VILNIUS UNIVERSITY
FACULTY OF MEDICINE

Medicine

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INTEGRATED STUDY MASTER THESIS

ALK-1 Mutated Skin Tumors: A Case Report and Literature Review

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Vilnius, 2026

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2. Abbreviations

5A4 – ALK antibody clone (used in immunohistochemistry)

ABCG2 – ATP-binding cassette sub-family G member 2

ACLC – Anaplastic large cell lymphoma

ALK – Anaplastic lymphoma kinase

ALKAL – ALK and LTK ligand

ATP – Adenosine triphosphate

CARS – Cysteinyl-tRNA synthetase

CD30 – Cluster of differentiation 30

CD34 – Cluster of differentiation 34

CLIP2 – CAP-Gly domain containing linker protein 2

COL1A1 – Collagen type I alpha 1

CREB – cAMP response element-binding protein fusion

D5F3 – ALK antibody clone (used in immunohistochemistry)

DCTN1 – Dynactin subunit 1

Desmin – Muscle-specific intermediate filament protein

DFSP – Dermatofibrosarcoma protuberans

DNA – Deoxyribonucleic acid

EFH – Epithelioid fibrous histiocytoma

EGF – Epidermal growth factor

EIMS – Epithelioid inflammatory myofibroblastic sarcoma

EML4 – Echinoderm microtubule-associated protein-like 4

EMA – Epithelial membrane antigen

ERK – Extracellular signal-regulated kinase

EWSR1 – Ewing sarcoma breakpoint region 1

FDA – U.S. Food and Drug Administration

FISH – Fluorescence in situ hybridization

FLNA – Filamin A

FUS - Fused in sarcoma

HMB-45 – Human melanoma black 45

HPF – High power fields

IHC – Immunohistochemistry

IMT – Inflammatory myofibroblastic tumor

IPT-like FDCS – Inflammatory pseudotumor-like follicular dendritic cell sarcoma

JAK – Janus kinase (protein tyrosine kinase family)

LTK – Leukocyte tyrosine kinase

miRNA – MicroRNA

MRT – Magnetic Resonance Tomography

MSA – Muscle-specific actin

MYH9 – Myosin heavy chain 9

NGS – Next-generation sequencing

NPM – Nucleophosmin

NSCLC – Non-small cell lung cancer

NTRK – Neurotrophic tyrosine receptor kinase

PanCK – Pancytokeratin

PDGFRA – Platelet-derived growth factor receptor alpha

PI3K – Phosphatidylinositol 3-kinase

PLEKH2 – Pleckstrin homology domain-containing family H member 2

PRKAR2A – Protein kinase cAMP-dependent type II regulatory subunit alpha

RANBP – RAN binding protein

RNA – Ribonucleic acid

ROS1 – ROS proto-oncogene 1 (receptor tyrosine kinase)

RRBP1 – Ribosome binding protein 1

RT-PCR – Reverse transcription polymerase chain reaction

S100 – S100 calcium-binding protein

SMA – Smooth muscle actin

SOX10 – SRY-box transcription factor 10

SQSTM1 – Sequestosome 1

STAT – Signal transducer and activator of transcription

TFE3 – Transcription factor E3

TGF – Transforming growth factor

TIMP3 – Tissue inhibitor of metalloproteinases 3

TKI – Tyrosine kinase inhibitor

TPM3 – Tropomyosin 3

USP – Ubiquitin-specific peptidase

VCL – Vinculin

WHO – World Health Organization

3. Summary

Anaplastic lymphoma kinase (ALK)-mutated tumors of the skin represent a rare and heterogeneous group of mesenchymal neoplasms that present significant diagnostic challenges due to their overlapping morphology and immunophenotypic features. This thesis aims to provide a structured overview of their histopathological morphology, molecular genetics, clinical implications, diagnostic approaches, and therapeutic aspects.

The structured literature review was conducted using PubMed and Google Scholar, focusing on studies on ALK-positive neoplasms and their diagnostic strategies. Key diagnostic modalities, including immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and next-generation sequencing (NGS), were evaluated for their utility and limitations. In addition, it examines key entities, including inflammatory myofibroblastic tumor (IMT), epithelioid fibrous histiocytoma (EFH), and epithelioid inflammatory myofibroblastic sarcoma (EIMS).

The case report describes a 22-year-old female presenting with a spindle cell tumor of the neck. Initial histopathological and immunohistochemical findings suggested a low-grade mesenchymal neoplasm, but a definitive diagnosis remained uncertain due to overlapping features with other entities. In the end, a TPM3::ALK fusion was identified with genetic testing, supporting the diagnosis of an ALK-rearranged tumor most consistent with an inflammatory myofibroblastic tumor. No recurrence or metastasis was observed during 2-year follow-up after complete surgical excision.

The findings underscore the important role of genetic and molecular testing in the diagnostics and classification of ALK-positive neoplasms. Although immunohistochemistry is a valuable screening tool, it can yield false-positive or false-negative results, which require confirmation through molecular testing such as fluorescence in situ hybridization or next-generation sequencing, especially in cases of unresectable or advanced disease.

In conclusion, the expanding spectrum of ALK-rearranged neoplasms requires a structured diagnostic approach, including histopathological, immunohistochemical, and molecular data. Improved characterization, classification, and long-term follow-up data are essential to refine diagnostic criteria and optimize treatment strategies for these rare tumors in the future.

4. Santrauka

Odos navikai su anaplastinės limfomos kinazės mutacijomis sudaro retą ir nevienalytę mezenchiminių navikų grupę, kuri kelia didelių diagnostinių sunkumų dėl tarpusavyje panašios morfologijos ir imunofenotipinių savybių. Šioje disertacijoje siekiama pateikti sistemingą jų

histopatologinės morfologijos, molekulinės genetikos, klinikinių pasekmių, diagnostikos metodų ir gydymo aspektų apžvalgą.

Struktūruota literatūros apžvalga buvo atlikta naudojantis duomenų bazėmis „PubMed“ ir „Google Scholar“, daugiausia dėmesio skiriant tyrimams apie ALK-teigiamus navikus ir jų diagnostikos strategijas. Buvo įvertintas pagrindinių diagnostikos metodų, įskaitant imunohistocheminį tyrimą (IHC), fluorescencinę in situ hibridizaciją (FISH) ir naujos kartos sekvenavimą (NGS), naudingumas ir trūkumai. Be to, šiame darbe aptariami pagrindiniai navikai, įskaitant uždegiminį miofibroblastinį naviką (IMT), epitelioidinę fibrozinę histiocitomą (EFH) ir epitelioidinę uždegiminę miofibroblastinę sarkomą (EIMS).
Šiame klinikinio atvejo aprašyme analizuojamas 22 metų moters paviršinis kaklo srities mezenchiminis navikas, turintis ryšį su oda. Pirminiai histopatologiniai ir imunohistocheminiai tyrimų rezultatai leido įtarti žemo piktybiškumo mezenchiminės kilmės sarkomą. Tačiau atlikus išplėstinį likutinio naviko patologinį ištyrimą ir genetinius testus buvo nustatyta TPM3::ALK susiliejimas, patvirtinantis ALK-mutuoto naviko diagnozę, kuri labiausiai atitiko uždegiminį miofibroblastinį naviką. Per 2 metus trukusį stebėjimo laikotarpį po radikalaus chirurginio pašalinimo naviko recidyvų ar metastazių nepastebėta.
Šie duomenys pabrėžia genetinių ir molekulinių tyrimų svarbą diagnozuojant ir klasifikuojant ALK-teigiamus navikus. Nors imunohistocheminis tyrimas yra vertinga atrankos priemonė, jis gali duoti klaidingai teigiamus arba klaidingai neigiamus rezultatus, kuriuos būtina patvirtinti atliekant molekulinius tyrimus, pavyzdžiui, FISH arba NGS, ypač tais atvejais, kai liga yra neoperuojama arba pažengusi.
Apibendrinant galima teigti, kad dėl vis dažniau nustatomų ALK-mutuotų navikų būtinas kompleksinis ir sistemingas diagnostinis požiūris, apimantis histopatologinius, imunohistocheminius ir molekulinius duomenis. Siekiant ateityje patobulinti šių retų navikų diagnostinius kriterijus ir optimizuoti jų gydymo strategijas, būtina geriau apibūdinti šiuos navikus, juos klasifikuoti ir rinkti ilgalaikio stebėjimo duomenis.

5. Key words

“ALK”, “ALK in cutaneous neoplasms”, “ALK inhibitors”, “anaplastic lymphoma kinase”, “ALK-rearranged”, “inflammatory myofibroblastic tumor”.

6. Introduction

6.1 Why focus on ALK-mutated skin tumors?

ALK-rearranged mesenchymal neoplasms of the skin and subcutis constitute a rare, morphologically heterogeneous group of tumors that frequently pose diagnostic challenges due to overlapping histologic features with other spindle-cell and epithelioid lesions. (1) These tumors share oncogenic ALK fusions that drive constitutive kinase signaling through pathways like JAK/STAT, RAS/ERK, and PI3K/AKT, resulting in variable ALK protein expression detectable by immunohistochemistry. (2,3)

For several reasons, focusing on ALK-mutated skin tumors is clinically and scientifically justified. In recent years, numerous cytogenetic alterations and novel ALK mutations have been identified in various neoplastic processes, particularly cutaneous malignancies. (4) Consequently, the biological and clinical significance of ALK has become increasingly recognized. ALK expression can serve as a useful diagnostic marker for different cutaneous and noncutaneous mesenchymal neoplasms, including inflammatory myofibroblastic tumors and fibrous histiocytomas. (5) Since ALK can help distinguish between different neoplasms that may share similar morphology, it has increasingly become an important marker and diagnostic tool in pathology. Especially, dermatopathologists commonly use ALK for diagnosing soft tissue and cutaneous hematolymphoid neoplasms. Considering the skin may be a site with a wide spectrum of hematolymphoid and fibroblastic proliferations the use of ALK immunohistochemistry has been expanding. (6)

Since ALK-targeted therapies have already significantly improved the treatment outcomes for ALK-positive non–small cell lung cancer, they may also be a promising therapeutic approach for cutaneous neoplasms, such as melanoma and cutaneous lymphomas. (5) The heterogeneity and rarity of ALK-positive cutaneous tumors, together with substantial gaps in knowledge regarding the full spectrum of ALK-driven diseases, underline the need for further research to optimize diagnostic strategies and therapeutic approaches. (7) Overall, identifying ALK mutations or gene rearrangements in skin tumors has diagnostic, prognostic, and therapeutic implications.

To further close the knowledge gaps, this thesis provides a review of the clinicopathologic spectrum, molecular genetics, histopathology, and clinical implications of ALK-mutated soft tissue tumors of the skin. It will include a detailed analysis of the tumors' histopathological profiles, immunohistochemical and molecular diagnostic approaches, and a case-based perspective to illustrate core concepts. Additionally, the thesis will evaluate current therapeutic strategies, including the role of ALK inhibitors, and highlight areas for future research in the diagnosis and management of these emerging entities.

6.2 Aim of the thesis

This thesis aims to evaluate the current literature on ALK-mutated skin tumors, present a diagnostically challenging case report ultimately classified as TPM3: ALK inflammatory myofibroblastic tumor, and discuss implications for diagnosis, classification, and therapy.

7. Methodology

This thesis comprises a combined approach of a literature review and a single case report. The aim was to provide an overview of ALK-positive cutaneous and subcutaneous neoplasms, with a particular focus on their histopathological, immunohistochemical, and molecular characteristics.
The literature research was conducted using the electronic databases PubMed and Google Scholar. The search included articles published up to March 2026.
The following keywords and their combinations were used: “ALK”, “ALK in cutaneous neoplasms”, “ALK inhibitors”, “anaplastic lymphoma kinase”, “ALK-rearranged”, “inflammatory myofibroblastic tumor”, “epithelioid fibrous histiocytoma”, “mesenchymal neoplasms”, “soft tissue tumor”, “immunohistochemistry”, and “molecular characterisation of ALK”.
Studies were included if they consisted of original research articles, case reports, case series, comprehensive review articles, and clinical trial reports. Only articles published in English were considered. Articles that focused on ALK-rearranged mesenchymal tumors of the skin or soft tissues in humans were included. Studies were included if they described clinicopathological features, molecular alterations, diagnostic methods such as IHC, FISH, and NGS, and therapeutic approaches, with particular emphasis on ALK inhibitors and mechanisms of resistance.
The reference lists of key review articles were manually screened to identify additional relevant publications. Relevant studies were identified by reading the titles and abstracts, followed by full-text evaluation.
Studies were excluded if they were published before 2000 or were not relevant to the scope of this thesis. The majority of the research papers included have been conducted in the preceding 10 years.
The presented case was obtained from the National Center of Pathology, an affiliate of Vilnius University Hospital Santaros Klinikos, and selected for its relevance to ALK-mutated cutaneous neoplasms and its challenging histopathological differential diagnosis. An informed consent was obtained from the patient prior to the preparation of this case report. All patient data were anonymized.
The diagnostic evaluation included, apart from clinical examination, ultrasound and MRT imaging, microscopic histological analysis with hematoxylin and eosin staining, also, results from different

immunohistochemical panels, which were used for differential diagnostics. Final diagnosis was reached after ALK (D5F3) immunohistochemical reaction and comprehensive molecular testing.

8. Literature review

8.1 Epidemiology

The family of ALK mesenchymal neoplasms has been continuously expanding in recent years, while at the same time, many non-ALK fusions have been found in ALK immunoreactive neoplasms. In this case, strong ALK protein expression can be misleading in diagnostics, if not confirmed with molecular testing. (1) Additionally, literature is rare and currently consists of case reports or small case series. Hence, most conclusions are derived from small, heterogenous cohorts, reflecting the rarity of these entities.

Alterations of ALK, including activating mutations, amplifications, fusions, or rearrangements, occur in approximately 3.3% of cancers. (8) Because ALK alterations are present across multiple tumor types, each with distinct malignancies and genetic changes, the demographic distribution is correspondingly diverse. The reported age distribution is heterogeneous. ALK associated cutaneous tumors can occur across a wide age spectrum in both pediatric and adult patients. (7,9) Due to its rarity the exact prevalence of cutaneous ALK mutated tumors is hard to determine.

Reported frequencies of ALK expression in IMTs range from 36 to 61.8% in the literature. (2)

8.2 Background on soft tissue tumors of the skin

Soft tissue tumors represent a large and diverse group of mesenchymal neoplasms ranging from malignant entities such as dermatofibrosarcoma protuberans, atypical fibroxanthoma, and various sarcomas to intermediate and benign lesions including dermatofibroma and lipoma. They mostly arise from dermal and subcutaneous connective tissue including fibroblasts, myofibroblasts, adipocytes, smooth muscle cells, and vascular elements. The diagnosis of soft tissue tumors of the skin is primarily based on clinical presentation and histopathology. In appropriate cases immunohistochemical and molecular studies are employed. (10)

Due to genetic characterization and the discovery of gene rearrangements in different tumor types, diagnostically useful markers have been identified in the recent years. These advances as well as recent developments in molecular diagnostics has improved the accuracy of classification. (11,12)

Malignant soft tissue tumors are far rarer than benign soft tissue tumors and typically present with rapid growth, larger size, deeper location and ulceration. Due to their potential for local recurrence and metastasis they require prompt evaluation. (12–14)

The World Health Organization (WHO) updated the classification of soft tissue tumors in 2020. The new classification organizes neoplasm primarily by line of differentiation, integrating histopathological, immunohistochemical and molecular genetic features. The eleven major categories are based on the presumed cell of origin, such as adipocytic, fibroblastic or myofibroblastic.

Regarding biological behavior, neoplasms could be classified as benign, locally aggressive, rarely metastasizing or true malignant. Inflammatory myofibroblastic tumor, for example, is included in the rarely metastasizing tumors category within the fibroblastic and myofibroblastic category, while the fibrosarcoma belongs to the malignant entities category. (Table 1). (15)

Table 1 The WHO Classification of soft tissue tumors with a list of major entities under each category (15)

CATEGORY	BENIGN	LOCALLY AGGRESSIVE	RARELY METASTASISING	MALIGNANT
ADIPOCYTIC	Lipoma Lipomatosis Lipoblastoma Angiolipoma Myolipoma Chondroid Lipoma Spindle Cell Lipoma ***Atypical Spindle Cell/Pleomorphic Lipomatous Tumour*** Hibernoma	Atypical Lipomatous Tumour		Liposarcoma Well-differentiated Dedifferentiated Myxoid Pleomorphic ***Myxoid pleomorphic***
FIBROBLASTIC AND MYOFIBROBLASTIC	Nodular Fasciitis Proliferative Fasciitis Proliferative Myositis Elastofibroma Fibrous Hamartoma of Infancy Fibroma of Tendon Sheath Desmoplastic Fibroblastoma Myofibroblastoma Calcifying Aponeurotic Fibroma ***Ewsr1- Smad3 Positive Fibroblastic Tumour*** Angiomyofibroblastoma Cellular Angiofibroma ***Angiofibroma Of Soft Tissue*** Nuchal Fibroma Acral Fibromyxoma Gardner Fibroma	Solitary Fibrous Tumour Fibromatosis Palmar/Plantar Desmoid Type Lipofibromatosis Giant Cell Fibroblastoma	Dermatofibrosarcoma Protuberans Solitary Fibrous Tumour Inflammatory Myofibroblastic Tumour Myofibroblastic Sarcoma ***Superficial Cd34 Positive Fibroblastic Tumour*** Myxoinflammatory Fibroblastic Sarcoma Infantile Fibrosarcoma	Solitary Fibrous Tumour Fibrosarcoma Myxofibrosarcoma Low-grade fibromyxoid sarcoma Sclerosing epitheloid fibrosarcoma
SO-CALLED FIBROHISTIOCYTIC TUMOURS	Tenosynovial Giant Cell Tumour Deep Benign Fibrous Histiocytoma	Plexiform Fibrohistiocytic Tumour Giant Cell Tumour Of Soft Parts		Malignant Tenosynovial Giant Cell Tumour
VASCULAR TUMOURS	Hemangioma Epithelioid Hemangioma Acquired Tufted Hemangioma	Kaposiform Hemangioendothelioma	Retiform Hemangioendothelioma Papillary Intralymphatic Angioendothelioma Composite Hemangioendothelioma Kaposi Sarcoma Pseudomyxogenic Hemangioendothelioma	Epithelioid Hemangioendothelioma ***Epithelioid Hemangioendothelioma With Yap1- Tfe3 Fusion*** Angiosarcoma
PERICYTIC TUMOURS	Glomus Tumour Myopericytoma Angioleiomyoma			Malignant Glomus Tumour
SMOOTH MUSCLE TUMOURS	Leiomyoma	***Smooth Muscle Tumour Of Uncertain Malignant Potential***		Leiomyosarcoma ***Inflammatory Leiomyosarcoma***
SKELETAL MUSCLE TUMOURS	Rhabdomyoma			Rhabdomyosarcoma Embryonal Alveolar Pleomorphic Spindle Cell ***Ectomesenchymoma***

8.3 Relevance of ALK mutations in tumorgenesis

Anaplastic lymphoma kinase (ALK) is a transmembrane receptor tyrosine kinase and closely related to the leukocyte receptor tyrosine kinase, based on overall homology. ALK is not normally expressed in most adult tissues, but is active during fetal development. (16) The human ALK gene encodes a polypeptide consisting of 1,620 amnio acids and is located on the 2p23 chromosomal segment. Following post-translational modifications, the polypeptide yields a mature ALK protein comprising an extracellular domain, a transmembrane domain and an intracellular domain containing a distinct number of amino acids. Following ligand binding to its extracellular domain the transmembrane tyrosine kinase receptor undergoes dimerization and subsequent autophosphorylation of the intracellular kinase domain. (2,3)

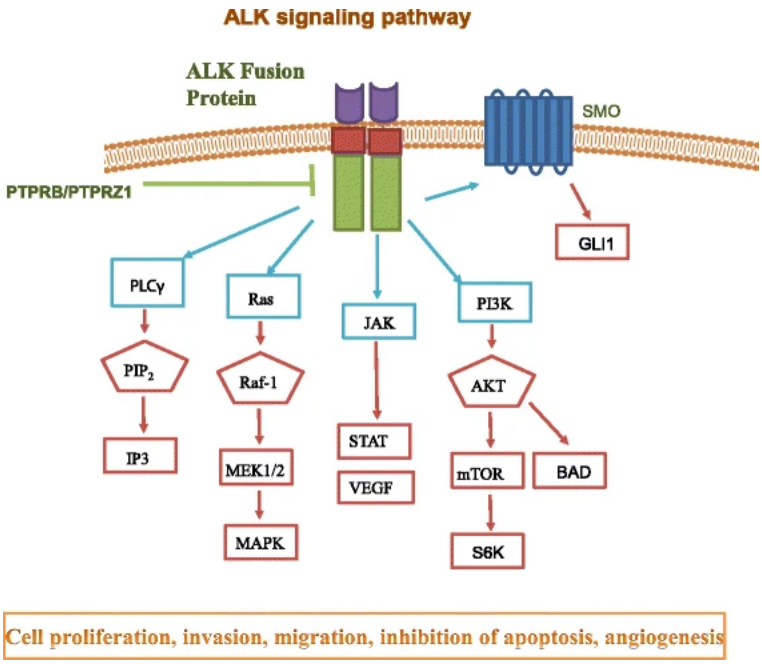

Figure 1. ALK signaling pathway (3)

Anaplastic lymphoma kinase (ALK) plays a crucial role in multiple intracellular signaling pathways that regulate essential processes of cell growth and survival. Aberrant ALK activation, particularly through the formation of ALK fusion proteins, leads to constitutive signaling that promotes tumorigenesis. These fusion proteins activate downstream pathways involved in cellular proliferation, invasion, and migration, while simultaneously inhibiting apoptosis and promoting angiogenesis.

ALK activation occurs exclusively upon ligand-induced homodimerization. In the absence of ligand binding, however, the receptor is inactivated through dephosphorylation by the beta and zeta complex of the receptor tyrosine kinase.
ALK rearrangements as well as activating mutations and gene amplification, have been observed in various malignancies. Initially ALK was identified in anaplastic large cell lymphoma (ACLC) due to a chromosomal rearrangement. Since then, ALK has also been found in neuroblastomas, non-small cell lung cancer (NSCLC), lymphomas as well as in inflammatory myofibroblastic tumors. (3,17) The multiple breakpoints and ALK fusion partner genes identified result in a molecular heterogeneity of proteins. These proteins subsequently exhibit distinct oncogenic properties that influence proliferation, invasion, tumorgenicity, and activation of diverse downstream signaling pathways. (3)

8.4 Molecular and Genetic Background

8.4.1 Structure and function of the ALK gene

The ALK gene is structurally similar to the leukocyte tyrosine kinase (LTK) and encodes for a protein consisting of three Main Domains: an extracellular region, a single transmembrane helix, and an intracellular tyrosine kinase domain (Figure 2). ALK is normally involved in the regulation and development of the nervous system and is expressed during embryogenesis. (18) The extracellular region has a unique architecture among cytokine receptors, which includes a permuted TNF-like module and a glycine-rich subdomain with polyglycine type II helices, and an EGF-like domain that influences ligand specificity. (19)

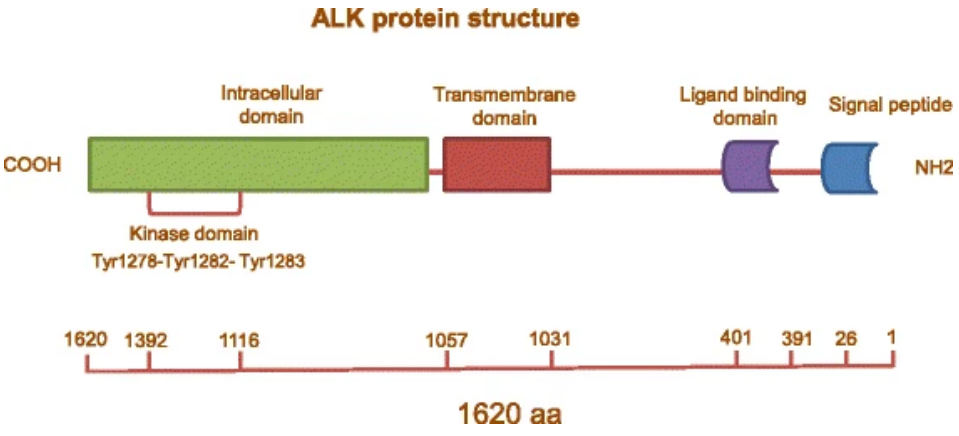

Figure 2. ALK Protein Structure (3)

The receptor can be activated by binding of the Cytokines ALKAL1 and ALKAL2 on to the extracellular region. This is followed by dimerization, which triggers a conformational change in the receptor. ALKAL1 and ALKAL2 are cognate cytokines composed of monomeric three-helix bundles. (19,20) Previously, midkine and pleiotrophin were reported to activate ALK. However, more recent studies have not confirmed these findings. Nevertheless, the endogenous interactions between ALK and its ligands remain incompletely understood and require further investigation. (7)

8.4.2 Known ALK translocations

Due to its involvement in the pathogenesis of several cancer types, the ALK locus is a hotspot for translocations. Twenty-two different translocation partners have been identified. (7) Nevertheless, the reason why ALK forms fusion proteins more frequently than other proteins remain unclear. Like most fusion partners, ALK is a large, structurally unstable, and acidic protein, which increases its likelihood of fusing with other genes. (21)

The first known ALK was identified in anaplastic large-cell lymphoma (ALCL) as a fusion oncogene (NPM-ALK) with nucleophosmin, resulting from a translocation involving chromosomes 5q and 2p. TPM3-ALK and TGF-ALK are other fusion proteins that were later identified in ALCL and inflammatory myofibroblastic tumors. (22,23) The most common ALK fusion in non-small cell lung cancer (NSCLC) is EML4-ALK, which arises from intrachromosomal inversions on the short arm of chromosome 2p. This inter-arm interchange results in a fusion protein with tumorigenic activity that activates downstream signaling pathways, leading to the development of NSCLC. (18) TPM3-ALK and TPM4-ALK are fusion proteins formed by the fusion of the N-terminal coiled-coil domains of the tropomyosin genes (TPM3 or TPM4) to the C-terminal kinase domain of ALK. These oncoproteins lead to tyrosylphosphorylation and constitutive kinase activity. (23)

ALK translocations are common in soft-tissue tumors. Especially in conventional IMTs, more than 50% harbor ALK gene rearrangements and overexpression. (24) ALK fusions define distinct molecular subsets within mesenchymal neoplasms, including both morphologically well-characterized entities as well as a growing group of unclassified or emerging tumors with variable spindle, epithelioid, or foamy/pseudolipogenic morphology. This is mainly due to the growing use of next-generation sequencing (NGS) technologies. (1) Identifying the specific fusion partner (e.g., EML4) is continuously very important as it can aid in diagnosis and may have prognostic or therapeutic relevance.

8.4.3 Mechanisms of ALK-driven oncogenesis

A specific combination of ALK fusion with partner genes leads to the generation of oncogenes, and the mechanisms of ALK-driven oncogenesis are variable. (21) Multimerization and autophosphorylation of ALK occur due to different oncogenic translocations. This leads to constitutively active ALK.

Key mechanisms of the constitutively active ALK fusion proteins include activation of the Janus protein tyrosine kinase (JAK)/signal transducer and activator of transcription (STAT), Ras/extracellular signal-regulated kinase (ERK), and phosphatidylinositol 3-kinase (PI3K) pathways. Collectively, this leads to cell proliferation, survival, and resistance to apoptosis. (25) Additionally, transcriptional and epigenetic regulation contribute to the maintenance of cancer stem cell–like properties and tumor progression, as seen in sarcomas, for example. ALK fusion proteins can influence transcriptional regulation by interacting with nuclear proteins and thereby modulating gene expression. This leads to upregulation of stem cell–associated markers and epigenetic alterations, such as changes in DNA methylation patterns and microRNA (miRNA) expression. (25,26) In particular, ALK overexpression in sarcomas has been shown to enhance cancer stem cell–like properties. This is associated with the upregulation of stem cell–related markers such as c-Myc, which interacts directly with ALK, thereby increasing its binding to the ABCG2 promoter and further promoting stem cell-like properties and tumorigenicity. (26) Another mechanism supporting the aggressive tumor behavior of ALK-positive neoplasms is the phosphorylation of ALK. In soft tissue tumors, especially in myxoid sarcoma, phosphorylated ALK is associated with a higher risk of metastasis. (27)

In summary, two fundamental factors appear to be essential for ALK-driven oncogenesis. First, ALK must be capable of constitutive activation, either through oligomerization-induced autophosphorylation, as in the case of ALK fusion proteins, or through ligand-independent receptor activation, as in the case of activating point mutations. Second, ALK expression must be enabled either endogenously through activating mutations or ectopically via the promoter of the fusion partner gene (Figure 3). (25)

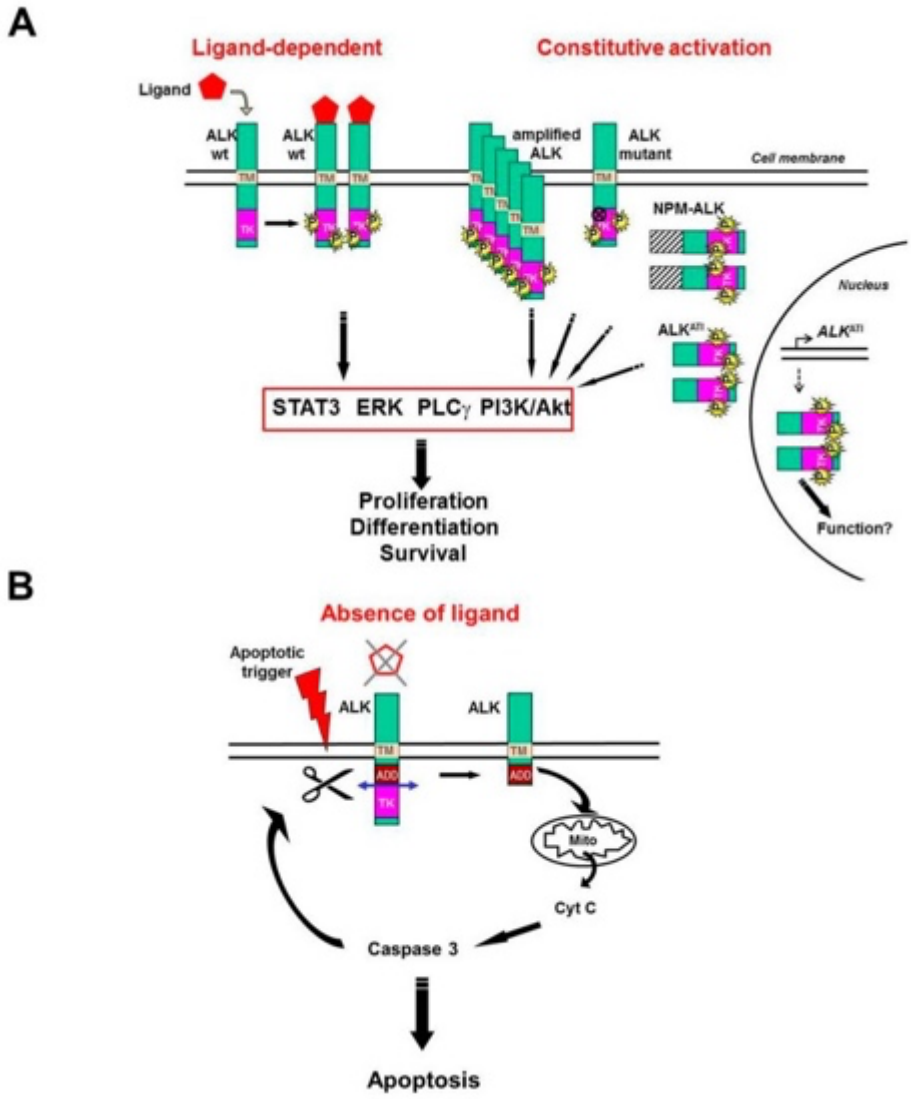

Figure 3. Model for Anaplastic Lymphoma Kinase positive and negative signaling. (28)

8.4.4 ALK rearrangement detection methods

There are several methods currently available for the detection of ALK rearrangements. Fluorescence in situ hybridization (FISH) is the only assay approved by the U.S. Food and Drug Administration (FDA) and is considered the gold standard for ALK testing. However, FISH is not widely available because of its high cost and the technical expertise required for interpretation. Other specialized techniques, including immunohistochemistry (IHC), and reverse transcription polymerase chain reaction (RT-PCR), are also widely used in laboratories to detect ALK alterations. (29)

IHC is widely used as a rapid, cost-effective screening tool for the detection of ALK alterations. It identifies ALK protein expression in formalin-fixed, paraffin-embedded tissue sections using monoclonal or polyclonal antibodies. The resulting antigen-antibody reactions are visualized through chromogenic or fluorescent markers and evaluated using a conventional light or

fluorescence microscope. In this approach, the detection markers are either directly conjugated to the primary antibody or attached to an appropriate secondary antibody. (30,31) However, atypical or rare ALK fusion variants may not be detected by IHC, and technical artifacts or low-level ALK expression can further complicate interpretation of results. (30,32) The immunohistochemical staining pattern of ALK depends on the specific ALK fusion partner. It can exhibit cytoplasmic, membranous, perinuclear or dot-like staining pattern (Figure 4). The most common ALK immunostaining pattern observed in IMTs is a diffuse cytoplasmic pattern. The highly sensitive ALK antibody clones (5A4 and D5F3) can even improve the ALK protein detection in IMTs. (17)

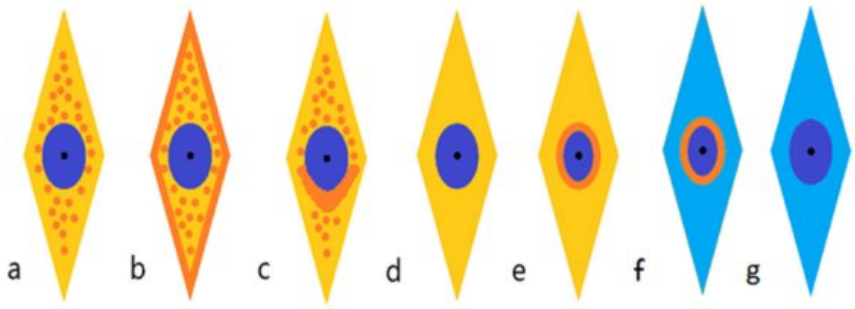

Figure 4. Interpretation of anti-ALK Immunohistochemistry. ALK positive staining patterns: **(a)** granular cytoplasmic **(b)** granular cytoplasmic with distinct staining of the cytoplasmic membrane **(c)** granular cytoplasmic with paranuclear accentuation **(d)** smooth cytoplasmic **(e)** smooth cytoplasmic with perinuclear accentuation **(f)** nuclear membranous **(g)** ALK-negative staining (33)

In comparison, FISH is technically demanding but presents with higher sensitivity and specificity. (32) The key principle of FISH is the hybridization of a labeled DNA probe to a complementary target DNA sequence. Before hybridization, both the probe and the target DNA are denatured into single-stranded DNA and labeled. Labeling can be done either directly with flurophone or indirectly using a hapten. When the probe and target are combined the complementary DNA sequences anneal. By using fluorescence microscopy hybridization signals are detected and analyzed. (34)
ALK fusion transcripts can be detected at the RNA level by RT-PCR, which provides direct evidence of gene rearrangement and fusion partner identity. Although sensitivity may be lower than with FISH or IHC, due to RNA-Degradation in formalin-fixed tissue and the diversity of possible fusion partners, RT-PCR can identify common fusions with high specificity. (30,35)
By sequencing DNA or RNA from tumor samples, next-generation sequencing (NGS) provides high sensitivity and specificity for the detection of ALK rearrangements. Because NGS can identify rare and novel fusion partners while assessing multiple genetic alterations simultaneously, it represents a particularly valuable diagnostic approach. In ALK-positive tumors, NGS is often used to map the neoplastic molecular landscape and identify atypical fusions that targeted assays may miss. (35)

FISH remains the reference standard for detecting gene rearrangements. IHC serves as a practical screening tool, and RT-PCR and NGS provide molecular confirmation and enable identification of fusion partners. Because each technique has distinct advantages and limitations, the choice of diagnostic method depends on tissue availability, tumor type, and the specific clinical question.

8.5 Histopathology and Classification

8.5.1 Key histological types

As already mentioned, ALK-mutated soft tissue tumors exhibit a wide range of histopathological features and represent a heterogeneous group of mesenchymal neoplasms (Annex 1). Molecular findings and immunohistochemistry are increasingly defining the classification. The category of ALK-rearranged mesenchymal neoplasms has recently expanded, including a range of unclassified, benign, low-grade, and aggressive neoplasms. These neoplasms exhibit diverse morphologies and immunophenotypes but still share unique clinicopathological and genetic traits. (1) Because architectural patterns, stromal appearances, and cytoplasmic characteristics often overlap, making an accurate diagnosis is difficult. Consequently, the context, including anatomic site, patient's age, and sex, remains vital for differential diagnosis. (12) Key entities which will be described in the following include classical IMT, EIMS, EFH, Spitzoid Tumors and others.

8.5.2 Inflammatory myofibroblastic tumor

Inflammatory myofibroblastic tumor (IMT) was the first ALK-rearranged mesenchymal neoplasm that has been defined. It most commonly occurs in children, adolescents, and young adults, predominantly involving the genitourinary tract, intraabdominal soft tissues, and the head and neck region; however, it may arise at any age or any anatomic site. (1,36) Cutaneous IMTs are particularly rare with fewer than 30 reported cases until today. (37) This influences the high genetic and molecular heterogeneity of the soft tissue tumor. Metastasis in IMT is rare, resulting in a generally favorable prognosis, despite its tendency for local invasion and recurrence. Macroscopically, most cases appear as well-circumscribed multinodular masses that are white to gray or yellow in color. (17) Classically, IMT is characterized by spindled myofibroblastic cells embedded in a variable hyalinized to myxoid stroma, accompanied by an admixed inflammatory infiltrate composed predominantly of mononuclear and eosinophilic cells, as illustrated in Figure 4A. The paucicellular neoplastic cell population is composed of elongated plump cells that often have variable dendritic cytoplasmic processes. Occasionally larger ganglion-like cells with prominent nucleoli can be found. (1) Histologically, IMTs exhibit three dominant growth patterns, including angiomyxoid inflammatory areas, a cellular spindle cell pattern with fascicular and

storiform architecture, and a dense hypocellular fibrous pattern resembling desmoid-type tumors or scar tissue. (1,17,36) Necrosis is absent in IMTs and mitotic activity is usually low.

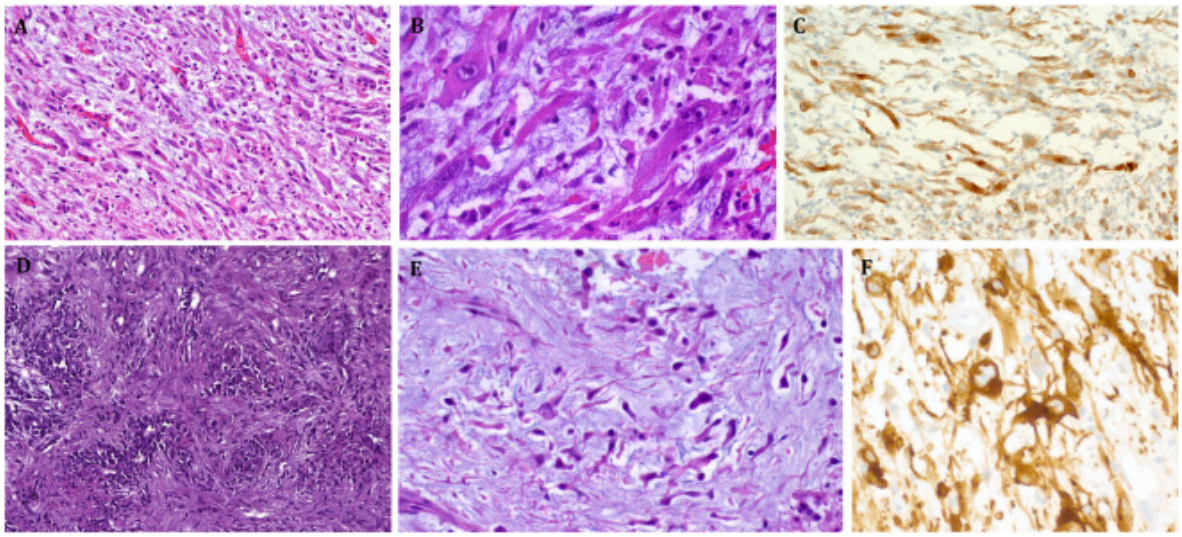

Figure 5. Examples of inflammatory myofibroblastic tumor (IMT). (**A**) myxoid pattern with scattered inflammatory cells. (**B**) high power shows elongated plump myofibroblasts with moderately eosinophilic cytoplasm and vesicular nuclei containing prominent nucleoli. (**C**) diffuse cytoplasmic ALK. (**D**) this IMT closely mimicked IgG4-related disease histologically. (**E**) This *ALK::TIMP3* positive highly myxoid dermal neoplasm closely mimicked myxoid "fibroblastic" tumors and showed extensive cytoplasmic ALK highlighting multiple dendritic cell processes (**F**). (1)

Immunohistochemically, IMTs frequently show membranous expression of muscle-specific actin (MSA), smooth muscle actin (SMA), and broad-spectrum cytokeratins. Approximately 50-60% of IMTs harbor ALK immunoreactivity, which is characterized by a variety of staining patterns depending on the specific ALK-fusion partner. Most typically IMT presents with a diffuse cytoplasmic ALK expression. (1,17) Wherever a granular cytoplasmic pattern is specifically associated with a CLTC: ALK fusion. ALK-negative IMTs harbor ROS1 and NTRK3 gene rearrangements at similar frequencies. Because of the considerable morphologic overlap with other tumors and molecular and genetic heterogeneity, accurate diagnosis of IMT based on immunohistochemical analysis is important. Nevertheless, a correlate between ALK immunoreactivity and recurrence has not been described. (17) Additionally, FISH probes mapped to the gene locus or diverse NGS tools can confirm the fusion events.

One of the most important differential diagnoses for IMT is IgG4-related inflammatory pseudotumor, given its continued underdiagnosis, particularly in the presence of SMA-prominent, positive, reactive background myofibroblastic cells. In addition, several differential diagnoses should be considered, which can be divided into two groups. Those that share histologically similar entities but lack ALK expression, such as NTRK fusion neoplasms, IPT-like FDCS, and

inflammatory rhabdomyoblastic tumors. Conversely, those that exhibit similar morphology and occasionally express ALK. (1)
Treatment of IMT usually aims for a complete surgical resection with negative surgical margins to minimize the risk of recurrence. Systemic Therapy for unresectable IMTs with ALK fusions includes ALK inhibitors such as crizotinib. (17)
The detection of ALK-mutated skin tumors has been remarkably difficult due to the morphological overlap with different ALK-negative and positive soft tissue tumors. Recent molecular genetic and clinicopathologic findings reveal atypical histopathological characteristics and unique molecular profiles in cutaneous IMTs – especially in the head and neck region. (37)

8.5.3 Epithelioid fibrous histiocytoma

Epithelioid fibrous histiocytoma (EFH) is likewise predominantly driven by ALK gene rearrangements. (38) It most commonly affects young to middle-aged adults, similar to inflammatory myofibroblastic tumor (IMT), but typically arises on the extremities. Macroscopically, EFH presents as a well-circumscribed, exophytic lesion, often with an epidermal collarette at the periphery. Histologically, EFH is composed of a relatively monotonous proliferation of polygonal cells with moderate amounts of eosinophilic to amphophilic cytoplasm. Tumor cells frequently exhibit binucleation or multinucleation, with vesicular nuclei and small nucleoli. The stroma is richly vascularized, consisting of both thin- and thick-walled vessels. In contrast to conventional fibrous histiocytoma, EFH typically lacks a prominent infiltrate of lymphocytes and foamy histiocytes, as well as lateral entrapment of collagen. (39,40)
In previous studies an accordant ALK expression and ALK gene rearrangement was revealed in most cases of EFH. The most common ALK fusions in EFH are SQSTM1:ALK (50 %) and VCL:ALK (30 %). (1) The soft tissue tumor can be distinguished from other histologic mimics and variants of fibrous histiocytoma with immunohistochemistry because it demonstrates with diffuse cytoplasmic ALK expression pattern. (39–41) There is subtle evidence which indicates certain correlations between the tumor morphology and fusion partner and the ALK immunoreactivity pattern, even though no general phenotype-genotype correlation has been pointed out yet. For instance, SP100 fusions can drive a nuclear dot pattern, and PRKAR2A fusions have been associated with dot-like perinuclear expression.

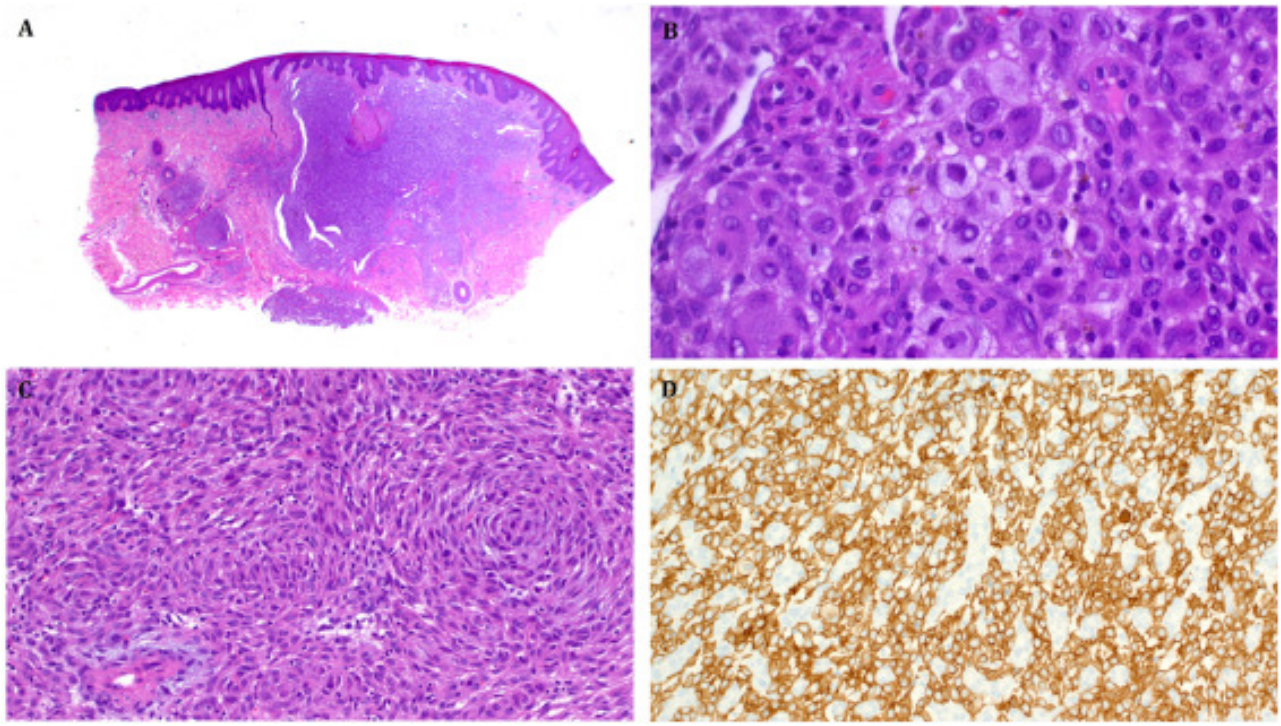

Figure 6. **(A)** this epithelioid fibrous histiocytoma shows multiple lobules at low power. (**B**) large epithelioid cells with amphophilic to pale-eosinophilic cytoplasm, focal foamy cells and binucleation. (**C**) superficial ALK-associated myxoid spindle cell neoplasm (SAMS) showing prominent concentric whorls. (**D**) intense cytoplasmic ALK (same case as in A, B). (1)

Immunohistochemically, EMA and infrequently CD30 and CD34 can be other positive markers for EFH. (1) In comparison to IMT EFH is usually negative for SMA, desmin and CD31. Nevertheless, there is limited list of differential diagnosis which includes common dermatofibroma with variable epithelioid plump compact cells, syncytial myoepithelioma, non-pigmented dermal nevi with epithelioid cytology, which are newly called as melanocytomas, and ALK-rearranged epithelioid hemangioma. (1) Although most EFH cases harbor ALK gene fusions, rare instances of ALK immunoreactivity without detectable fusion have also been reported, suggesting possible fusion-independent transcriptional activation. (42) Even though over 90% of EFH cases express ALK the sensitivity of detection remains clone-dependent and is even higher with D5F3 or 5A4 clones then with the ALK-1 clone. To date specific ALK fusion partner and tumor morphology or clinical behavior have no clear correlation. (39,41,43)

8.5.4 Epithelioid Inflammatory Myofibroblastic Sarcoma

Another soft tissue tumor frequently associated with ALK rearrangements is epithelioid inflammatory myofibroblastic sarcoma (EIMS). EIMS represents a rare but aggressive entity within the spectrum of ALK-rearranged mesenchymal neoplasms and can be distinguished from other

ALK-driven tumors by its characteristic morphology and distinct molecular features. Similar to the other soft tissue tumors discussed above, EIMS affects a broad age range, including pediatric and adult patients but is typically characterized by younger age of onset. (44,45) With a median onset of 39 years it primarily affects males. EIMS predominantly originates in deep and superficial soft tissue sites, most frequently in visceral locations like omentum and mesentery of the small intestine, presenting with abdominal pain and ascites. However, they may also rarely occur in the lung, stomach, and brain. These tumors often present as large intra-abdominal masses with a reported median size of approximately 15 cm, ranging from 8 to 26 cm. At the time of diagnosis about 50% of EIMS cases are already multifocal. (17) Given that the clinical course of EIMS is more aggressive and the prognosis is generally poorer than that of conventional inflammatory myofibroblastic tumor, accurate diagnosis is crucial. After complete surgical excision some patients with localized disease may achieve long-term survival (44,46)

Histologically, EIMS presents with sheets or nests of round to epithelioid cells with vesicular nuclei, prominent large nucleoli and amphophilic to pale eosinophilic cytoplasm, embedded within a variable myxoid stroma (Figure 6). EIMS is often characterized by a prominent neutrophilic infiltrate, with plasma cells often being absent. Increased mitotic activity and tumor necrosis are common high-grade features, particularly in deeply seated tumors. (1) Hereby, focal necrosis is identified in about 50% of cases and the median mitotic rate is 4 per high power field. (17)

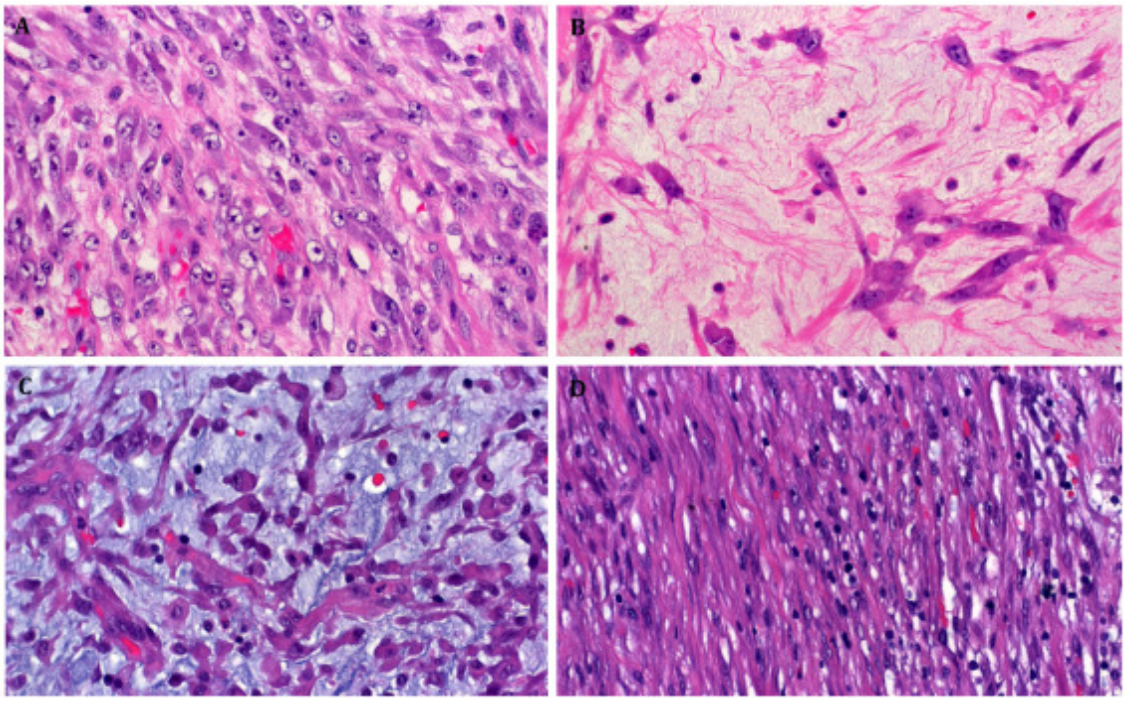

Figure 7. Epithelioid inflammatory myofibroblastic sarcoma is composed of large epithelioid cells with vesicular nuclei and large nucleoli (**A**) Myxoid areas with cell culture-like (**B**) or epithelioid/ rhabdoid looking cells within angiomyxoid stroma (**C**) are seen. (**D)** minor IMT-typical foci may be present as a clue to diagnosis. (1)

All EIMS samples are ALK-positive by immunohistochemistry. (17) ALK::RANBP2 is the most common ALK gene rearrangement in EIMS, resulting in constitutive activation of ALK and driving oncogenesis. In tumors harboring this gene fusion, ALK protein expression is characteristically localized to the nuclear membrane, resulting in a nuclear membranous staining pattern on IHC. In contrast, cases with an RRBP1-ALK fusion demonstrate predominantly cytoplasmic ALK expression with perinuclear accentuation. So, immunohistochemically, the staining pattern of ALK expression in EIMS correlates with the fusion partner. (46)

CD34 and S100 may be additionally useful markers, although their expression is variable. SOX10 is typically negative, which facilitates the differential diagnosis from other epithelioid neoplasms. (45) A few thoracic presentations of ALK positive tumors showed that the absence of pan-cytokeratin and smooth muscle actin could be diagnostically useful. (8)

Treatment options for EIMS currently focus on ALK inhibition. These therapies have demonstrated benefits like partial response and disease stabilization in ALK-rearranged neoplasms. Nevertheless, outcomes may vary depending on disease burden and tumor biology. (8,45) Shortly after surgical resection, most cases of EIMS experienced at least one local recurrence. (1)

Since the differential diagnosis of EIMS is broad, ALK immunoreactivity alone may not be specific enough, molecular confirmation of ALK rearrangement is essential for guiding therapy. (1,47)

Especially high-grade malignancies like ALCL, malignant melanoma, epithelioid leiomyosarcoma, epithelioid rhabdomyosarcoma, high-grade myxofibrosarcoma, dedifferentiated liposarcoma and undifferentiated carcinoma must be considered in the differential diagnosis. These neoplasms frequently exhibit a predominance of large epithelioid tumor cells. In particular, the sarcomatoid variant of ALCL shows significant morphologic and immunophenotypic overlap with EIMS. However, ALCL typically expresses cytotoxic T-cell markers and epithelial membrane antigen (EMA) and lacks the characteristic perinuclear ALK staining pattern. In contrast, EIMS commonly expresses CD30 and EMA but does not express cytotoxic markers. Moreover, the absence of the specific perinuclear ALK pattern is an additional helpful feature in distinguishing ALCL from EIMS.

The differential diagnosis between EIMS and IMT requires careful evaluation of epithelioid and ganglion cell-like elements. Additionally, distinction from peritoneal unclassified epithelioid or round cell sarcomas harboring EWSR1 or FUS:CREB gene fusions and showing aberrant ALK expression is essential. (1)

Table 2 Reported distinct ALK immunoreactivity patterns and their molecular correlates in certain ALK fusion neoplasms. (1)

Entity	ALK staining pattern	Fusion partner	Reference
Epithelioid fibrous histiocytoma	Nuclear only	ETV6	36]
Epithelioid fibrous histiocytoma	Perinuclear dots	*PRKAR2A*	40
Epithelioid fibrous histiocytoma	Nuclear dots	*SP100*	41
Epithelioid fibrous histiocytoma	Diffuse cytoplasmic (-/+ nuclear)	All other reported *ALK* partners	35, 36
Epithelioid inflammatory myofibroblastic sarcoma	Nuclear membrane/ perinuclear	*RANBP2*	26
IMT, classical	Diffuse cytoplasmic only	All reported *ALK* partners	27,-29
ALK+ with non-ALK fusions	Diffuse cytoplasmic only	Non-ALK fusions: *FET::CREB* fusions, *FET::TFCP* fusions, *EWSR1::PBX3*	42_43

8.5.5 Spitzoid tumors

Another interesting group of neoplasms harboring ALK fusions is Spitzoid melanocytic tumors. (7) Spitz melanocytic neoplasms define a distinct subset of Spitz tumors, including Spitz nevi, atypical Spitz tumors, and Spitzoid melanomas. ALK-rearranged Spitz tumors typically present as rapidly growing, dome-shaped, or polypoid nodules on the extremities of children or adolescents. (48) Histologically, the tumors are typically compound lesions with both junctional and dermal components. They display a characteristic wedge-shaped or plexiform silhouette formed by intersecting fascicles of large, plump spindled to fusiform melanocytes. Infiltrative growth pattern is commonly seen at the periphery, and an associated inflammatory infiltrate is frequently present. ALK immunohistochemistry reveals strong, diffuse cytoplasmic positivity. S100 is commonly expressed, along with other neural and more specific melanocytic markers. Common ALK gene fusions include NPM1 and TPM3. Generally, ALK-fused Spitz tumors behave indolently after complete excision. Because of morphological overlap with conventional Spitz tumors or Spitzoid melanoma, ALK IHC can serve as a sensitive differential tool. (49)

8.5.6 Other spindle cell or epithelioid neoplasms

Beyond inflammatory myofibroblastic tumor (IMT), epithelioid fibrous histiocytoma (EFH), and epithelioid inflammatory myofibroblastic sarcoma (EIMS), a growing spectrum of skin-based lesions has shown ALK rearrangements or over-expression. Superficial ALK-rearranged myxoid spindle cell neoplasm displays myxoid stroma, spindle cell morphology, an epidermal collarette and perivascular hyalinization. ALK is altered in 79% of cases with a cytoplasmic ALK pattern. ALK fusions include several, such as FLNA, MYH9, and DCTN1. Plaque-like ALK-fusion dermal neoplasms are CD34-positive, lack S100, and harbor DCTN1, PLEKH2 or CLIP2 fusions. The behavior of these neoplasms presents as indolent with no reported recurrence or metastasis. (1) Collectively, these entities demonstrate the growing, morphologically diverse group of ALK-altered skin tumors and emphasize the crucial role that fusion-specific molecular testing plays in precise classification and treatment targeting.

8.6 Prognosis and therapeutic approaches

Since ALK-rearranged cutaneous neoplasms span a spectrum of clinical behavior, the prognosis and therapeutic approaches differ. For unresectable, recurrent, or metastatic disease, ALK-directed tyrosine kinase inhibitors (TKIs) are increasingly used. Because ALK has been characterized as a driver mutation in cancer pathogenesis, these targeted ALK therapies have been introduced. Theoretically, ALK inhibition for cancer treatment should exhibit high selectivity for tumor cells and cause few systemic toxicities, as ALK is physiologically expressed in only a few neuronal tissues.

Crizotinib has demonstrated objective responses and disease stabilization in ALK-positive cutaneous ALCL and ALK-mutated NSCLC. (7) It works by inhibiting ALK by competing for the ATP-binding site of the ALK kinase domain, thereby suppressing aberrant activation of critical oncogenic signaling pathways. ALK-targeted therapy with crizotinib has demonstrated clinical efficacy in ALK-positive IMTs, particularly in unresectable or recurrent disease. This provides a justification for precision therapy in this patient population.

Second- and third-generation ALK inhibitors were developed because of different adverse effects and various resistance mechanisms. The resistance to ALK inhibitors is based on various, complex, and multifaceted mechanisms. A major obstacle is the interdependence of these mechanisms. Together they can create a dynamic and multilayered resistance network, which allows tumor cells to avoid targeted therapy. (50)

9. Case Report

A 22-year-old female presented to her family doctor with a 1 cm palpable lump with multicolored surface on the left lateral aspect of her neck. The initial ultrasonographic findings suggested a cystic formation a measuring 1.8 x 1.0 x 0.5 cm linked to a hair follicle, due to this reason the patient was treated under the suspicion of an atheroma, which later lead to non-radical excision biopsy. Additionally, the patient underwent magnetic resonance imaging (MRI) in the private clinic. The imaging showed a contrast-enhancing lesion on the left side of the neck, within the dermal-subcutaneous soft tissues, adjacent to the lateral border of the left sternocleidomastoid muscle (Figure 8).

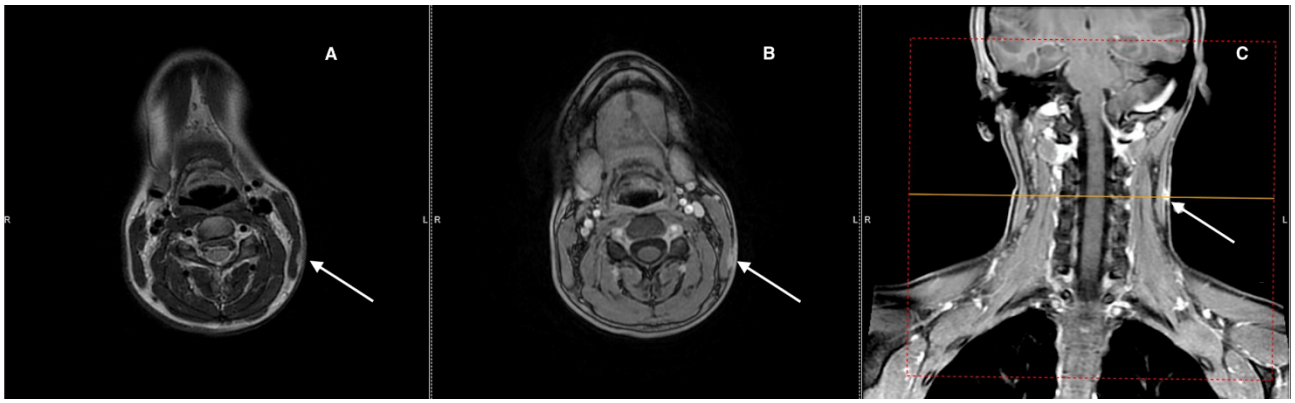

Figure 8. Magnetic resonance imaging showing a contrast-enhancing lesion (arrows): **(A)** T2-weighted axial image; **(B)** T1-weighted axial image; **(C)** T1-weighted frontal image.

The preliminary histopathological examination was conducted at a local hospital. The resected specimen revealed, on macroscopic examination, a fragmented, heterogeneous, grey elastic dermal and hypodermal formation.

Microscopically, the lesion was composed predominantly of monomorphic spindle-shaped cells arranged in a diffuse growth pattern. The tumor cells exhibited eosinophilic cytoplasm and elongated nuclei with prominent nucleoli. Mitotic activity was present, with up to 4 mitoses per 10 high-power fields (HPFs). Within the tumor stroma, a scattered mononuclear inflammatory infiltrate was present. Hemorrhages were additionally observed.

Immunohistochemical analysis demonstrated focal smooth muscle actin (SMA) positivity in approximately 40% of tumor cells. L-Caldesmon showed strong cytoplasmic positivity in 90% of cells, which are non-specific markers and, in many cases, expressed in cells from fibro-, myofibro-, and fibroblastic lineage. The Ki-67 proliferation index was estimated at approximately 20%. The tumor cells were negative for desmin, epithelial membrane antigen (EMA). Negative reactions for S100 and HMB-45 excluded possibility of neural and melanocytic origin. CD34-negativity easily excluded most common dermal sarcoma – dermatofibrosarcoma protuberans (DFSP).

Based on immunohistochemical and histomorphological findings, the initial diagnosis was consistent with a low-grade mesenchymal tumor, most compatible with a myofibroblastic sarcoma. However, due to overlapping morphological and immunophenotypic features, a leiomyosarcoma could not be definitively excluded. The non-radical excision margins were indicative of incomplete tumor removal. Consequently, a definitive diagnosis was deferred pending further surgical excision and additional histopathological evaluation.

Two and a half months later, the patient was admitted to the referring oncological center.

She underwent wide surgical re-excision of the tumor, accompanied by a sentinel lymph node biopsy. Macroscopically the tissue specimen measured 4.2 x 1.8 x 1.6 cm. The surgical scar was 2.0 x 0.3 cm large and located 0.6 cm from the closest resection margin. The lymph node that was submitted measured 1.0 x 1.0 x 0.5 cm. The tumor presented as a well-circumscribed nodule, which was predominantly placed in the hypodermis with minimal involvement of the dermis and bordering on deeper striated muscle.

Histology revealed monotonous, intertwined spindle cell bundles with several scattered lymphocytes and slight extravasation of erythrocytes. The tumor structures were surrounded by adjacent nerves and blood vessels. Foreign-body-type multinucleated giant cells were present due to the previous intervention. The lymph node presented with sinus histiocytosis and paracortical hyperplasia, which was consistent with reactive changes without tumor metastasis.

Immunohistochemical staining demonstrated focal positivity for fibrohistiocytic markers, including CD68, CD163 and microphthalmia-associated transcription factor (MiTF). Conversely, desmin and H-Caldesmon were entirely negative, which excluded a myocytic origin of the tumor. The Ki-67 proliferation index was also low, measuring approximately 5 %, which was even lower than in the initial histological examination. The tumor cells were negative for pancytokeratins (PanCK), S100 protein and CD34. In conclusion, the histological and immunohistochemical findings suggested a possibility of nodular fasciitis.

The young age of the patient and the superficial dermal-subcutaneous location underlined the possibility for nodular fasciitis. Nevertheless, this was excluded when FISH revealed a lack of USP6 gene rearrangement (17p13.2).

An additional immunohistochemical panel was carried out to further assess alternative fibro-histiocytic neoplasms. Unexpectedly the tumor cells displayed a strong, homogeneous cytoplasmic ALK (D5F3) positivity.

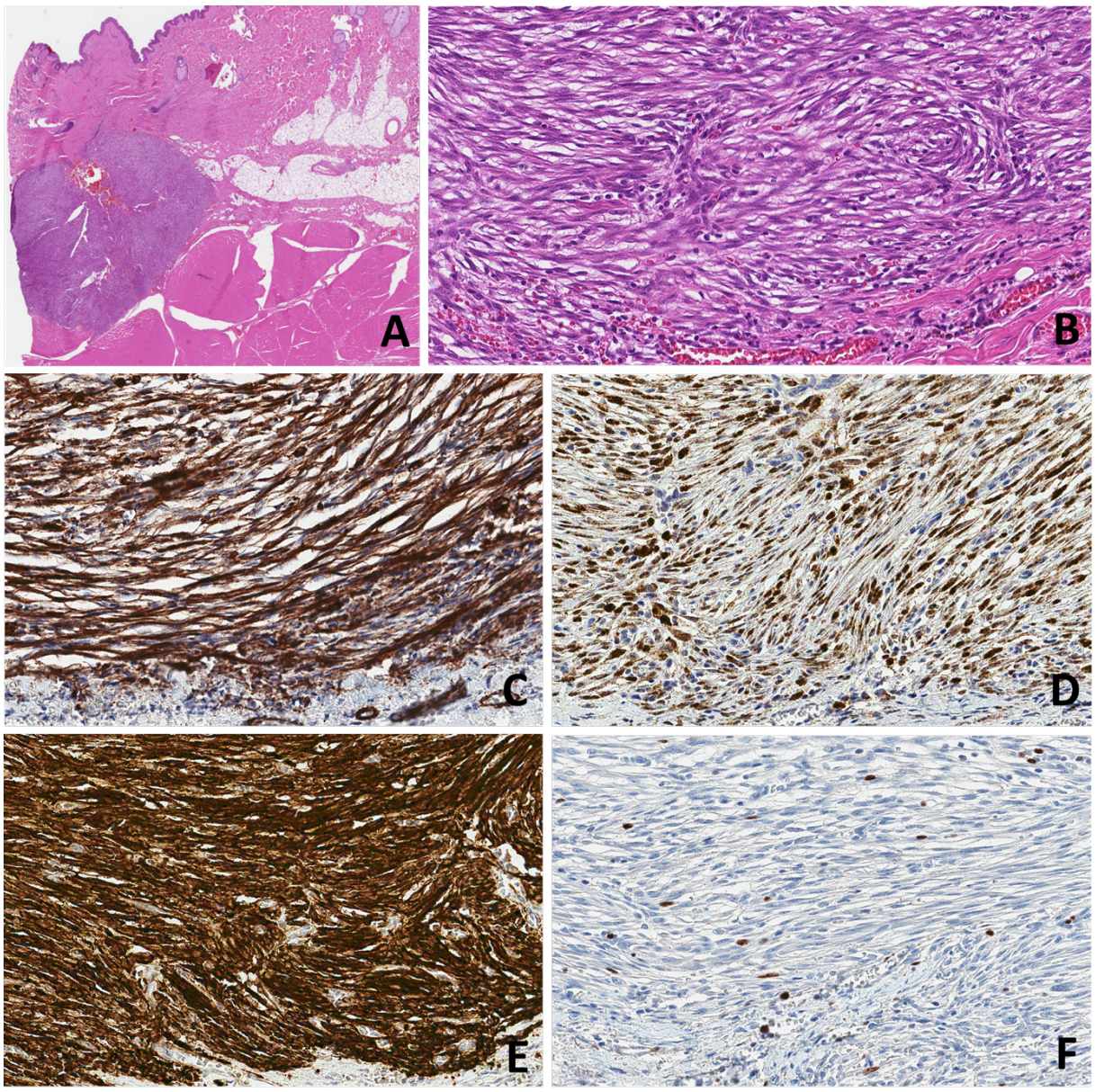

Figure 9. Microscopic slides from scar re-excision with residual tumor: **(A)** panoramic view of residual tumor nodule, placed underneath cutaneous scar, haematoxylin and eosin (HE), magnification ×10; **(B)** monotonous intertwined spindle cell bundles with several scattered lymphocytes and slight extravasation of erythrocytes, HE, x200; **(C)** strong diffuse cytoplasmic immunohistochemical reaction for smooth muscle actin, SMA, x200; **(D)** focal nuclear immunohistochemical reaction for microphtalmia-associated transcription factor, MiTF, x200; **(E)** strong diffuse cytoplasmic immunohistochemical reaction for ALK (D5F3), x200; F. low Ki67 proliferative index in the tumor cells, magnification ×200.

In conjunction with the spindle cell, myofibroblastic tumor morphology located in the dermis and subcutis, the differential diagnosis was shifted towards IMT as the most likely diagnosis. However, EFH with the prominent spindle cell component and other rare ALK-positive neoplasms could not be completely excluded. Accordingly, NGS was undertaken as further molecular testing. An oncogenic chimeric RNA transcript, resulting in a TPM3–ALK fusion, through a t(1;2) (q25;p23) translocation was identified.

Considering the relevant literature, a final diagnosis of TPM3-ALK-rearranged inflammatory myofibroblastic tumor with a nodular fasciitis-like histological pattern without typical prominent inflammatory infiltration and unusual superficial location was made, based on the combined histological, immunohistochemical, and molecular findings. After the patient underwent a complete wide local excision with tumor-free margins, no adjuvant therapy was administered because no aggressive histological features were identified, and no lymph node metastasis was present. Over two years, the patient showed no evidence of local recurrence and no regional or distant metastasis at follow-up.

The case demonstrates several features that are consistent with previously reported inflammatory myofibroblastic tumors. These include occurrence in a young adult patient, predominant spindle cell morphology, immunohistochemical positivity for SMA, diffuse ALK overexpression, and molecular confirmation of a TPM3::ALK fusion. However, the lesion also illustrates the diagnostic complexity described in the literature. The key factor that led to the final diagnosis was the strong, homogenous cytoplasmic ALK (D5F3) positivity, seen in IHC.

10. Discussion

10.1 Comparison from literature and the case

ALK mutations in cutaneous and melanocytic neoplasms have been expanding, presenting with diverse features and different clinical and histological presentations. (49) As previously noted, cutaneous inflammatory myofibroblastic tumors are extremely rare, with around 30 reported cases in the literature, including two new cases involving the oral mucosal site and one affecting the skin and subcutaneous tissue of the limb girdle. (37,51–55) Although the majority of reported cases have occurred on the extremities, there are also documented instances of tumors located in the head and neck region as well as the trunk and genitals (Table 3). (55) One case was even reported with occurrence on the digit. (56) They mostly are less than 10 cm in size, presenting as multilobulated, solid, and tan-white masses. (55) Consolidated data about the 21 cutaneous IMTs is showing a balanced sex distribution with 11 males and 10 female cases. The age ranges from 10-89 years. The increasing use of NGS reveals recurrent ALK rearrangements but molecular heterogeneity exists. Like already mentioned above, CARS has been identified as a fusion partner of ALK rearrangements in cutaneous IMT. (37) Nevertheless, a recent case from the literature described a PDGFRA::USP8 fusion in a cutaneous IMT, highlighting the expanding spectrum of kinase gene fusions in soft tissue neoplasms. (51) A case series from Xiong et al. additionally underlined the molecular diversity by identifying three superficial ALK-rearranged IMTs, two of them with DCTN1::ALK fusion. The superficial IMTs were found in the oropharyngeal mucosa, on the

forearm dermis/subcutis, and on the tongue. All presented as well-circumscribed, firm, gray-white lesions, varying from 1.3–3.5 cm. Histology mirrored classic IMT patterns with low mitoses and absent necrosis. Next to the two DCTN1::ALK cases, the third case harbored a TIMP3::ALK fusion. All patients remained disease-free after the excision. (37)

Table 3 Cutaneous inflammatory myofibroblastic tumors in the literature (37,51,55)

Author	Age	Gender	Location (size, cm)	ALK gene fusion	Treatment and follow up
Hurt et al	19-64	2 F 2M	Neck (1,3) Right arm (0,8) Right calf (0,8) Left arm (1,3)	Not tested	Excision No recurrence at average follow up at 4 years
Yang	44	M	Right dorsal hand (4,4)	Not tested	Excision No recurrence at 8 Months
Vadmal and Pellegrini	49	F	Left elbow (4)	Not tested	Excision No recurrence at 6 weeks
Nakajima et al	25	F	Thigh (no size reported)	Not tested	Excision No follow-up data
El Shabrawi-Caelen	15-89	2F 1M	Shoulder (1) Scalp (2) Arm (2)	Not tested	Excision No recurrence at average follow up at 12 months
Yung et al	33	M	Shoulder (2,4)	Not tested	Patient declined further excision after two biopsies No recurrence at 35 Month
Saricaoglu et al	57	M	Cheek (4)	Not tested	Excision No recurrence at 36 months
Pagni	63	M	Arm (1)	Not tested	Excision No follow-up data
Gonzales-Vela et al	10	M	Arm (0,7)	Not tested	Excision No follow-up data
Pellegrino et al	38	F	Labia (2)	Not tested	Excision No recurrence at 6 months
Son et al	26	M	Mid-back (NR)	Not tested	Excision No recurrence at 11 months
West et al	36	M	Right-digit (NR)	Positive for ALK by FISH	Excision No follow-up data
Collum et al	38	F	Scalp	CARS::ALK fusion by NGS	Excision No recurrence at 8 months
Vernemmen et al	24	M	Upper leg (7)	PDGFR::USP8 fusion by NGS	Excision No recurrence at 4 months
Xiong et al	27	F	Left forearm (3,5)	DCTN1::ALK fusion by NGS	Excision No recurrence at 4 to 40 months
This case	22	F	Neck (2)	TPM3::ALK fusion by NGS	Excision No recurrence at 2 years

Abbreviations: F – female, M – male, NR – not reported.

From the 21 reported case the tumors show excellent prognosis, with very low recurrence risk after excision. However, due to the lack of follow-up data it is difficult to make a generalized statement. Histologically, they display infiltrative fascicles of spindle/ovoid or stellate myofibroblasts in a myxoid stroma, with a prominent lymphoplasmacytic infiltrate and low mitotic activity; necrosis

and calcification are uncommon. (37,55) This overlaps with the findings from our presented case. However, cutaneous IMTs may present with more compact cellularity and less prominent inflammation, as in our case. (37) Cytoplasmic ALK is observed in approximately 50%–60% of cases. Expression in the reported cases ranged from mostly strong and diffuse to perinuclear dot-like in a few cases, similar to our case, in which IHC revealed strong, homogeneous ALK positivity. (16,37,55,57)

By contrast, seven ALK-rearranged CD34-positive spindle-cell neoplasms resembling dermatofibrosarcoma protuberans that were reported in 2022 displayed storiform growth, uniform bland cells, CD34 positivity, and diverse ALK partners. However, they lacked PDGFB rearrangements, which are commonly present in DFSP. Methylome profiling separated them from IMT and angiomatoid fibrous histiocytoma, underscoring a separate clinicopathologic entity. The ALK immunoreactivity in these cases enabled these tumors to be distinguished from conventional DFSP, which harbor PDGFB fusions, and underlines ALK as a reliable screening biomarker to identify tumors with possible ALK fusions. (57)

Helm et al. reported a single VCL::ALK cutaneous spindle-cell tumor which displayed a pedunculated myxoid pattern with focal S100 positivity. The strongly positive ALK immunostaining raised suspicion for an ALK-rearranged tumor, which later was confirmed using NGS. Other differential diagnoses like EFH were ruled out by different factors like the age and morphology, but also the negative TFE3 fusion and negative desmin and SMA. The molecular result classified the lesion as a low-grade sarcoma with potential for local recurrence and rare metastasis. The patient subsequently underwent complete wide excision of the tumor. At follow-up, 1.5 years later, there was no evidence of recurrence or metastasis. (16)

In the study from Yeh et. al., Spitzoid tumors with ALK fusion demonstrated distinct histopathological features. The ALK-rearranged neoplasms also showed positive ALK IHC, consistent with the genetic findings. The study revealed characteristic cytologic features in these ALK-fused Spitz tumors. In some cases, darkly pigmented exophytic papules mostly presented on the extremities. Over 90% displayed a fascicular growth pattern, and over 80% fibrillar cytoplasm with large nuclei and prominent nucleoli. Nevertheless, Spitz tumors demonstrate a high genetic diversity, which makes it difficult to produce reliable diagnostic criteria for these tumors. (48) Our case could be differentiated from Spitzoid tumors by clinical and histological presentation. Nevertheless, ALK-rearranged Spitz tumors represent an important differential diagnosis for cutaneous IMTs.

This case of a young woman with a confirmed TPM3::ALK fusion-positive spindle cell tumor illustrates the diagnostic challenges and complexities associated with ALK-rearranged mesenchymal neoplasms. This exemplifies the findings from the literature. Initially, diagnostic

uncertainty arose when the lesion was considered a low-grade myofibroblastic sarcoma but could not be definitively distinguished from leiomyosarcoma based solely on morphology and initial IHC. This diagnostic challenge is consistent with the literature's description of overlapping histological and immunophenotypic features among different spindle cell tumors. Making a definitive diagnosis remains difficult in the absence of molecular confirmation.

Even though the diagnosis was confirmed by the molecular confirmation of the TPM3::ALK fusion, this case highlights the important role of ALK as a biomarker. It supports the existing literature highlighting the relevance of routinely incorporating ALK immunostaining into the diagnostic and differential evaluation of superficial cutaneous soft-tissue tumors. Nevertheless, the role of molecular testing is essential for confirming gene rearrangements and establishing a precise diagnosis.

The case supports the growing concept that molecular characterization is essential in soft tissue tumor classification and that ALK-rearranged mesenchymal tumors represent a distinct biologic subset rather than merely a morphologic variant.

The morphological heterogeneity and variable inflammatory infiltrate characteristic of IMT often complicate histopathological diagnosis, thereby underscoring the critical role of molecular genetic testing in establishing a definitive diagnosis. This is consistent with the findings in the literature on ALK-mutated skin tumors. The diagnosis of these tumors remains difficult due do the broad and continuously expanding differential diagnosis.

10.2 Clinical significance of ALK mutations in diagnosis and therapy

For a growing number of malignancies, ALK is influencing both diagnosis and therapy. First, it serves as a crucial biomarker, as it is normally silent in adult tissue. Therefore, the detection can be a strong indicator of a neoplastic process. (28) As already discussed, ALK immunohistochemistry is used as a diagnostic tool and is essential for differentiating between soft tissue tumors with similar morphologies. (5) The specific IHC staining pattern can further refine the diagnosis. For example, the distinctive perinuclear/nuclear membrane pattern, together with an RANBP2::ALK fusion, is nearly defining in all cases for EIMS. This helps distinguish it from many typical IMTs, which often express diffuse cytoplasmic staining. (1)

Therapeutically, ALK can serve as a concrete target, as it is a validated oncogenic driver. (7) The first ALK inhibitor, crizotinib, showed a therapeutic response in ALK-fusion-positive IMT patients. In unresectable, recurrent, or refractory ALK-aberrant IMTs, the FDA approved crizotinib for treatment. (58) In ALK-rearranged NSCLCs, crizotinib as a first- or second-line therapy had even better outcomes than standard chemotherapy. (3) ALK-driven lung cancers have been shown to be dependent on the ALK signaling pathway, which makes them highly susceptible to ALK-specific

inhibitors. (22) Even though acquired resistance to these inhibitors is already a major challenge, it has prompted the development of second- and third-generation inhibitors to maintain therapeutic control. (7)

The mechanisms of resistance can broadly be classified into ALK-dependent, or target modifications, and ALK-independent, or bypass pathways. In ALK-dependent resistance, the oncogenic role of ALK is maintained, and alterations to the ALK gene itself are associated with resistance. This can happen due to ALK amplification or ALK mutations (Figure 9). Bypass pathways mediate resistance by activating alternative survival pathways. For example, this can involve activating the EGFR or insulin-like growth factor pathways. (3)

Response rates to ALK inhibitors for neoplasms bearing ALK fusions or rearrangements are normally in the range of 50–85%. Nevertheless, ALK alterations in cancers apart from IMTs, NSCLCs, and ALCLs are extremely rare, being found in only around 0.2% of patients. This makes studies of this disease incredibly challenging due to the lack of cases and the difficulty of reaching the feasibility threshold of prospective treatment trials. (58)

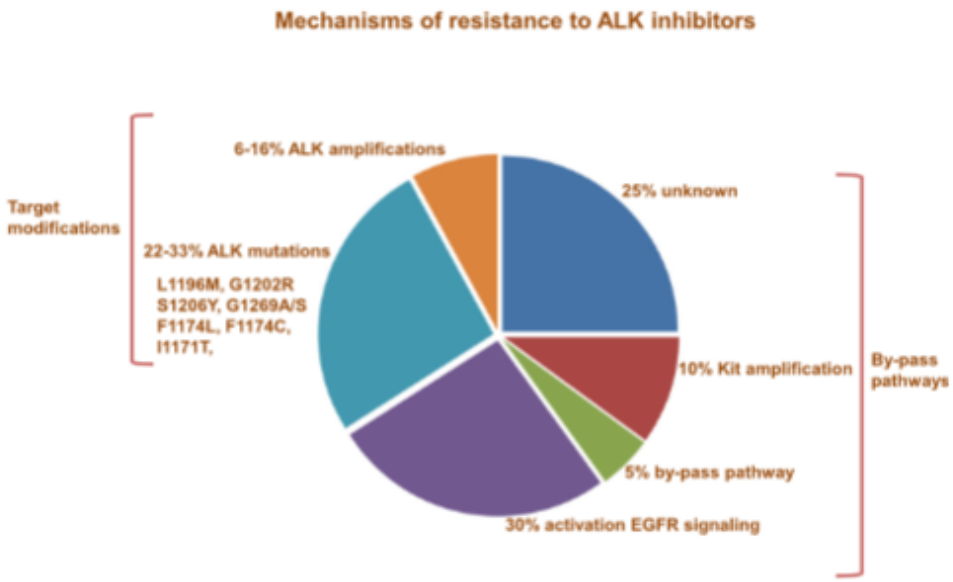

Figure 10. Mechanisms of resistance to ALK inhibitors. Resistance to ALK inhibitors may be mediated by acquired secondary mutations in the ALK kinase domain (F1174 L, F1174C, L1196 M, I1171T, G1202R, S1206Y, G1269S, and G1269A) or ALK gene amplification, indicating the persistence of ALK dependency. Resistance can also be mediated by activation of alternative survival pathways such as EGFR or the insulin-like growth factor pathways. (3)

10.3 Diagnostic limitations and pitfalls

The clinical presentation of ALK-rearranged cutaneous neoplasms is often nonspecific, posing significant diagnostic challenges. These tumors can masquerade as common benign or malignant skin lesions, for example, the ovoid-spindle cell neoplasm caused by a VCL::ALK fusion, which presents clinically as a pink-red papule resembling a pyogenic granuloma. (16) This clinical uncertainty could mean that, without a high degree of suspicion, the necessary molecular tests may not be performed, which can lead to delayed or incorrect diagnoses.

Although ALK immunohistochemistry is valuable for diagnosing various mesenchymal tumors, it may yield misleading results. High ALK reactivity does not necessarily confirm the presence of an ALK fusion, since certain mesenchymal neoplasms lacking ALK fusions can also exhibit substantial reactivity. Conversely, neoplasms bearing ALK positivity are highly heterogeneous, and ALK staining may be absent even in cases with true fusions. (1) Also, antibody sensitivity varies significantly by clone; for instance, the D5F3 clone generally demonstrates higher sensitivity for low-level protein expression compared to ALK1 or 5A4. Poor pre-analytical tissue fixation in IHC can also compromise staining quality and sensitivity. (30) Therefore, there is a risk for false positive and false negative diagnoses. Given the ongoing expansion of the non-ALK fusion category, this development may represent a significant pitfall. (1) In cases of neuroendocrine differentiation, ALK expression in IHC unrelated to fusion events has been observed, highlighting the necessity for molecular validation in these contexts. (59)

On the other hand, technique-specific limitations can hinder molecular confirmation. FISH may fail to detect cryptic or intrachromosomal rearrangements, leading to false-negative results. (57) NGS sequencing offers comprehensive bioinformatics but requires specialized bioinformatics and may be unavailable in many labs. (1) The literature is predominantly composed of case reports and small series with limited follow-up, which precludes definitive conclusions about recurrence rates, metastatic potential, and overall prognosis. The shortage of data also aggravates the establishment of clear genotype-phenotype correlations. The exact influence of specific fusion on tumor morphology and clinical aggressiveness is not yet fully understood. This uncertainty extends to therapeutic management. Even though first-generation TKIs have shown significant efficacy in IMTs, data on cutaneous tumors are rare.

10.4 Recommendations for clinical practice or future studies

To address the significant knowledge gaps, future research must prioritize the creation of prospective, large cohorts and registries. Different approaches are essential to navigate the pitfalls arising from the expanding number of neoplasms harboring ALK fusions. Firstly, a multimodal

diagnostic algorithm should be adopted. The morphology of suspicious cases should be assessed carefully, and IHC for ALK could be used to identify potential cases. Positive or uncertain IHC findings should subsequently be confirmed using molecular testing like FISH, or targeted RNA sequencing and NGS. A standardized, integrated diagnostic work-up should be used in every case. To ensure diagnostic consistency, some cases may benefit from review at specialized pathology laboratories.

For future research, novel ALK fusions across diverse tumor types should be identified and undergo molecular characterization to expand the spectrum of ALK-positive neoplasms. To better predict the course of ALK-positive neoplasms, long-term clinical outcomes would need to be monitored. Only through systematic and large-scale data collection can classification be refined, reliable prognostic biomarkers be established, and tailored therapeutic strategies be developed for patients with these complex malignancies.

10.5 Strengths and Limitations

The main strengths of this case report and literature review lie in the case's rarity and clinical relevance. Cutaneous presentations of inflammatory myofibroblastic tumors are uncommon, and documenting such cases can contribute valuable data to the limited existing literature. Another strength is the multimodal diagnostic approach by integrating histopathology, immunohistochemistry, and molecular analysis. Comparing the lesion with morphologically and molecularly overlapping entities such as IMT variants, EFH, and ALK-rearranged Spitzoid neoplasms, offering a systematic differential diagnostic discussion. This can enhance the practical value for diagnostic pathology.

The main limitation, on the other hand, is the nature of a single case report, which inherently limits the generalizability of the findings. Due to the limited number of case reports and data, the comprehensive study of ALK-mutated skin tumors was difficult. It is not possible to draw general conclusions about morphology, histology, molecular analysis, diagnosis, treatment, or outcome. Additionally, the field is still rapidly evolving. The classification system is still changing, so some tumors may be reclassified in the future. This makes exact categorization difficult right now. Finally, there is a potential for selection bias, since not all published cases or studies may have been included.

11. Conclusion

In summary, the expanding spectrum of neoplasms with anaplastic lymphoma kinase-rearrangements requires a multimodal diagnostic algorithm. Although anaplastic lymphoma kinase

expression is quite reliable in the appropriate histological and clinicopathological context, it is insufficient for morphologically unclassified tumors and for neoplasms occurring at unusual ages or in unusual locations. The presented case, together with the findings in the literature, has shown that initial screening should combine morphological assessment with immunohistochemistry, followed by molecular confirmation using methods such as next-generation sequencing to resolve uncertainties and identify specific fusion partners. As the case study highlights, molecular confirmation is critical for definitive diagnosis, especially, in rare tumors like cutaneous inflammatory myofibroblastic tumors. Further research should focus on identifying and characterizing novel anaplastic lymphoma kinase fusions to improve our understanding of these rare tumors.

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13. Annexes

Annex 1 Well-established and emerging *ALK*-rearranged mesenchymal neoplasms and neoplasms of unknown histogenesis. (1)

Entity	Age	M:F	Main sites	Key histology features	IHC+	ALK pattern	% *ALK*-altered	Fusion partner/s	Behavior
Inflammatory myofibroblastic tumor, classical	3 mo – 46y (median: 9y)	1: 1.3	Intraabdominal >>GU>> GI>>*H&N*>> extremities	Spindle cells, hyaline to myxoid stromal, mononuclear & eosinophilic cells	MSA, SMA, CK	Cytoplasmic	50–60 %*	*TPM3, TPM4, SEC31A, TFG, CLTC, FN1, LMNA, PRKAR1A, CARS, ATIC, SEC31L1, PPFIBP1, EML4, DCTN1, HNRNPA1*	Metastasis in <5 %. Local recurrence in 15–25 %
Epithelioid inflammatory myofibroblastic sarcoma	7 mo-63y (median: 39 y)	10: 1	Intraabdominal mesentery + omentum	Sheets of round-to-epithelioid cells, vesicular nuclei, large nucleoli, amphophilic-to-pale eosinophilic cytoplasm. Minor spindle cell areas, Variable myxoid stroma. Prominent neutrophils	Desmin, variable SMA and CD30. Negative for H-caldesmon, keratins, EMA, S100	Perinuclear/ nuclear membrane	Definitional	*RANBP2*	Highly aggressive
ALK+ myxoid laryngeal neoplasms	15–65y (median: 32)	6: 1	Superficial vocal cord	Large cells with intranuclear and intracytoplasmic vacuoles (resembling ganglion cells) forming alternating myxoid and more compact areas, delicate curvilinear vasculature	Most are SMA-negative	Cytoplasmic, dendritic	Definitional	*TIMP3*	No local recurrence or metastasis reported
Epithelioid fibrous histiocytoma	8 – 74 (median: 40)	1: 1	Lower >> upper extremities >> trunk and H&N	Uniform plump epithelioid cells, vesicular nuclei, small nucleoli, abundant pale eosinophilic to amphophilic cytoplasm, frequent binucleation	Variable EMA and CD30, negative for CD31, SMA, and desmin	Cytoplasmic**	90 %−100 %	*SQSTM1, VCL, TMP3, PRKAR2A, MLPH, EML4, DCTN1, CLTC, SPECC1L, PPFIBP1, AP3D1, COL1A, LRRFIP2, ETV6, PRKAR1A, PRKAR2A*	Benign
Superficial ALK-rearranged myxoid spindle cell neoplasm	6 to 82 y (median: 39)	1: 1	Lower >> upper extremities >> trunk and H&N	Whorled growth, myxoid stroma, spindle cell morphology, epidermal collarette, perivascular hyalinization, amianthoid collagen, + variable EFH-like areas	Variable CD34, EMA, S100	Cytoplasmic	79 %	*FLNA, MYH10, HMBOX1,* SQSTM1, VCL, COL1A2, DCTN1, EML4, FXR1, MPRIP, PLEKHH2, PRKAR1A, SPECC1L, TLN2	benign
Plaque-like ALK fusion dermal neoplasm	8 mo - 76 y	1: 6	Arm, scalp, eyelid, thigh, abdomen, shoulder	Spindle cells with storiform pattern, plaque-like	CD34	Cytoplasmic	Definitional	*DCTN1, PLEKHH2, CLIP2, FLNA*	No local recurrence or metastasis reported
Pseudolipogenic ALK+ unclassified neoplasms	46 – 69y (median: 63)	1: 2	superficial soft tissue sites, H&N	Large hydropic to foamy, pseudolipogenic, faintly granular epithelioid cells	Variable CathepsinK, focal EMA, desmin, MyoD1 and SMA	Cytoplasmic	Definitional	*RND3, SQSTM1, desmin*	Indolent
Other unclassified ALK neoplasms	10 to 78 (median: 42)	2: 1	Soft tissue >> viscera	Variable including LG-MPNST-like, tyrosine kinase-like features, variable high-grade morphology	CD34, S100, variable CD30, EMA, SMA	Cytoplasmic	Definitional	*EML4, HMBOX1, VCL, PRRC2B, MYH10, STRN, KLC1, EML4, DCTN1, PLEKHH2, TIMP3, HMBOX1, FMR1*	Variable indolent to aggressive metastasizing
ALK-altered nonneural granular cell tumor	5 to 73 (median: 23)	1: 2	Extremities > back, neck	Medium-to-large vesicular oval nuclei, conspicuous nucleoli, abundant pale basophilic cytoplasm, prominent granularity, focal spindling and storiforming	CD68, CD63 (NKI/C3) (S100 negative)	Weak cytoplasmic	60 %	*DCTN1* and *SQSTM1*	Indolent, subset with nodal metastasis, no further progression
ALK-associated histiocytosis	Infant & children, rarely adults	*F* > *M*	Hematopoietic organs, skin, any other based on disease pattern	True histiocytes, foamy histiocytes, Touton giant cells, ± emperipolesis	CD68, CD163, variable S100, negative for CD1a, langerin	Cytoplasmic	Definitional	*Mostly KIF5B, less common CLTC, TPM3, TFG, EML4, DCTN1, COL1A2, and TRIM33*	Mostly indolent. Rarely fatal