cell-rich neoplasms were in this study mainly represented by GCTB and ABC. GCTB were correctly identified in 74% of the cases and other studies report even higher accuracies (100%).[81] In most cases, FNAC from GCTB show a cellular diagnostic yield with recognizable pattern.[82] However, the diagnosis of GCTB can be more challenging when the tumors show an altered morphology, e.g. in case of ABC-like changes or malignant

transformation, as we show in this study that contained one and three of those cases, respectively. ABC were identified in 65% of the cases and this result is congruent to other reports.[81] In contrast to GCTB, material obtained by FNA from ABC is often sparse and tends to be mainly haemorrhagic. Thus, the diagnosis is sometimes difficult to establish on cytology and should generally only be rendered in conjunction with imaging results. In doubtful cases the driver mutation in most cases, a rearrangement of *USP6*, can be addressed by FISH or immunohistochemistry.

Suspected metastatic disease is probably the most common application for bone FNAC. We report a diagnostic accuracy of 74% for FNAC from bone metastases to the bone, which is in line with other reports with accuracies between 79-83%.[74,81]

Bone engagement of hematopoietic tumors was represented mainly by plasma cells neoplasias but also by a variety of lymphomas and leukemias as well as a few cases of histiocytosis (Langerhans cell and mast cell). The overall diagnostic accuracy in this group was 73% and was highest for Langerhans cell histiocytosis, B-cell lymphomas (other than diffuse large B-cell lymphoma) and plasma cell neoplasias (80%, 80% and 78%, respectively). Data on accuracies of bone engagement of hematopoietic tumors are sparse but has been reported as high as 58%.[74]

We report the lowest accuracy among vascular tumors (0%) and "other" mesenchymal tumors, according to the recent WHO classification.[13] The study included only three primary vascular tumors with histological follow-up and none of the two hemangiomas or the only angiosarcoma was diagnosed by FNAC. The main reason was the scantiness of the material, as also reported by others.[78]

Among "other" mesenchymal tumors, our study included eight cases of fibrous dysplasia, none of those identified on FNAC. We and others do not think that cytological material can be reliably obtained from these tumors by FNA.[83]

Furthermore, we report five cases of undifferentiated pleomorphic sarcoma (UPS). All of those were identified as pleomorphic malignancies, but as it is a diagnosis of exclusion which requires a comprehensive immunohistochemical work-up, none of those tumors were diagnosed on FNAC.

A few words regarding the role of genetic analyses on FNA material from bone tumors: the majority of genetic analyses in this context (as described, in only 2% of the cases) were FISH analyses in suspected Ewing sarcomas. This illustrates, that genetical analyses were, in our hands, only performed in a few selected cases. The most important explanation for that is, that we in most cases did not rely on FNAC alone but had access to histological material (e.g. CNB in 73% of the cases) for the diagnostic work-up. Genetic analyses were performed on histological material in 46%, most common were genomic arrays (62%, see Article 3), FISH analyses (32%), targeted MPS-based methods (12%) and chromosome banding analysis (10%).

For our proposal for reporting bone cytopathology, we distributed all diagnostic results into one of six categories: (I) Non-diagnostic, (II) Benign, (III) Atypia, (IV) Bone neoplasm of uncertain malignant potential (BNUMP), (V) Suspicious for malignancy and (VI) Malignant. The results are shown in Table 2.

The non-diagnostic category (I) had a ROM of 48%, which should encourage resampling. We reported a comparable result in Article I in our proposal for reporting cytopathology of soft tissue tumors. The ROM in the benign category was 6.7%. The majority of cases were either non-neoplastic conditions or benign neoplasms (mainly GCTB and ABC), but also all 12 FN cases, as discussed above. The ROM in this category seems relatively high and brings us back to the question how to treat non-neoplastic cytological diagnoses (which are in this proposal part of the benign category). When redistributing all cases with non-neoplastic FNA results to the non-diagnostic category, the ROM in the benign category and the non-diagnostic category would drop to 4% and 28%, respectively. There was a 69% ROM in the intermediate atypical category (III). Most cases in this category were sparsely sampled metastases or hematopoietic tumors. In our BNUMP category (IV) we report a 28% ROM. Most cases in this category were chondroid neoplasms with no or low-grade atypia with its already discussed diagnostical limitations. Most cases of Langerhans cell histiocytosis were also distributed to this category. Those tumors can be reliably diagnosed by FNAC, but the biological behaviour is difficult to predict. We further evaluated two malignant categories; (V) suspicious for malignancy and (VI) malignant with a respective ROM of 93% and 100%.

Table 2. Proposed reporting system for bone cytopathology (ROM in grey shade)

	I. Non-diagnostic	II. Benign	III. Atypia	IV. BNUMP	V. Suspicious for malignancy	VI. Malignant
n (of total)	101 (14%)	180 (25%)	36 (5%)	25 (3%)	29 (4%)	350 (48%)
n (within category)						
Dx Benign	53 (52%)	167 (93%)	11 (31%)	14 (56%)	2 (6.9%)	0
Dx Malignant	48 (48%)	12 (6.7%)	25 (69%)	7 (28%)	27 (93%)	350 (100%)
Dx UMP	-	1 (0.6%)	-	4 (16%)	-	

Abbreviations: ROM=risk of malignancy, UMP=tumor of unknown malignant potential, Dx=final histopathological diagnosis, BNUMP=bone neoplasm of uncertain malignant potential

At the time of writing, there was only one article with a comparable approach, published by Saoud et al. and based on 341 cases.[74] The authors tested their study cohort on five categories with the corresponding ROM in each category: Benign (ROM 0%), Atypical (ROM 51.7%), Neoplasm of unknown malignant potential (ROM 46.7%), Suspicious for malignancy (ROM 100%) and Malignant (ROM 99.1%). The group chose not to include non-neoplastic lesions and the benign category included consequently only benign neoplasms.

In conclusion FNAC is a useful diagnostic tool for bone lesions and can differentiate between benign and malignant entities with high sensitivity and specificity. Various tumor entities can reliably be diagnosed on cytology. Furthermore, the study provides valuable information for a standardised reporting system for bone cytopathology.

Article 3

The morphologic heterogeneity among STBT is reflected by an extensive genetic variation, including copy number aberrations (CNA). This class of mutations has been described in numerous tumor entities but is only exceptionally used as diagnostic tool for soft tissue and bone tumors.[13] In this study, we examined the diagnostic value of SNP array analysis on core needle biopsies (CNB) from 171 soft tissue and bone lesions. The technical failure rate was only 2%, and thus much lower than the reported 19% for chromosome banding on CNB specimens.[84] Hence, SNP array analysis is a technically robust method for small preoperative tissue samples. The occurrence and complexity

of CNAs varied among the different soft tissue and bone lesions. CNBs from biopsies, diagnosed as non-neoplastic/non-representative, benign and malignant lesions showed CNAs at SNP array in 15%, 28% and 85% of the cases, respectively. The total number of CNAs and the number of chromosomes displaying CNAs were significantly higher in malignant than in benign lesions (Wilcoxon test, $p<0.001$).

The clinical implication, however, was difficult to evaluate due to several reasons. A diagnosis is a consensus of various clinical, morphologic and genetic features, supporting certain diagnoses and arguing against potential differential diagnoses. As we have shown in Article 1, and as has been reported by others, the examination of CNB material does not necessarily result in a specific diagnosis or a diagnosis can be wrong.[60] This does not only concern morphological mimics among neoplasms but also neoplasms versus reactive conditions. In our study, this was reflected by three cases diagnosed as non-neoplastic/not representative, but with detectable CNAs. One of those was a case of inflammatory leiomyosarcoma, diagnosed as not representative, on CNB. Possibly, a biopsy used for morphologic examination can be non-representative whereas the biopsy taken for genetic analysis is, or vice-versa. Finally, due to the morphologic heterogeneity among STBT, an interobserver variability, even among sarcoma specialists, has been reported.[85,86]

Some genetic features strongly indicate a certain tumor entity. This is especially true for tumors that are driven by fusion genes but applies also to characteristic CNA profiles, such as 12q amplifications in atypical lipomatous tumors.[50,87] However, genetic features are, at least to date, not diagnostically sufficient on their own and must be evaluated in a clinical context. For example, the CNA profile of a parosteal osteosarcoma is indistinguishable from the profile found in atypical lipomatous tumors.[49] With all these considerations in mind, we decided to designate the CNA profiles as *compatible with* and the stronger *indicative of* specific diagnoses or diagnostic groups. Of all analysed 168 cases, the CNA profiles of 87% were compatible with the corresponding CNB diagnoses. Of those, 41% cases did not show CNAs and thus did not provide any further diagnostic information. The majority of the discrepant cases could be explained by false-negative results at SNP array, non-representative material or contamination with a critical amount of non-tumoral cells. In a few cases, discrepant results occurred due to false morphologic diagnoses and were

corrected after SNP array analysis. The aberrant genomic profiles of 70 cases were designated as indicative of a certain tumor entity, or a broader group of neoplasms. The former included genomic profiles, regarded as characteristic of one of the following six tumor types: undifferentiated pleomorphic sarcoma (UPS)/myxofibrosarcoma (MFS), liposarcoma, osteosarcoma, leiomyosarcoma or gastrointestinal stromal tumor and chondrosarcoma. Broader diagnostic groups were based on the occurrence and complexity of CNAs, however without a profile that pointed to a certain tumor entity. Those groups included high-grade sarcoma not otherwise specified (NOS), sarcoma NOS and neoplasm NOS. All in all, the SNP array results were in agreement with the corresponding CNB and final diagnoses in 83% and 86% of the cases. Discrepant cases were identified among UPS/MFS and liposarcomas. Cases among the former were diagnosed as other types of pleomorphic, and thus genomically complex, sarcomas. Two dedifferentiated liposarcomas showed the genomic profiles of well-differentiated liposarcomas at SNP array, presumably due to sampling from the well differentiated part of the tumor.

For 95 cases (138 samples) we had the possibility to generate segmentation files, that were used for cluster analyses, using the Jaccard similarity index, in order to evaluate the similarity between the genomic profiles. A Jaccard score can range between 0 and 1; a score that equals 0 indicates no intersection, and a score that equals 1 indicates complete overlap of the copy number profiles. The cases were distributed in three major clusters; two clusters included mainly high-grade sarcomas with complex genome-wide copy number shifts and one cluster with genomically less complex cases including the vast majority of benign tumors. For 43 cases with segmentation files, we had the possibility to compare the similarity of the CNA profile of the CNB with that of the resected lesion. With this analysis, we attempted to evaluate the representativeness of CNB biopsies for the whole tumor. Most malignancies show an intra-tumoral heterogeneity regarding the mutational spectrum, including CNAs and it is supposed to be unlikely that the whole range of mutations is represented in a small biopsy.[88] The genomic similarity among those paired samples was, however, generally high (median Jaccard score 0.9). As expected, cases with less complex copy number shifts, as well- or dedifferentiated liposarcomas, scored generally higher (about 0.98) than cases with complex genomic profiles (< 0.9). The majority of sample pairs (77%) were located next to each other in the cluster analysis showing very high similarity indices (median score 0.96),

indicating that CNBs in the majority of cases are representative for the whole lesion. Even among the ten sample pairs that did not cluster with each other, six revealed relatively high Jaccard scores (0.83-0.97). Only one case of an undifferentiated pleomorphic sarcoma with very complex genomic profile showed distinctive differences in the samples from the CNB and the resection specimen. In a single case, a different ploidy assumption on each of the paired samples lead to the separation in the plot. The discrepancies in the remaining cases could be explained by a contamination with a critical amount of non-tumoral cells or a suboptimal technical quality.

Although generally a robust method on CNB material, our article highlights some drawbacks of SNP array analysis. Genomic profiles without any CNAs do not provide any diagnostic information and can be found among both benign and some malignant tumor entities.[13] When lacking copy number shifts, the representativeness of the analysed material cannot be confirmed by SNP array analysis; in this regard other approaches, like WGS, have the advantage of identifying SNV or structural variants, that could support if a sample originates from a neoplasm or not. Furthermore, SNP array results can be obscured by a critical amount of non-tumoral cells, that can affect the evaluation of both copy number shifts and ploidy level. Finally, ploidy levels are difficult to estimate in very complex aneuploid genomic profiles, although this might be overcome by alternative bioinformatical approaches like allele-specific copy number analysis of tumors (ASCAT).[89,90]

In conclusion, we show that SNP array analysis is a technically robust method for CNB with very low failure rate. The genomic copy number profiles were in the majority of cases compatible with the morphologic diagnoses and in the majority of the cases representative for the whole lesion.

Article 4

This study was performed to get new insights in the genetic/genomic alterations in a specific family of tumor entities, the dermatofibrosarcoma protuberans family of tumors (DPFT). The driver mutation in the vast majority of these tumors - a *COL1A1*::*PDGFB* fusion - was already known.[91] The aim of my study was to get a better understanding of (i) the origin of the underlying gene

fusion and (ii) to characterize secondary genomic aberrations and how those differ with age and tumor progression.

We analysed 42 tumor samples from 39 patients diagnosed with a tumor belonging to the DPFT with various genetic approaches, i.e. chromosome banding, FISH analysis, SNP array analysis and various MPS based methods (MPS-based gene panel, WES and RNA-seq).

At chromosome banding analysis all analysed tumor samples (n=32) showed abnormal karyotypes, which were compared with previously described cases (n=84).[42] The structural chromosomal changes were in agreement with previously described aberrations.[91–93] The *COL1A1*::*PDGFB* gene fusion occurred either through a balanced t(17;22), an unbalanced der(22)t(17;22), or on one or more ring chromosomes. By interphase FISH analysis on nine cases, we could detect one fusion event on each der(22)t(17;22) and up to three fusion events on ring chromosomes. The cytogenetic features of the analysed tumors followed an age-associated distribution. The only balanced t(17;22) in our series was detected in the tumor of a young child (age 7 years). The unbalanced der(22)t(17;22) predominated in adolescents and younger adults whereas ring chromosomes were mainly identified in adults including elderly. Recurrent secondary genomic changes preferentially included numerical chromosomal aberrations, the most frequent being trisomy 8 (n=7), mono- or trisomy 22 (n=6), and trisomy 5 and 18 (each n=4). We did not identify recurrent structural rearrangements other than those involving chromosomes 17 and 22 in our series, although add(5)(p13) has to date been described in two tumors.[42,94]

SNP array analysis revealed copy number aberrations in all analyzed samples (n=16). Genomic gains on 17q21-pter of 1-2 copies were found in tumors with balanced/unbalanced translocations and gains of 1-4 copies in tumors harbouring ring chromosomes. On 22q13-qter, genomic losses were found in tumors with t(17;22)/der(22)t(17;22) while tumors with ring chromosomes showed 1-4 extra copies of 22q11-q13. The copy number shifts were in or near the *COL1A1* and *PDGFB* gene loci, respectively. A recent study with available copy number profiles of DFSP samples, extracted from WGS data, shows a comparable copy number pattern.[95] However, the copy number profiles were not interpreted in context with karyotypes.

Although the age-associated cytogenetic differences and genomic imbalances in DPFT have already been described, underlying mechanisms of tumorigenesis across the age groups remain still to be elucidated.[96] However, the allelic status combined with cytogenetic features in the current project gave some new insights into the origin of the fusion gene. The disomic parts of chromosomes 17 and 22 in tumors with der(22)t(17;22) and ring chromosomes always showed retained heterodisomy at SNP array. Hence, and in contrast to DPFT with balanced t(17;22), it is most likely that the genetic error in tumors with der(22)t(17;22) and ring chromosomes occurred after DNA synthesis, i.e. in the S-G2 phase of the cell cycle, with the der(22) and der(17) being distributed to different daughter cells. Other possible mechanisms for the development of a der(22)t(17;22) would result in uniparental isodisomy of the disomic part of chromosome 17 in at least a part of the tumor cells (Figure 10).

Mechanisms of progression to high-grade DPFT are still poorly understood. We had the possibility to analyse six such tumors. Neither numerical nor structural aberrations were clearly associated with tumor grade. However, genomic loss on chromosome arm 9p at SNP array was found in two of three high-grade and only one of 12 low-grade tumors at SNP array. This is an interesting finding, as *CDKN2A* is located in the deleted part. *CDKN2A* deletion is in various malignancies linked to tumor progression and poor prognosis.[97–99] In DPFT, *CDKN2A* deletion has been reported by others in high-grade and high-grade metastatic tumors and might contribute to tumor progression.[100–102] Further analysis of the potential link between 9p deletions and tumor progression is thus warranted, not least because an altered *CDKN2A/INK4A/p16* pathway could be addressed therapeutically.[102]

We could not, by RNA-seq of ten tumors, identify other gene fusions than *COL1A1*::*PDGFB*, although other driver mutations have recently been described in a subset of DPFT. Those include *COL6A3*::*PDGFD* gene fusions, especially in tumors located in the breast, and *EMILIN2*::*PDGFD* gene fusions; the latter is associated with sarcomatous dedifferentiation and *CDKN2A* loss.[100,103] Gene expression analysis revealed differentially expressed genes compared to other soft tissue tumors, including *PRKCA*, *TBX2*, *MSI2* on chromosome 17 and *PDGFB*, *PRAME* and *SOX10* on chromosome 22, partly also described by others.[104] From a clinical point of view, *PRAME* is particularly

interesting. This gene is expressed in various malignancies and might be a target for immune therapy also in sarcoma subtypes.[105,106]

We could identify only one potentially pathogenic SNV, a mutation in the *PTEN* gene by targeted sequencing. A recent study, utilizing WGS, confirms a relatively low mutational burden in DFSP (TMB 3.4). Interestingly, the TMB of regular DFSP and those with fibrosarcomatous transformation did not significantly differ. Nonetheless, an average number of 20 small genetic variants, mostly SNV, were identified per analysed sample; among the most frequently mutated genes were *MUC4*, *MUC6* and *BRCA1* but not *PTEN*.[95]

In conclusion, we show that the mechanisms behind the origin of the *COL1A1*::*PDGFB* fusion are age-associated and that the gene fusion probably arises after DNA-synthesis in most cases. There was no clear impact of secondary changes on tumor aggressiveness, but chromosome number and 9p deletions tended to be associated with high-grade lesions. Some of the transcriptionally upregulated genes in the amplified regions of chromosomes 17 and 22 could potentially also be of clinical significance.

Beyond our study and from a diagnostic point of view it could be mentioned, that the *COL1A1*::*PDGFB* fusion gene is regarded as pathognomonic for DPFT. However, the same fusion gene has recently been identified in a rare fibrosarcomatous tumor in the uterine cervix and corpus, which may show morphological similarities to DFSP.[107–110] As DPFT are cutaneous tumors, this should not cause any diagnostical problems but should be kept in mind in case of a metastatic disease.

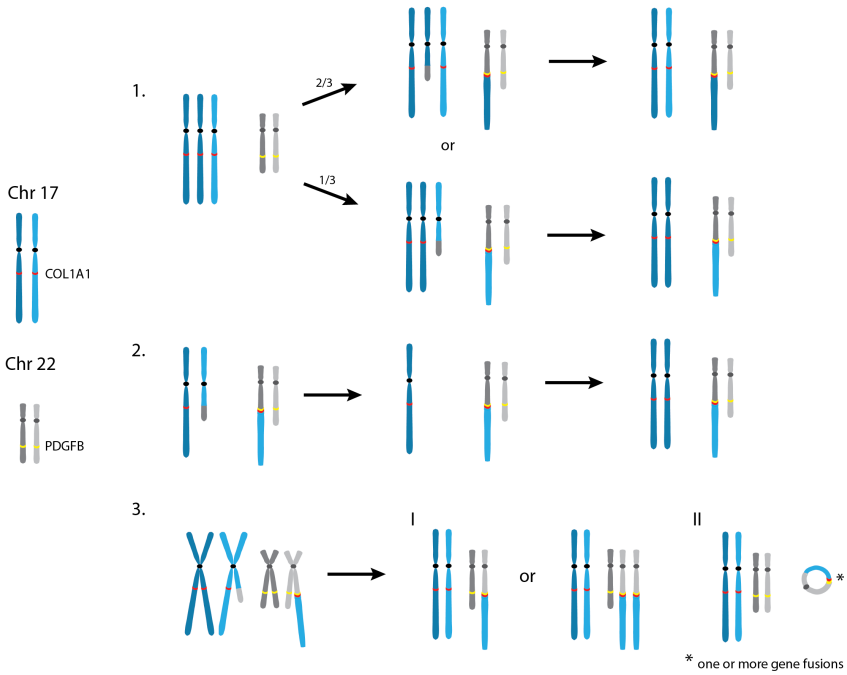


Figure 10. Possible outcomes of a t(17;22) in different phases of a cell cycle
The two parental copies of chromosomes 17 and 22 are shown in different shades of blue and gray, respectively. (1) Trisomy 17 is followed by t(17;22) in G0-G1 and loss of der(17), resulting in uniparetal isodisomy (UPID) for the disomic part of chr 17 in 1/3 of the cases. (2) t(17;22) in G0-G1 with subsequent loss of der(17) and duplication of the normal homologue, always leading to UPID of the disomic parts of chr 17. (3) Recombinations in G2 would consistently result in retained heterodisomy for the disomic parts of chromosomes 17 and 22 in dermatofibrosarcoma protuberans cases with (I) der(22)t(17;22) or (II) ring chromosomes.

Context, Conclusion and Perspective

Soft tissue and bone pathology is a diagnostic field that is rapidly changing in various aspects. According to the main approaches in this thesis I want to emphasize two of those: morphology, including (interventional) cytopathology, and molecular pathology.

Morphological and interventional aspects

In the perception of many pathologists, cytopathology is a diagnostic discipline, that only exists at the edge of histopathology. But cytopathology has gained more attention in the recent years, not least since the International Academy of Cytology (IAC) and the International Agency for Research on Cancer (IARC)/WHO joined forces to publish a complete Cytopathology Reporting System series alongside the series of *blue books*, that serve as reference books for pathologists in their daily diagnostic work. As already mentioned, the first two volumes of this new series have already been published.[72,73]

We presented a first draft of a research project, that finally led to Article 1, at the European Congress of Cytology (ECC) 2018 in Madrid and caused a subsequent discussion about soft tissue and bone cytopathology in general but also about a possible reporting system in this diagnostic field. One year later, in 2019, we presented the almost finished study, including our proposal for a soft tissue cytopathology reporting system on the ECC in Malmö. Subsequently, the IAC-IARC decided to include soft tissue cytopathology in their series. Article 1 was influential in this process and an integral part in the development of a refined reporting system, that will be published as the first

version of the WHO Reporting System for Soft Tissue Cytopathology. Recently, more studies subsequently emerged, to test this refined final system, partly on more defined groups of soft tissue tumors.[111–113] Bone cytopathology is not part of this first version but will be integrated in the future. As a logical succession, we drafted a comparable approach regarding bone cytopathology (adequate data were missing at that time and are still sparse) and adopted the six categories that are now finally utilized for the WHO Reporting System for Soft Tissue Cytopathology. To date, the work of Saoud et al. and Article 2 deliver the only available data that address this topic and provide the basic information for the next version of the WHO reporting system. Both reporting systems need to be extensively tested, ideally in prospective approaches, in order to evaluate the clinical use (if any) in patient management. A task for every medical discipline involved.

While working with cytopathology of soft tissue and bone tumors, I realized that there are still many tumor entities, that are not or not well characterized in terms of the basic cytomorphology. This concerns even relatively common neoplasms like spindle cell/pleomorphic lipomas with the largest series to date describing 12 of those tumors.[114] Those observations emphasise that basic morphological research, even nowadays with the scientific interest in the field of STBT strongly focussing on molecular pathology, is still valuable and necessary. In Lund, we luckily have access to a large archive, with cases of STBT that were approached by FNAC dating back to the 1970s. Research projects elucidating the cytomorphology of selected STBT entities is thus comparably easy to perform and are already planned for years to come.

Since pathologists began to perform FNA in Lund, the puncture procedures were executed palpation-guided. This is sufficient in many situations but has obviously always the caveat that the needle during sampling is not visualized which carries the risk that the obtained material is not representative. This is especially true when dealing with small, deeply seated lesions or lesions in close proximity to delicate structures. Already since the early 2000s, reports about ultrasound-guided FNA and even CNB, performed by the cytopathologist were accumulating.[115,116] There is growing and already strong evidence, that ultrasound-guided sampling by the cytopathologist enhances the diagnostic process.[117–119] Consequently, this approach is increasingly adopted in the diagnostic workflow in several countries. Trainings in ultrasound-guided FNA

for board certified pathologists exist, among others in the USA and in Switzerland. To follow that path is a logical development in our FNA clinic. This is going to be especially useful for soft tissue and head & neck lesions, not only to safely hit the region of interest but also to avoid necrotic, cystic or hypervascular areas. Although this project (which is already initiated) is more logistic than scientific, it is for sure one of the, if not the, most essential development at our FNA clinic for the next years.

Molecular aspects

In the past decades, an increasing number of driver mutations have been discovered in STBT, that characterize (more or less) specifically certain tumor entities.[13] Targeting those mutations either directly with molecular analyses or indirectly via immunohistochemistry is an integrated part of the diagnostic workup. To date, however, specific driver mutations have been identified in only about one third of STBT. Morphology and immunohistochemical stains to determine the cell lineage remain the most important diagnostic approach by which the lesion can be identified in most instances or at least narrowed down to few possible differential diagnoses.

One ambition in this thesis was to evaluate molecular approaches, that could support the diagnostic process of STBT without necessarily targeting specific genetic aberrations. In Article 3 we evaluated the diagnostic use of CNA analysis. Copy number profiles can also be extracted from a variety of MPS-based methods, among those WGS, WES, methylation assays but also from gene panels.[120–124] With the rapid decline in cost of the various MPS-based sequencing methods, SNP array analyses will in this context most likely become obsolete in the future. CNAs, however, remain an essential class of mutations, both to unravel mechanisms of tumor development and progression and for diagnostical purposes.[120,125]

A further interesting approach is the evaluation of the diagnostic use of gene expression profiles from RNA-seq data. Transcriptomic profiles have been analysed in a multitude of tumor entities and can discriminate between tumor entities and define subentities, including among sarcomas.[126–129] However, this technique has to date not been evaluated for usage as a sarcoma classifier in a

larger cohort. In addition, the identification of differentially expressed genes provides prognostic information and can unveil therapeutic targets.[55,130,131] In an ongoing project, we have identified more than 800 STBTs that were analysed by RNA-seq. The sequencing data were shared with a company, that is specialized in tumor classifying based on transcriptomic profiles (www.qlucore.com). The collaboration is still ongoing and is expected to come to fruition in 2025/26.

Lastly, applying molecular approaches on cytological material (*molecular cytology*) in the diagnostics of STBT, is a discipline that seems to be a logical succession for this thesis. Various genetic analyses, among those FISH and MPS-based methods are widely performed in daily routine on cytological material for diagnostic and predictive purposes, e.g. in lung tumors.[132–134] The situation is different though when it comes to STBT cytology. Immunohistochemical analyses, that serve as surrogate markers for underlying mutations, as well as FISH analyses and RT-PCR are the main approaches in this context and have been part of the diagnostic catalogue for quite a while.[135–137] However, data on MPS-based approaches on cytology specimens from STBT are surprisingly sparse. One exception, at least in our practice, is currently the application of an MPS-based DNA panel for diagnostics and predictive testing of GISTs, that works well even on cytological material. According to some authors, MPS-based methods in the diagnostics of STBT are to date of limited value.[137] This might be true at the moment. But with MPS-based methods getting more and more accessible, approaches like transcriptomic sequencing or methylation assays would in my eyes be a valuable addition to the diagnostic toolbox, even on cytological material.

Neoplasms are a fascinating class of diseases, that have always been and will always be a part of us. Tumors are "stitched into our genome", as Siddhartha Mukherjee puts it in his book *The Emperor of All Maladies – A Biography of Cancer*.[138] Although life without cancer is an imagination out of reach, diagnostic and therapeutic approaches are continuously improving. I hope that this thesis is a small contribution. From time to time, it seems difficult to finish research projects as an addressed question commonly raises a new one, without coming to an end. But this thesis does. Here.

Die Ente bleibt draußen.

Other Publications

- Ibstedt S, Piccinelli P, Sydow S, Köster J, Mertens F. Structural Variants in the SMC1A Gene Associated With Near-Haploidy in Undifferentiated Pleomorphic Sarcomas. *Genes Chromosomes Cancer.* 2024;63:e23255.

- Sydow S, Piccinelli P, Mitra S, Tsagkozis P, Hesla A, Bedeschi Rego de Mattos C, Köster J, Magnusson L, Nilsson J, Ameur, A, Wardenaar R, Foijer F, Spierings D, Mertens F. *MDM2* amplification in rod-shaped chromosomes provides clues to early stages of circularized gene amplification in liposarcoma. *Commun Biol* 2024; 606

- Ibstedt S, Bedeschi Rego de Mattos C, Köster J, Mertens F. A cryptic *EWSR1*::*DDIT3* fusion in myxoid liposarcoma: Potential pitfalls with FISH and cytogenetics. *Genes Chromosomes Cancer.* 2023;62:167-170.

- Arbajian E, Köster 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.

POPULÄRVETENSKAPLIG SAMMANFATTNING (SVENSKA)

Tumörer uppstår genom ackumulerande fel i genomet av en cell. Detta kan medföra att den drabbade cellen kan undvika mekanismer i kroppen, som reglerar celldelning och differentiering (ändring i cellens funktion och utseende), och cellen kan mångfaldigas mer eller mindre okontrollerat. Mjukdels- och bentumörer är en stor och heterogen grupp av neoplasmer som uppstår i bind-, fett-, muskel- och nervvävnad, i blod/lymfkärl samt brosk- och benvävnad. Det finns både godartade och elakartade tumörer, de sistnämnda kallas i den medicinska terminologin för sarkom och är jämfört med andra elakartade tumörtyper sällsynta. Diagnosen av tumörer ställs vanligtvis av en patolog, genom en makro- och mikroskopisk analys av cell- eller vävnadsprov. Diagnostiken av mjukdelstumörer kan vara mycket utmanande, framför allt på små vävnadsprov, så kallade biopsier. Anledningen för detta är att det finns med >150 beskrivna entiteter många olika tumörtyper med varierande kliniskt beteende och behandlingskrav, som dock delvis kan visa ett likartat utseende i mikroskopet. Tvärtom kan dessutom en och samma tumörentitet visa olika mikroskopiska utseenden. I den diagnostiska processen får patologer därför hjälp av olika ytterligare undersökningar, bland dessa genetiska analyser av tumörprov.

Syftet med denna avhandling var att förbättra diagnostiken av mjukdels- och bentumörer genom optimering av morfologiska metoder men framför allt genom att förbättra genetiska diagnosverktyg.

I Artikel 1 jämförde vi morfologiska diagnostiska resultat av finnålspunktat (fine needle aspiration cytology, FNAC) och grovnålsbiopsier (core needle biopsies, CNB) från 828 mjukdels- och benförändringar. FNA-analyser är ett robust verktyg för att skilja mellan god- och elakartade förändringar, dock är

CNB-analyser bättre när det gäller att ställa en specifik diagnos. Dessutom föreslog vi ett klassifikations-system för en standardiserad rapportering av FNA-diagnoser för att förenkla hanteringen av patienter med mjukdelstumörer. Detta system hade sju kategorier: (I) Icke-diagnostisk, (II) Icke-neoplastisk, (III) Oklar atypi, (IVa) Benign tumör, (IVb) Tumör av oklar malignitetspotential, (V) Misstanke på malignitet och (VI) Malignt. Malignitetsrisken i varje kategori beräknades: (I) 42%, (II) 0%, (III) 46%, (IVa) 3%, (IVb) 27%, (V) 72% och (VI) 97%. Denna studie är redan ett värdefullt bidrag till det kommande WHO systemet för rapportering av mjukdelstumörer, som är annonserad för sen 2024.

Artikel 2 ägnade sig åt bentumörer med en jämförbar approach som i Artikel 1, här dock med fokus på cytologiska analyser och ett lätt förenklat rapporteringssystem. Studien omfattade 721 benförändringar. Cytologiska analyser kunde i de flesta fall skilja mellan godartade och elakartade förändringar. Den diagnostiska precisionen varierade mycket mellan olika tumörtyper och vi kunde visa att några tumörer kan diagnosticeras förhållandevis säkert på cytologi och andra inte. Fallen i kohorten fördelades i sex kategorier: (I) Icke-diagnostisk, (II) Benign, (III) Atypi, (IV) Bentumör av oklar malignitetspotential, (V) Misstanke på malignitet och (VI) Malignt. Malignitetsrisken i de olika kategorierna var: (I) 48%, (II) 6.7%, (III) 69%, (IV) 28%, (V) 93% och (VI) 100%. Denna studie kommer att kunna bidra till det kommande WHO systemet för rapportering av bentumörer.

I Artikel 3 undersöktes, om genomiska array-analyser, specifikt Single Nucleotide Polymorphism (SNP) arrays, lämpar sig som diagnostiskt verktyg för mjukdels- och ben-tumörer på CNB-material. SNP-arrays detekterar överskott och förlust av genetiskt material i hela genomet med hög upplösning i form av relativa kopietalsförändringar (copy number aberrations, CNA). Vi kunde visa, att SNP-arrays är en tekniskt robust metod på små biopsier. CNA-profilerna i en stor del av de undersökta tumörerna (87%) passade väl med de morfologiska diagnoserna. Några entiteter visade till och med tumörkarakteriserande CNA-profiler. Dessutom är SNP-array-resultat på CNB-material i de flesta fall (77%) representativa för hela tumören.

I Artikel 4 undersökte vi en särskild tumörfamilj i huden, dermatofibrosarcoma protuberans tumörfamiljen (DPFT), med olika genetiska analyser för att få en bättre förståelse av sjukdomsorsak och -förlopp. Vi kunde beskriva att den

kända *COL1A1*::*PDGFB*-genfusionen, som orsakar tumörsjukdomen, har sitt ursprung mest sannolikt efter DNA-syntesen i cellcykeln. Tydliga genetiska skillnader mellan vanliga och mer aggressiva varianter av DPFT kunde inte identifieras, dock tror vi att förlust av genetiskt material på kromosom 9p är oftare förknippad med aggressiva tumörer. Dessutom identifierade vi i DPFT ett antal uppregulerade gener, som kan vara av terapeutiskt intresse.

Populärwissenschaftliche Zusammenfassung (Deutsch)

Tumoren entstehen durch akkumulierende Fehler im Genom einer Zelle. Dies kann zur Folge haben, dass sich die betroffene Zelle normalen Regelkreisläufen des Organismus, vor allem Zellteilung und -differenzierung (d.h. Veränderungen in Zellfunktion und -aussehen), entziehen, und sich unkontrolliert vervielfältigen kann.

Weichteil- und Knochentumoren sind eine große und heterogene Gruppe von Neoplasmen, die in Binde-, Fett-, Muskel- und Nervengewebe, in Blut- und Lymphgefäßen, sowie in Knochen- und Knorpelgewebe entstehen. Sie umfassen sowohl gutartige als bösartige Entitäten; letztere werden in der medizinischen Terminologie als Sarkome bezeichnet und kommen verhältnismäßig selten vor. Tumordiagnosen werden normalerweise von Pathologen nach Analyse von Zell- oder Gewebeproben gestellt. Vor allem Weichteiltumore stellen hierbei häufig eine diagnostische Herausforderung dar, vor allem, wenn nur sehr sparsame Zell- und Gewebeproben für die Diagnostik vorliegen. Dies hat mehrere Gründe. Zum einen gibt es sehr viele (> 150) verschiedene Tumorentitäten, die sich in ihrem klinischen Verhalten und damit auch den erforderlichen Behandlungsstrategien stark unterscheiden, sich in ihrem mikroskopischen Aussehen jedoch sehr ähneln können. Umgekehrt kann aber auch ein und dieselbe Tumorentität unterschiedliche morphologische Gesichter aufweisen. Während des diagnostischen Prozesses bedienen sich Pathologen daher neben rein morphologischen Untersuchungen häufig weiterer Methoden, darunter genetische Analysen, um sichere Diagnosen stellen zu können.

Ziel der vorliegenden Dissertation war es die Diagnostik von Weichteil- und Knochentumoren zu verbessern, sowohl hinsichtlich morphologischer, aber auch genetischer Herangehensweisen.

In Artikel 1 verglichen wir die morphologischen diagnostischen Resultate von zytologischen Feinnadelpunktionen (fine needle aspiration, FNA) und Stanzbiopsien (core needle biopsy, CNB) von 828 Raumforderungen in Weichteilen und Knochen. Wir konnten zeigen, dass Feinnadelpunktionen ein robustes Werkzeug zum Differenzieren zwischen gut- und bösartigen Tumoren darstellen. Stanzbiopsien waren jedoch sensitiver und spezifischer hinsichtlich des Stellens von exakten Diagnosen. In einem weiteren Schritt schlugen wir ein Klassifikationssystem zum standardisierten Befunden von zytologischen Analysen vor, um die Handhabung von Patienten mit Weichteiltumoren zu optimieren. Dieses System umfasste die folgenden sieben Kategorien: (I) Nicht diagnostisch, (II) Nicht neoplastisch, (III) Atypi mit unklarer Bedeutung, (IVa) Gutartige Tumore, (IVb) Tumore mit unklarem Malignitätspotential, (V) Verdacht auf Malignität, (VI) Malignität. Das Risiko in jeder Kategorie ergab sich wie folgt: (I) 42%, (II) 0%, (III) 46%, (IVa) 3%, (IVb) 27%, (V) 72% und (VI) 97%. Die Resultate haben sich bereits als sehr wertvoll für die Entwicklung des offiziellen WHO-Klassifikationssystems für die Befundung der Zytopathologie von Weichteiltumore herausgestellt. Die Publikation dieses Systems ist für Ende 2024 angekündigt.

Artikel 2 folgte einer ähnlichen Herangehensweise wie Artikel 1, jedoch mit Fokus auf FNA und der Etablierung eines Klassifikationssystems für zytologische Analysen ausschließlich von Knochenraumforderungen. Die Studie umfasst 721 Fälle. Auch hier konnten wir zeigen, dass eine Differenzierung zwischen gut- und bösartigen Tumoren durch FNA relativ zuverlässig möglich ist. Darüber hinaus lassen sich einige Knochentumorentitäten sicher durch FNA diagnostizieren, andere hingegen nicht. Der Vorschlag eines Klassifikationssystem wurde im Vergleich zu dem in Artikel I etwas vereinfacht und umfasst hier sechs Kategorien: (I) Nicht diagnostisch, (II) Gutartig, (III) Atypi, (IV) Knochentumore mit ungewissem Malignitätspotential, (V) Verdacht auf Malignität und (VI) Malignität. Auch hier wurde das Malignitätsrisiko für jede Kategorie berechnet: (I) 48%, (II) 6.7%, (III) 69%, (IV) 28%, (V) 93%, (VI) 100%. Die Ergebnisse stellen eine

solide Basis für ein kommendes WHO-Klassifikationssystem für die Befundung der Zytopathologie von Knochentumoren dar.

In Artikel 3 untersuchten wir, ob genomische Arrayanalysen, mehr spezifisch Single Nucleotide Polymorphism (SNP) Arrays, ein geeignetes diagnostisches Werkzeug für Stanzbiopsien von Weichteil- und Knochentumoren darstellen (n=171). SNP-Arrays detektieren genomweit und mit hoher Auflösung den Verlust und Überschuss von genetischem Material in Form von Veränderungen in der relativen Anzahl von genomischen Kopien (copy number aberrations, CNA). Wir konnten zeigen, dass SNP-Arrays ein technisch robustes Werkzeug auch bei der Analyse von kleinen Biopsien darstellen. Die CNA-Profile sind in einem Großteil der untersuchten Fälle (87%) kompatibel zu den histologischen Diagnosen und wurden als charakteristisch für verschiedene spezifische Tumorentitäten identifiziert. Darüber konnten wir zeigen, dass CNA-profile von Stanzbiopsien in den meisten Fällen (77%) repräsentativ für den gesamten Tumor sind.

In Artikel 4 untersuchten wir bestimmte Tumorentitäten in der Haut, die der Familie der sogenannten Dermatofibrosarcoma protuberans (dermatofibrosarcoma family of tumors, DPFT) angehören. Verschiedene genetische Verfahren wurden genutzt, um ein besseres Verständnis von Krankheitsursache und -verlauf zu bekommen. Wir konnten zeigen, dass die bekannte *COL1A1*::*PDGFB*-Genfusion, die dieser Tumorfamilie zugrunde liegt, in unterschiedlichen Altersklassen ihren Ursprung in unterschiedlichen chromosomalen Abweichungen hat in den meisten Fällen wahrscheinlich nach der DNA-Replikation im Zellzyklus hat. Deutliche genetische Unterschiede zwischen gewöhnlichen und aggressiveren Varianten von DPFT konnten wir nicht identifizieren, tendenziell scheinen aber Verluste von genetischem Material auf Chromosom 9p eher mit aggressiven Tumoren in Verbindung zu stehen. Darüber hinaus sind in DPFT einige Gene heraufreguliert, die von therapeutischem Interesse sind.

Acknowledgements

Obviously, a lot of people were involved in this work, and I want to thank you all for your work and support on the way!

First of all, my supervisor **Fredrik**. Thank you for your rock-solid guidance, support and perpetual willingness for discussion. The award for being the humblest great cancer researcher and expert sarcoma geneticist is yours. I really enjoy that you always want to share "this interesting case that came in the other day", no matter how stressful your clinical duties are.

Henryk, my co-supervisor and colleague. Your devotion and expertise for cytology and sarcoma diagnostics as well as your endurance to always keep on writing one more cytology book is very inspiring. Thank you and **Annie** for being one of the first anchors in my social network, when I came to Sweden.

The marvelous Mertens group. **Jenny** and **Linda**, thank you very much for your work and patience (with my doubtfully smart questions). I really would have loved to do some more lab work with you! **Paul**, thank you for reliably solving all data-related obstacles and for finding applicable R packages within five minutes that I was chasing for several days. **Saskia**, thanks a lot, especially for keeping my degrading emigrant German running. **Elsa** and **Jakob**, the seniors, thank you for your work and support. **Nils**, many thanks for your input and your sharp eyes. Thank you, **Maria** and good luck with your thesis.

Many thanks to all the other good people at the **Division of Clinical Genetics**. Due to my rather intermittent presence at the BMC, I never got to know every one of you as well as it would have been appropriate. But it is of course never too late! **Anette**, thank you for your tremendous administrative work!

My colleagues at the **pathology department**. I really enjoy working with you! **Pehr** and **Emilia**, the sarcoma comrades. Unlikely, that anyone turns pages more efficiently than we do, trying to find a tumor in the books that looks like this never-seen-before one under the microscope. Many thanks to you and the **cytology group** for backing me up during my absences and for sharing my visions for the FNA clinic. **Gunilla**, **Margareta** and **Anders** thank you for your support and that it never has been a problem to release me from my clinical duties to do research.

Many thanks to the **sarcoma team** at Skåne University Hospital in Lund. You are part of a good, super interesting and friendly working environment. Despite the dimmed light during our tumor conferences, it is hard to get sleepy.

Min egen familj. **Pipi**, **Jacob** och **Kaspar**. Ni är bäst! Framför allt sedan ni, pojkar, tycker också att det är rimligt att stå upp först efter kl 5. Underbart att ni alla finns och jag ser fram emot allt som vi framöver kommer att hitta på tillsammans!

Meine deutsche Familie. **Els** und **Gerrit**, **Kathrin** und **Anna**. Ihr seid eine große Freude und Stütze in allen Lebenslagen! Danke für alles!

Στη **Θεώνη** και τον **Ελευθέριο**, την ελληνική οικογένειά μου, ένα μεγάλο ευχαριστώ για την υποστήριξή σας!

Meine alten **Freunde aus Aachen**, wo auch immer ihr mittlerweile abhängt. Ich verdanke euch viel und genieße unsere, mittlerweile leider seltenen, Begegnungen sehr.

I also want to sincerely thank my opponent **Raf Sciot** and the examination board **Ana Carneiro**, **Jacob Engellau** and **Anand Loya**. I hope that this book was not just a boring read. Thanks to **Anna Hagström** for chairing my defense.

esistderfehler
derevolutionmögl
ichmachtdennperfekt
ionerfordertkeineve
ränderungmutatione
nsindseitjeher
quellundtr
iebkr
af
tdesneu
enlebensund
ervielfaltvierb
austeinefüreinganz
esuniversumdochdiek
omplexitätdesleben
shataucheinekehrs
eitemutatione
nsindseit
jehe
rauchq
uellundtri
ebkraftvonkran
kheitzelluläreran
archiekonzertierten
fehlverhaltensorgan
isiertemmolekularen
chaosglitchesspr
üngerausche
nundehr
für
chtigefa
szinationmor
phologischeabwe
ichungandersartigk
eitnichtmehrnurüber
schussschwarzergall
eabernochimmertie
fundunergründ
etjenseit
sde
rstagna
tionortsver
ändertentwurfu
ndgegenentwurfdem
zufallentwachsenest
rategieevolutionimm
ikrokosmosdeskörpe
rsvierbaustein
efüreingan
zesuniv
ersu
mkomplexe
slebenohnetum
oreistundenkbarxx

References

1. Wu S, Zhu W, Thompson P, Hannun YA. Evaluating intrinsic and non-intrinsic cancer risk factors. *Nat Commun* 2018;9:3490.
2. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell* 2011;144:646–674.
4. Hanahan D. Hallmarks of Cancer: New Dimensions. *Cancer Discov* 2022;12:31-46.
5. Chen X, Zhang T, Su W, Dou Z, Zhao D, Jin X et al. Mutant p53 in cancer: from molecular mechanism to therapeutic modulation. *Cell Death Dis* 2022;13:974.
6. Sporn MB. The war on cancer. *Lancet* 1996;347:1377–1381.
7. Ai L, Xu A, Xu J. Roles of PD-1/PD-L1 Pathway: Signaling, Cancer, and Beyond. *Adv Exp Med Biol* 2020;1248:33–59.
8. Malone ER, Oliva M, Sabatini PJB, Stockley TL, Siu LL. Molecular profiling for precision cancer therapies. *Genome Med* 2020;12:8.
9. Liu ZL, Chen HH, Zheng LL, Sun LP, Shi L. Angiogenic signaling pathways and anti-angiogenic therapy for cancer. *Sig Transduct Target Ther* 2023;8:198.
10. Pellestor F, Gaillard JB, Schneider A, Puechberty J, Gatinois V. Chromoanagenesis, the mechanisms of a genomic chaos. *Semin Cell Dev Biol* 2022;123:90–99.
11. Voronina N, Wong JKL, Hübschmann D, Hlevnjak M, Uhrig S, Heilig CE et al. The landscape of chromothripsis across adult cancer types. *Nat Commun* 2020;11:2320.
12. Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 1990; 250:1233–1238.

13. WHO Classification of Tumours Editorial Board. Soft Tissue and Bone Tumours. (5th edn). Lyon:IARC Press; 2020.
14. Anderson WJ, Hornick JL. Immunohistochemical correlates of recurrent genetic alterations in sarcomas. *Genes Chromosomes Cancer* 2019;58:111–123.
15. Wakefield C, Hornick JL. Update on immunohistochemistry in bone and soft tissue tumors: Cost-effectively replacing molecular testing with immunohistochemistry. *Hum Pathol* 2024;147:58–71.
16. Italiano A, Di Mauro I, Rapp J, Pierron G, Auger N, Alberti L et al. Clinical effect of molecular methods in sarcoma diagnosis (GENSARC): a prospective, multicentre, observational study. *Lancet Oncol* 2016;17:532–538.
17. Diamantis A, Magiorkinis E, Koutselini H. Fine-needle aspiration (FNA) biopsy: historical aspects. *Folia Histochem Cytobiol* 2009;47:191–197.
18. Valent P, Groner B, Schumacher U, Superti-Furga G, Busslinger M, Kralovics R et al. Paul Ehrlich (1854-1915) and His Contributions to the Foundation and Birth of Translational Medicine. *J Innate Immun* 2016;8:111-120.
19. Kaufmann S. Paul Ehrlich: founder of chemotherapy. *Nat Rev Drug Discov* 2008;7:373.
20. Martin HE, Ellis EB. Biopsy by Needle Puncture and Aspiration. *Ann Surg* 1930;92:169.
21. Mannheim EP. Die Bedeutung der Tumorpunktion für die Tumordiagnose. *Z Krebsforsch* 1931;34:574–593.
22. Rosa M. Fine-needle aspiration biopsy: A historical overview. *Diagn Cytopathol* 2008;36:773–775.
23. Brunnström H, Darai-Ramqvist E, Domanski HA. Interventional and EBUS cytology in Sweden. Semin Diagn Pathol 2022;39:458–462.
24. Linsk JA. Aspiration cytology in sweden: The karolinska group. *Diagn Cytopathol* 1985;1:332–335.
25. Fraznén S, Zajicek J. Aspiration biopsy in diagnosis of palpable lesions of the breast. Critical review of 3479 consecutive biopsies. *Acta Radiol Ther Phys Biol* 1968;7:241–262.
26. Eneroth CM, Franzén S, Zajicek J. Cytologic diagnosis on aspirate from 1000 salivary-Gland tumours. *Acta Otolaryngol* 1967;63:168–172.

27. Engzell U, Esposti PL, Rubio C, Sigurdson Å, Zajicek J. Investigation on tumour spread in connection with aspiration biopsy. *Acta Radiol Ther Phys Biol* 1971;10:385–398.
28. Zajicek J. Aspiration Biopsy Cytology. Basel:Karger;1974.
29. Akerman M, Rydholm A, Persson BM. Aspiration cytology of soft-tissue tumors. The 10-year experience at an orthopedic oncology center. *Acta Orthop Scand* 1985;56:407–12.
30. Willén H, Akerman M, Carlén B. Fine needle aspiration (FNA) in the diagnosis of soft tissue tumours; a review of 22 years experience. *Cytopathology* 1995;6:236–247.
31. Akerman M, Berg NO, Persson BM. Fine needle aspiration biopsy in the evaluation of tumor-like lesions of bone. *Acta Orthop Scand* 1976;47:129–136.
32. Domanski HA, Åkerman M. Fine-needle aspiration of primary osteosarcoma: a cytological-histological study. *Diagn Cytopathol* 2005;32:269–275.
33. Akerman M, Domanski HA. The cytology of soft tissue tumours. Monogr Clin Cytol Basel:Karger;2003.
34. Domanski HA, Åkerman M, Engellau J, Gustafson P, Mertens F, Rydholm, A. Fine-needle aspiration of neurilemoma (schwannoma). A clinicocytopathologic study of 116 patients. *Diagn Cytopathol* 2006;34:403–412.
35. Domanski HA. Fine-needle aspiration cytology of soft tissue lesions: Diagnostic challenges. *Diagn Cytopathol* 2007;35:768–773.
36. Domanski HA, Åkerman M, Carlén B, Engellau J, Gustafson P, Jonsson K et al. Core-needle biopsy performed by the cytopathologist: A technique to complement fine-needle aspiration of soft tissue and bone lesions. *Cancer* 2005;105:229–239.
37. Stewart FW. The Diagnosis of Tumors by Aspiration. *Am J Pathol* 1933;9:801.
38. Grohs HK. The interventional cytopathologist. A new clinician/pathologist hybrid. *Am J Clin Pathol* 1988;90:351–354.
39. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998;279:577–580.

40. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N et al. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003;299:708–710.
41. Lasota J, Miettinen M. Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal tumours. *Histopathology* 2008;53:245–266.
42. Mitelman F, Johansson B, Mertens F (Eds.). Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. 2024. https://mitelmandatabase.isb-cgc.org
43. Mertens F, Johansson B, Fioretos T, Mitelman F. The emerging complexity of gene fusions in cancer. *Nat Rev Cancer* 2015;15:371–381.
44. Schram AM, Chang MT, Jonsson P, Drilon A. Fusions in solid tumours: Diagnostic strategies, targeted therapy, and acquired resistance. *Nat Rev Clin Oncol* 2017;14:735–748.
45. Tang YC, Amon A. Gene copy-number alterations: A cost-benefit analysis. *Cell* 2013;152:394–405.
46. Amary MF, Pauwels P, Meulemans E, Roemen GM, Islam L, Idowu B et al. Detection of β-catenin mutations in paraffin-embedded sporadic desmoid-type fibromatosis by mutation-specific restriction enzyme digestion (MSRED): an ancillary diagnostic tool. *Am J Surg Pathol* 2007;31:1299–1309.
47. Bartuma H, Nord KH, Macchia G, Isaksson M, Nilsson J, Domanski HA et al. Gene expression and single nucleotide polymorphism array analyses of spindle cell lipomas and conventional lipomas with 13q14 deletion. *Genes Chromosomes Cancer* 2011;50:619–632.
48. Santarius T, Shipley J, Brewer D, Stratton MR, Cooper CS. A census of amplified and overexpressed human cancer genes. *Nat Rev Cancer* 2010;10:59–64.
49. Gisselsson D, Pålsson E, Höglund M, Domanski HA, Mertens F, Pandis N et al. Differentially amplified chromosome 12 sequences in low- and high-grade osteosarcoma. *Genes Chromosomes Cancer* 2002;33:133–140.
50. Lee ATJ, Thway K, Huang PH, Jones RL. Clinical and molecular spectrum of liposarcoma. *J Clin Oncol* 2018;36:151–159.
51. Sullivan LM, Folpe AL, Pawel BR, Judkins AR, Biegel JA. Epithelioid sarcoma is associated with a high percentage of SMARCB1 deletions. *Mod Pathol* 2013;26:385–392.

52. Brennan B, Stiller C, Bourdeaut F. Extracranial rhabdoid tumours: what we have learned so far and future directions. *Lancet Oncol* 2013;14:e329-36.
53. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors guideline from the college of American pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Arch Pathol Lab Med* 2018;142:321–346.
54. Charville GW, Longacre TA. Surgical Pathology of Gastrointestinal Stromal Tumors: Practical Implications of Morphologic and Molecular Heterogeneity for Precision Medicine. *Adv Anat Pathol* 2017;24:336–353.
55. van IJzendoorn DGP, Szuhai K, Briaire-De Bruijn IH, Kostine M, Kuijjer ML, Bovée JVMG. Machine learning analysis of gene expression data reveals novel diagnostic and prognostic biomarkers and identifies therapeutic targets for soft tissue sarcomas. *PLoS Comput Biol* 2019;15:e1006826.
56. Maitra A, Ashfaq R, Saboorian MH, Lindberg G, Gokaslan ST. The role of fine-needle aspiration biopsy in the primary diagnosis of mesenchymal lesions: a community hospital-based experience. *Cancer* 2000;90:178–185.
57. Nagira K, Yamamoto T, Akisue T, Marui T, Hitora T, Nakatani T et al. Reliability of fine-needle aspiration biopsy in the initial diagnosis of soft-tissue lesions. *Diagn Cytopathol* 2002;27:354–361.
58. Lima PM, Oliveira MP, da Silva HJ, de Mello RJV. The role of cytology in the diagnosis of musculoskeletal neoplasms: systematic review. *Acta Ortop Bras* 2012;20:48–52.
59. Hirachand S, Lakhey M, Singha AK, Devkota S, Akhter J. Fine needle aspiration (FNA) of soft tissue tumours (STT). *Kathmandu Univ Med J (KUMJ)* 2007;5:374–377.
60. Kasraeian S, Allison DC, Ahlmann ER, Fedenko AN, Menendez LR. A Comparison of Fine-needle Aspiration, Core Biopsy, and Surgical Biopsy in the Diagnosis of Extremity Soft Tissue Masses. *Clin Orthop Relat Res* 2010;468:2992–3002.
61. Liu K, Layfield LJ, Coogan AC, Ballo MS, Bentz JS, Dodge RK. Diagnostic accuracy in fine-needle aspiration of soft tissue and bone

lesions. Influence of clinical history and experience. *Am J Clin Pathol* 1999;111:632–40.
62. Kilpatrick SE, Ward WG, Chauvenet AR, Pettenati MJ. The role of fine-needle aspiration biopsy in the initial diagnosis of pediatric bone and soft tissue tumors: an institutional experience. *Mod Pathol* 1998;11:923–928.
63. Yang YJ, Damron TA. Comparison of needle core biopsy and fine-needle aspiration for diagnostic accuracy in musculoskeletal lesions. *Arch Pathol Lab Med* 2004;128:759–764.
64. Klijanienko J, Caillaud JM, Lagacé R. Fine-needle aspiration of primary and recurrent benign fibrous histiocytoma: classic, aneurysmal, and myxoid variants. *Diagn Cytopathol* 2004;31:387–391.
65. Domanski HA, Mertens F, Panagopoulos I, Åkerman M. Low-grade fibromyxoid sarcoma is difficult to diagnose by fine needle aspiration cytology: a cytomorphological study of eight cases. *Cytopathology* 2009;20:304–314.
66. Olson MT, Ali SZ. Myxofibrosarcoma: Cytomorphologic Findings and Differential Diagnosis on Fine Needle Aspiration. *Acta Cytol* 2012;56:15–24.
67. Woyke S, Kapila K, Goswami KC. Atypical lipoma as a potential pitfall in the cytodiagnosis of subcutaneous tumors. A report of two cases. *Acta Cytol* 1997;41:897–902.
68. Kapila K, Ghosal N, Gill SS, Verma K. Cytomorphology of Lipomatous Tumors of Soft Tissue. *Acta Cytol* 2003;47:555–562.
69. Sugiyama K, Washimi K, Sato S, Hiruma T, Sakai M, Okubo Y et al. Differential diagnosis of lipoma and atypical lipomatous tumor/well-differentiated liposarcoma by cytological analysis. *Diagn Cytopathol* 2022;50:112-122.
70. Ali SZ, Baloch ZW, Cochand-Priollet B, Schmitt FC, Vielh P, Vanderlaan PA. The 2023 Bethesda System for Reporting Thyroid Cytopathology. *Thyroid* 2023; 33: 1039–1044.
71. Faquin W, Rossi E, Batoch Z, Barkan G, Foschini M, Kurtycz D et al. The Milan System for Reporting Salivary Gland Cytopathology. New York;Springer:2023.
72. International Academy of Cytology - International Agency for Research on Cancer - World Health Organization Joint Editorial Board. WHO Reporting System for Lung Cytopathology. (1st edn). IARC;Lyon:2022.

73. International Academy of Cytology – International Agency for Research on Cancer – World Health Organization Joint Editorial Board. WHO Reporting System for Pancreaticobiliary Cytopathology. (1st edn). IARC;Lyon:2022.
74. Saoud C, Lam H, Gross JM, Ali SZ. Fine needle aspiration of bone lesions: A tertiary care centre experience. *Cytopathology* 2023;34:562–572.
75. Bommer KK, Ramzy I, Mody D. Fine-needle aspiration biopsy in the diagnosis and management of bone lesions: a study of 450 cases. *Cancer* 1997;81:148–156.
76. Chambers M, O'Hern K, Kerr DA. Fine-needle aspiration biopsy for the diagnosis of bone and soft tissue lesions: a systematic review and meta-analysis. *J Am Soc Cytopathol* 2020;9:429–441.
77. Chaudhary A, Sharma SK, Sharma N, Sharma R, Lal M. Diagnostic accuracy of fine needle aspiration cytology of bone lesions: A study of Tertiary Care Institute. *J Cancer Res Ther* 2024;20:133–138.
78. Jorda M, Rey L, Hanly A, Ganjei-Azar P. Fine-needle aspiration cytology of bone: accuracy and pitfalls of cytodiagnosis. *Cancer* 2000;90:47–54.
79. Layfield LJ. Cytologic diagnosis of osseous lesions: A review with emphasis on the diagnosis of primary neoplasms of bone. *Diagn Cytopathol* 2009;37:299–310.
80. Wahane RN, Lele VR, Bobhate SK. Fine needle aspiration cytology of bone tumors. *Acta Cytol* 2007;51:711–20.
81. Vangala N, Uppin SG, Pamu PK, Hui M, Nageshwara Rao K, Chandrashekar P. Fine-Needle Aspiration Cytology in Preoperative Diagnosis of Bone Lesions: A Three-Year Study in a Tertiary Care Hospital. *Acta Cytol* 2021;65:75–87.
82. Vetrani A, Fulciniti F, Boschi R, Marino G, Zeppa P, Troncone G et al. Fine needle aspiration biopsy diagnosis of giant-cell tumor of bone. An experience with nine cases. *Acta Cytol* 1990;34:863-867.
83. Huening MA, Reddy S, Dodd LG. Fine-needle aspiration of fibrous dysplasia of bone: a worthwhile endeavor or not? *Diagn Cytopathol* 2008;36:325–330.
84. Walther C, Domanski HA, von Steyern FV, Mandahl N, Mertens F. Chromosome banding analysis of cells from fine-needle aspiration biopsy samples from soft tissue and bone tumors: is it clinically meaningful? *Cancer Genet* 2011;204:203–206.

85. Hasegawa T, Yamamoto S, Nojima T, Hirose T, Nikaido T, Yamashiro K et al. Validity and reproducibility of histologic diagnosis and grading for adult soft-tissue sarcomas. *Hum Pathol* 2002;33:111–115.
86. Skeletal Lesions Interobserver Correlation among Expert Diagnosticians (SLICED) Study Group. Reliability of histopathologic and radiologic grading of cartilaginous neoplasms in long bones. *J Bone Joint Surg Am* 2007;89:2113–2123.
87. Mertens F, Antonescu CR, Mitelman F. Gene fusions in soft tissue tumors: Recurrent and overlapping pathogenetic themes. *Genes Chromosomes Cancer* 2016;55:291–310.
88. Dentro SC, Leshchiner I, Haase K, Tarabichi M, Wintersinger J, Deshwar AG et al. Characterizing genetic intra-tumor heterogeneity across 2,658 human cancer genomes. Cell 2021;184:2239-2254.e39.
89. Van Loo P, Nordgard SH, Lingjærde OC, Russnes HG, Rye IH, Sun W et al. Allele-specific copy number analysis of tumors. *Proc Natl Acad Sci USA* 2010;107:16910–16915.
90. Mitra S, Farswan A, Piccinelli P, Sydow S, Hesla A, Tsagkozis P et al. Transcriptomic profiles of myxofibrosarcoma and undifferentiated pleomorphic sarcoma correlate with clinical and genomic features. *J Pathol* 2024. doi:10.1002/path.6347.
91. Pedeutour F, Simon MP, Minoletti F, Barcelo G, Terrier-Lacombe MJ, Combemale P et al. Translocation, t(17;22)(q22;q13), in dermatofibrosarcoma protuberans: a new tumor-associated chromosome rearrangement. *Cytogenet Cell Genet* 1996;72:171–174.
92. Terrier-Lacombe MJ, Guillou L, Maire G, Terrier P, Vince DR, de Saint Aubain Somerhausen N et al. Dermatofibrosarcoma protuberans, giant cell fibroblastoma, and hybrid lesions in children: Clinicopathologic comparative analysis of 28 cases with molecular data - a study from the french federation of cancer centers sarcoma group. *Am J Surg Pathol* 2003;27:27–39.
93. Sirvent N, Maire G, Pedeutour F. Genetics of dermatofibrosarcoma protuberans family of tumors: From ring chromosomes to tyrosine kinase inhibitor treatment. *Genes Chromosomes Cancer* 2003;37:1–19.
94. O'Brien KP, Seroussi E, Dal Cin P, Sciot R, Mandahl N, Fletcher JA et al. Various regions within the alpha-helical domain of the COL1A1 gene are

fused to the second exon of the PDGFB gene in dermatofibrosarcomas and giant-cell fibroblastomas. *Genes Chromosomes Cancer* 1998;23:187–193.
95. Peng C, Jian X, Xie Y, Li L, Ouyang J, Tang L et al. Genomic alterations of dermatofibrosarcoma protuberans revealed by whole-genome sequencing. *Br J Dermatol* 2022;186:997–1009.
96. Pedeutour F, Simon MP, Minoletti F, Sozzi G, Pierotti MA, Hecht F et al. Ring 22 chromosomes in dermatofibrosarcoma protuberans are low-level amplifiers of chromosome 17 and 22 sequences. *Cancer Res* 1995;55:2400–2403.
97. Rebouissou S, Hérault A, Letouzé E, Neuzillet Y, Laplanche A, Ofualuka K et al. CDKN2A homozygous deletion is associated with muscle invasion in FGFR3-mutated urothelial bladder carcinoma. *J Pathol* 2012;227:315–324.
98. Sledzinska P, Bebyn MG, Furtak J, Kowalewski J, Lewandowska MA. Prognostic and Predictive Biomarkers in Gliomas. *Int J Mol Sci* 2021;22:10373.
99. Lu Y, Li T, Chen M, Peng H, Du T, Qiu Y et al. Coamplification of 12q15 and 12p13 and homozygous CDKN2A/2B deletion: synergistic role of fibrosarcomatous transformation in dermatofibrosarcoma protuberans with a cryptic COL1A1-PDGFB fusion. *Virchows Arch* 2022;481:313–319.
100. Dadone-Montaudié B, Alberti L, Duc A, Delespaul L, Lesluyes T, Pérot G et al. Alternative PDGFD rearrangements in dermatofibrosarcomas protuberans without PDGFB fusions. *Mod Pathol* 2018;31:1683–1693.
101. Stacchiotti S, Astolfi A, Gronchi A, Fontana A, Pantaleo MA, Negri T et al. Evolution of Dermatofibrosarcoma Protuberans to DFSP-Derived Fibrosarcoma: An Event Marked by Epithelial-Mesenchymal Transition-like Process and 22q Loss. *Mol Cancer Res* 2016;14:820–829.
102. Eilers G, Czaplinski JT, Mayeda M, Bahri N, Tao D, Zhu M et al. CDKN2A/p16 Loss Implicates CDK4 as a Therapeutic Target in Imatinib-Resistant Dermatofibrosarcoma Protuberans. *Mol Cancer Ther* 2015;14:1346–1353.
103. Dickson BC, Hornick JL, Fletcher CDM, Demicco EG, Howarth DJ, Swanson D et al. Dermatofibrosarcoma protuberans with a

novel COL6A3-PDGFD fusion gene and apparent predilection for breast. *Genes Chromosomes Cancer* 2018;57:437–445.

104. Linn SC, West RB, Pollack JR, Zhu S, Hernandez-Boussard T, Nielsen TO et al. Gene Expression Patterns and Gene Copy Number Changes in Dermatofibrosarcoma Protuberans. *Am J Pathol* 2003;163:2383–2395.

105. Cazzato G, Mangialardi K, Falcicchio G, Colagrande A, Ingravallo G, Arezzo F et al. Preferentially Expressed Antigen in Melanoma (PRAME) and Human Malignant Melanoma: A Retrospective Study. *Genes (Basel)* 2022;13:545.

106. Roszik J, Wang WL, Livingston JA, Roland CL, Ravi V, Yee C et al. Overexpressed PRAME is a potential immunotherapy target in sarcoma subtypes. *Clin Sarcoma Res* 2017;7:11.

107. Croce S, Hostein I, Longacre TA, Mills AM, Pérot G, Devouassoux-Shisheboran M et al. Uterine and vaginal sarcomas resembling fibrosarcoma: a clinicopathological and molecular analysis of 13 cases showing common NTRK-rearrangements and the description of a COL1A1-PDGFB fusion novel to uterine neoplasms. *Mod Pathol* 2019;32:1008–1022.

108. Grindstaff SL, DiSilvestro J, Hansen K, DiSilvestro P, Sung CJ, Quddus MR. COL1A1-PDGFB fusion uterine fibrosarcoma: A case report with treatment implication. *Gynecol Oncol Rep* 2019;31:100523.

109. Rota S, Franza A, Fabbroni C, Paolini B, Greco FG, Alessi A et al. COL1A1::PDGFB fusion-associated uterine fibrosarcoma: A case report and review of the literature. *Cancer Rep (Hoboken)* 2024;7:e1969.

110. Lu Y, Chen X, Zeng W, Hua P, Shen Y, Qiu Y et al. COL1A1::PDGFB fusion uterine sarcoma with a TERT promoter mutation. *Genes Chromosomes Cancer* 2024;63:e23210.

111. Layfield LJ, Dodd L, Esebua M. World Health Organization Reporting System for Soft Tissue Cytopathology: Risk of malignancy and reproducibility of categories among observers. *Diagn Cytopathol* 2024;52:480-484.

112. Saoud C, Schowinsky J, Ali SZ. Myxoid Soft Tissue Tumors: A 20-Year Experience on Fine Needle Aspiration with Application of the Proposed WHO Reporting System for Soft Tissue Cytopathology. *Acta Cytol* 2023;67:468–481.

113. Gajdzis P, Brisse HJ, Klijanienko J. Diagnostic performance of fine-needle aspiration in soft tissue tumors: Application of the World Health Organization System for Reporting Soft Tissue Cytopathology and risk of malignancy assessment. *Cancer Cytopathol* 2024. doi:10.1002/cncy.22897.
114. Domanski HA, Carlén B, Jonsson K, Mertens F, Åkerman M. Distinct cytologic features of spindle cell lipoma. A cytologic-histologic study with clinical, radiologic, electron microscopic, and cytogenetic correlations. *Cancer* 2001;93:381–389.
115. Abele JS. The case for pathologist ultrasound-guided fine-needle aspiration biopsy. *Cancer* 2008;114:463–468.
116. Abele JS. Private practice outpatient fine needle aspiration clinic: A 2018 update. *Cancer Cytopathol* 2018;126:902–923.
117. Bellevicine C, Vigliar E, Malapelle U, Pisapia P, Conzo G, Biondi B et al. Cytopathologists can reliably perform ultrasound-guided thyroid fine needle aspiration: A 1-year audit on 3715 consecutive cases. *Cytopathology* 2016;27:115–121.
118. Paksoy N, Ozbek B. Cytopathologist-performed and ultrasound-guided fine needle aspiration cytology enhances diagnostic accuracy and avoids pitfalls: An overview of 20 years of personal experience with a selection of didactic cases. *Cytojournal* 2018;15:8.
119. Wu M, Choi Y, Zhang Z, Si Q, Salem F, Szporn A et al. Ultrasound guided FNA of thyroid performed by cytopathologists enhances Bethesda diagnostic value. *Diagn Cytopathol* 2016;44:787–791.
120. Öfverholm I, Wallander K, Haglund C, Chellappa V, Wejde J, Gellerbring A et al. Comprehensive Genomic Profiling Alters Clinical Diagnoses in a Significant Fraction of Tumors Suspicious of Sarcoma. *Clin Cancer Res* 2024;30:2647-2658.
121. Koelsche C, Stichel D, Griewank KG, Schrimpf D, Reuss DE, Bewerunge-Hudler M et al. Genome-wide methylation profiling and copy number analysis in atypical fibroxanthomas and pleomorphic dermal sarcomas indicate a similar molecular phenotype. *Clin Sarcoma Res* 2019;9:2.
122. de Ligt J, Boone PM, Pfundt R, Vissers LE, Richmond T, Geoghegan J et al. Detection of clinically relevant copy number variants with whole-exome sequencing. *Hum Mutat* 2013;34:1439–1448.

123. Yao R, Yu T, Qing Y, Wang J, Shen Y. Evaluation of copy number variant detection from panel-based next-generation sequencing data. *Mol Genet Genomic* Med 2019;7:e00513.
124. Van der Linden M, Raman L, Trappen A Vander, Dheedene A, De Smet M, Sante T et al. Detection of Copy Number Alterations by Shallow Whole-Genome Sequencing of Formalin-Fixed, Paraffin-Embedded Tumor Tissue. *Arch Pathol Lab Med* 2019;144:974–981.
125. Steele CD, Abbasi A, Islam SMA, Bowes AL, Khandekar A, Haase K et al. Signatures of copy number alterations in human cancer. *Nature* 2022;606:984–991.
126. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001;98:10869–10874.
127. Lilljebjörn H, Orsmark-Pietras C, Mitelman F, Hagström-Andersson A, Fioretos T. Transcriptomics paving the way for improved diagnostics and precision medicine of acute leukemia. *Semin Cancer Biol* 2022;84:40–49.
128. Nakayama R, Nemoto T, Takahashi H, Ohta T, Kawai A, Seki K et al. Gene expression analysis of soft tissue sarcomas: Characterization and reclassification of malignant fibrous histiocytoma. *Mod Pathol* 2007;20:749–759.
129. Beird HC, Wu CC, Ingram DR, Wang WL, Alimohamed A, Gumbs C et al. Genomic profiling of dedifferentiated liposarcoma compared to matched well-differentiated liposarcoma reveals higher genomic complexity and a common origin. *Cold Spring Harb Mol Case Stud* 2018;4:a002386.
130. Lesluyes T, Pérot G, Largeau MR, Brulard C, Lagarde P, Dapremont V et al. RNA sequencing validation of the Complexity INdex in SARComas prognostic signature. *Eur J Cancer* 2016;57:104–111.
131. Lucchesi C, Khalifa E, Laizet Y, Soubeyran I, Mathoulin-Pelissier S, Chomienne C et al. Targetable Alterations in Adult Patients with Soft-Tissue Sarcomas: Insights for Personalized Therapy. *JAMA Oncol* 2018;4:1398–1404.
132. Karadzovska-Kotevska M, Brunnström H, Kosieradzki J, Ek L, Estberg C, Staaf J et al. Feasibility of EBUS-TBNA for histopathological

and molecular diagnostics of NSCLC-A retrospective single-center experience. *PLoS One* 2022;17:e0263342.

133. Pisapia P, Pepe F, Iaccarino A, Sgariglia R, Nacchio M, Conticelli F et al. Next Generation Sequencing in Cytopathology: Focus on Non-Small Cell Lung Cancer. *Front Med (Lausanne)* 2021;8:633923.

134. Jurmeister P, Lenze D, Berg E, Mende S, Schäper F, Kellner U et al. Parallel screening for ALK, MET and ROS1 alterations in non-small cell lung cancer with implications for daily routine testing. *Lung Cancer* 2015;87:122–129.

135. Pagliuca F, Ronchi A, Cozzolino I, Montella M, Zito Marino F, Franco R. Mesenchymal neoplasms: Is it time for cytology? New perspectives for the pre-operative diagnosis of soft tissue tumors in the molecular era. *Pathol Res Pract* 2020;216:152923.

136. Dal Cin P, Qian X, Ciba ES. The marriage of cytology and cytogenetics. *Cancer Cytopathol* 2013;121:279–290.

137. Hornick JL. Limited biopsies of soft tissue tumors: the contemporary role of immunohistochemistry and molecular diagnostics. *Mod Pathol* 2019;32:27–37.

138. Siddhartha Mukherjee. The Emperor of All Maladies - A Biography of Cancer. London;FourthEstate:2011