European Review for Medical and Pharmacological Sciences 2020; 24: 3829-3838

The potential diagnostic and predictive role of anaplastic lymphoma kinase (ALK) gene alterations in melanocytic tumors

A. RONCHI[1], M. MONTELLA[1], I. COZZOLINO[1], G. ARGENZIANO[2], E. MOSCARELLA[2], V. PICCOLO[2], F. IOVINO[3], T. TROIANI[4], R. ALFANO[5], M.E. ERRICO[6], V. D'ONOFRIO[6], M. BERRETTA[7], R. FRANCO[1], F. ZITO MARINO[1]

[1]Pathology Unit, Department of Mental and Physical Health and Preventive Medicine, University of Campania "L. Vanvitelli", Naples, Italy
[2]Dermatology Unit, Department of Mental and Physical Health and Preventive Medicine, University of Campania "L. Vanvitelli", Naples, Italy
[3]Surgery Unit, Department of Translational Medical Sciences, University of Campania "L. Vanvitelli", Naples, Italy
[4]Oncology Unit, Department of Experimental and Clinical Medicine "F. Magrassi", University of Campania "L. Vanvitelli", Naples, Italy
[5]Department of Medical, Surgical, Neurological, Metabolic and Aging Sciences, Universiy of Campania "L. Vanvitelli", Naples, Italy
6Department of Pathology, Santobono-Pausilipon Children's Hospital, Naples, Italy
[7]Department of Medical Oncology, National Cancer Institute, Aviano, PN, Italy

Abstract. – OBJECTIVE: Anaplastic lymphoma kinase (ALK) gene has been demonstrated to be rearranged, mutated or amplified in several haematological and solid tumors. Moreover, the use of ALK inhibitors has recently revolutionized the treatment of ALK-rearranged patients affected by non-small cell lung carcinoma. Herein we review the genetic alterations of ALK in melanocytic neoplasms described in literature, focusing on their potential diagnostic and predictive role.

MATERIALS AND METHODS: The Authors reviewed the pertinent literature through research on PubMed server was performed typing the terms "ALK", "Anaplastic lymphoma kinase", "ALKATI", "Melanoma", "Spitz", "Spitzoid".

RESULTS: ALK translocations were demonstrated in melanocytic neoplasms, particularly in acral melanoma and spitzoid tumors. ALKATI was described in primary and metastatic melanoma, indicating its early occurrence in oncogenesis, with varying immunohistochemical expression of the protein.

CONCLUSIONS: The identification of the specific type of ALK mutations could be interesting for planning biologic therapy of melanoma patients. Further studies are needed to evaluate the possibility to introduce an ALK-targeted therapy in patients affected by malignant melanoma.

Key Words:
ALK, Spitz, Melanoma.

Introduction

The continuously found genetic alterations in all the malignancies have been providing not only information about the mechanism of tumorigenesis, but also useful tools for the diagnosis of distinct pathological entities and the development of specific drugs for target therapies[1]. The anaplastic lymphoma kinase (ALK) gene encodes for a transmembrane tyrosine kinase receptor (TKR), belonging to the insulin receptor superfamily[2]. ALK gene was first described as the fusion partner of nucleophosmin (NPM) in Anaplastic Large Cell lymphoma (ALCL), as a result of the t(2;5)(p23;q35) translocation[3,4]. Thus, ALK immunohistochemical overexpression, as well as the detection of *NPM-ALK* genes fusion, have been considered essential for the diagnosis of ALCL[5]. However, more recently, ALK gene has been observed as rearranged, mutated or amplified in several other neoplasms, including non-small cell lung cancer (NSCLC), renal carcinoma, neuroblastoma, inflammatory myofibroblastic tumour (IMT) and several cutaneous malignancies, including Merkel cell carcinoma, basal cell carcinoma, fibrous histiocytoma and melanocytic neoplasms[6-12]. In addition, since the development of specific targeted therapies with ALK inhibitors,

Corresponding Author: Renato Franco, MD; e-mail: renato.franco@unicampania.it

successfully used in the treatment of NSCLC, IMT and ALCL patients, the identification of the ALK gene status has also gained a therapeutic predictive significance[13,14].

Herein we discuss the potential application of ALK gene detection in melanocytic lesions.

ALK as Oncogene and Target for Biological Therapy

ALK gene, located on chromosome 2p23, encodes for a protein belonging to the insulin-TKR superfamily[15-17]. ALK receptor is involved in the cell migration, proliferation and survival, through the activation of multiple downstream pathways, such as Ras/Raf/MEK/ERK1/2, JAK/STAT, PI3K/Akt and PLC-γ[15-17]. ALK gene was first described in 1994 in ALCL as the fusion partner of NPM in the characteristic t(2,5)(p23;q35) inter-chromosomal translocation[3]. The native ALK protein of 1620 amino-acids includes an extracellular domain with the binding sites for the two ALK ligands (midkine and pleiotrophin), a single-chain transmembrane segment and an intracellular domain[18,19]. ALK protein seems to be inactive in adults, being physiologically expressed only during embryogenesis in the brain and peripheral nervous system[2,20]. In the recent years, Next Generation Sequencing (NGS)-based studies have identified more than 20 different ALK fusion partners across multiple malignancies, with a high variability of chimeric ALK proteins in terms of both sequence and frequency[21]. Indeed, in ALCL, *NPM1*-ALK is the most commonly encountered fusion gene, accounting for up to 80% of all ALK -translocated cases[22]. IMT was the first solid tumour found to harbour ALK rearrangements, observed in up to 50% of such cases[23]. IMT shows different ALK gene fusion partners, never related to NPM1-ALK[23]. In addition, ALK fusions have been detected in 3-7% of NSCLC patients, generally associated with non-smoking habitus, younger age, and adenocarcinoma histology[24,25]. ALK gene rearrangements involving *EML4* are far more frequent in NSCLC than the several ALK fusion genes described in the last years[24]. Moreover, EML4-ALK translocations include over 10 distinct variants, but, in most cases, ALK breakpoint at intron 19 is conserved[24]. At low frequency, ALK gene rearrangements have been detected in other neoplasms, including colorectal, breast, renal cell, oesophageal, ovarian, anaplastic thyroid carcinoma, diffuse large B-cell lymphoma and cutaneous malignancies. Several common points emerge when comparing all identified ALK rearrangements[21]. Indeed, in all ALK fusion variants, while the C-terminal ALK kinase domain is entirely preserved, the N-terminal partner is represented by a constitutively activated promoter of different genes enhancing the fusion protein overexpression[24]. Thus, the level of ALK expression depends upon the partner gene, as demonstrated by *in vitro* studies. Indeed, the effects of ALK fusion transcripts on proliferation and invasion ability in NIH3T3 cell line were seen to vary according to the type of the ALK fusion partner[26]. These findings could also explain the different sensitivity of different ALK fusion proteins to tyrosine kinase inhibitors (TKIs) observed in the clinical settings, as described below[27]. ALK fusion proteins constitutively activate multiple downstream pathways, including JAK/STAT, PI3K/AKT, and MEK/ERK, driving aberrant cellular proliferation and survival[28,29]. However, besides translocations, ALK gene could be involved in other genetic alterations. Indeed, ALK gene amplification and the consequent ALK protein overexpression have been demonstrated in several different tumours, including melanoma, NSCLC, neuroblastoma, glioblastoma, rhabdomyosarcoma, ovarian cancer, breast cancer, astrocytoma, Ewing's sarcoma and retinoblastoma[19]. On the other side, ALK point mutations are found in 7% of sporadic neuroblastomas and 50% of familial neuroblastomas, with a demonstrated oncogenetic behaviour in both *in vitro* and *in vivo* models[8,30]. Moreover, ALK gene point mutations have also been described in NSCLC and anaplastic thyroid carcinoma[31,32]. Finally, secondary ALK point mutations, responsible for the acquired resistance to crizotinib, often occur in NSCLC and IMT[33-34]. Fluorescence In Situ Hybridization (FISH) pattern and ALK mutational status are showed in Figure 1. ALK protein is rarely found to be overexpressed in some neoplasms, in absence of any demonstrable alteration of ALK gene. In these cases, ALK overexpression may be due to poorly known genetic and epigenetic alternative mechanisms.

ALK Fusion in Acral Melanoma

Acral melanomas are relatively rare neoplasms occurring in palms, soles and nail apparatus[35]. They are characterized by a low mutational burden, as expected in poorly sun-damaged locations, and a lower frequency of BRAF mutations

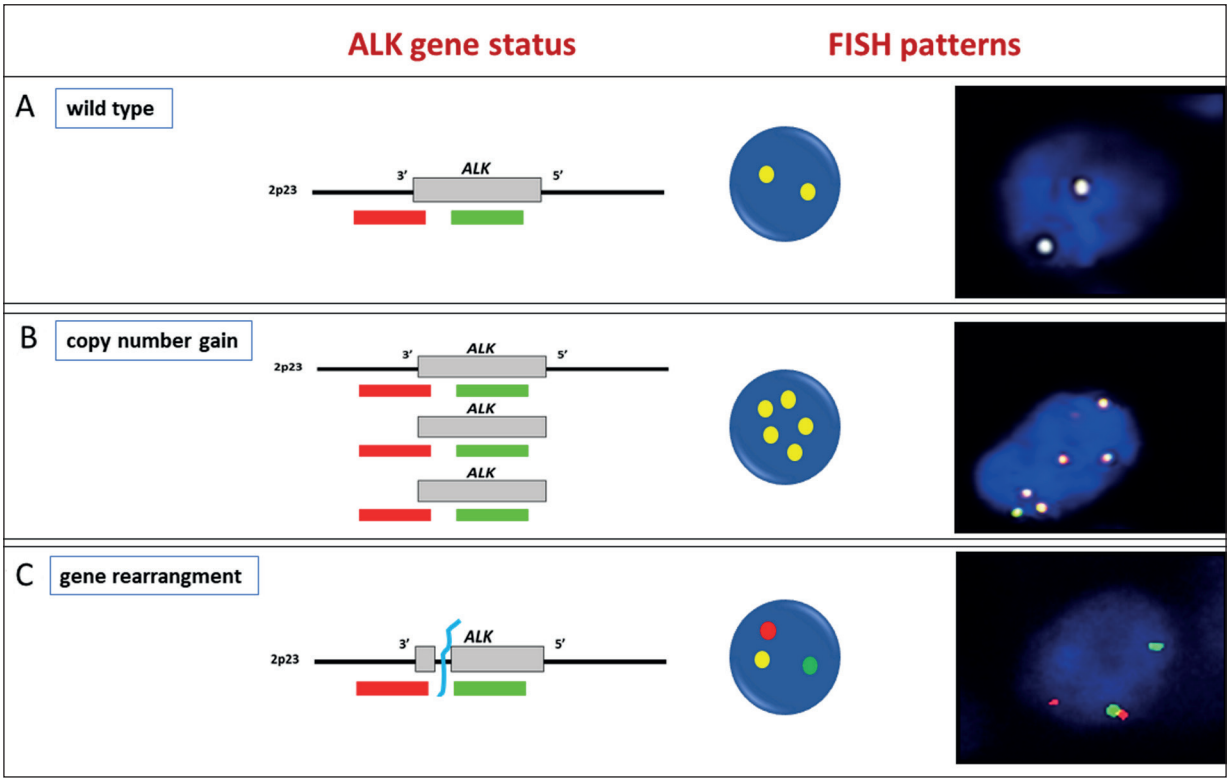

Figure 1. ALK gene status and FISH patterns. **A**, a normal cell with ALK gene wild type shows a signal pattern consisting of two orange/green fusion signal. **B**, a cell with ALK copy number gain or amplifications shows a signal pattern consisting of 3-6 orange/green fusion signal. **C**, a cell with ALK rearrangement shows a signal pattern consisting of one orange/green fusion signal, one orange signal, and a separate green signal. Abbreviations: ALK: anaplastic lymphoma kinase; FISH: fluorescent in situ hybridization.

than conventional melanoma[35]. Other genes resulted as mutated in acral melanoma include NRAS, KIT, CCND1, TERT, CDK4, GAB2, PAK1[36]. Few studies have recently investigated alterations of ALK in acral melanoma. In a cohort of acral melanomas from 30 southern Chinese patients, ALK translocation was demonstrated by fluorescent *in situ* hybridization in 4 out of 30 cases (6.9%), and one case showed a concurrent BRAF mutation[37]. The partners of the translocation were not investigated. All the ALK translocated cases resulted as positive by immunohistochemistry for ALK. Interestingly, the mean age of ALK-translocated patients was 46 years, i.e., significantly lower than non-translocated patients mean age. In addition, ulceration was observed in all ALK- translocated cases[37]. However, the data we currently have do not allow us to define the exact frequency of ALK alterations in acral melanomas. Indeed, Yeh et al[38] investigated the mutational status of a series of 122 acral melanomas through targeted deep-sequencing, and demonstrated ALK fusions only in one case (representing 0.8% of cases)[38]. In this case, the partner of the translocation was represented by SDHA[38]. The correlation between *ALK* mutational status and histological features in melanocytic lesions is summarized in Table I.

ALK Fusion in Spitz Tumors and in Melanocytic Myxoid Spindle Cell Tumor

Spitz neoplasms represent a spectrum of melanocytic lesions with different biological behaviour, including benign lesions as Spitz nevus, borderline lesions as atypical Spitz tumor and malignant lesions as Spitz melanoma. Regardless of their biological behaviour, all Spitz neoplasms are characterized by the presence of large cells with epithelioid or spindle morphology[39]. Approximately 50% of Spitz tumours harbour mutually exclusive gene fusions, involving TKR genes: ROS1 (in 17% of tumours), NTRK1 (16%), ALK (10%), BRAF (5%), and RET (3%)[40]. Remarkably, these gene alterations have been identified in all types of Spitz neoplasms, from

Table I. Correlation between ALK mutational status and histological features.

Melanocytic lesion	ALK mutational status	Associated histological features
Spitzoid tumors	ALK-R	Radial-oriented fascicular growth pattern
	ALK copy gain	Spitzoid melanoma
Acral melanoma	ALK-R	Ulceration
Nodular melanoma	ALKATI	Amelanotic
		Epithelioid or mixed (epithelioid and spindle-shaped cells) melanoma
Melanocytic Myxoid Spindle Cell Tumor	ALK-R	Dermal melanocytes with dermal expansion of spindle-shaped amelanotic cells floating in a myxoid stroma

Abbreviations: ALK-R: ALK rearrangement.

benign to malignant, particularly in 55% of Spitz nevi, 56% of atypical Spitz tumours and 39% of Spitz melanomas[40]. Currently, the histological categorization of Spitz neoplasms is particularly challenging for the pathologists, due to the lack of well-established, objective and reproducible morphological criteria for a correct classification of these lesions[41]. Hence, there is an impelling interest in the discovery of molecular markers useful in the differential diagnosis of Spitz neoplasms[42]. Differently from conventional melanoma, oncogenes such as NRAS, KIT, GNAQ, and GNA11 are not found mutated in Spitz neoplasms, while only a subset carries *BRAFV600E* and HRAS mutations[40,43,44]. Recently published studies have described the presence of the aforementioned, mutually exclusive activating kinase fusions in spitzoid neoplasms lacking mutations in BRAF or HRAS, all showing tumorigenic activity when transduced in cell lines[40,42]. There were several ALK gene partners observed in these fusions, including genes such as TPM3, NPM1, CAP-Gly domain-containing linker protein 1 (CLIP1), translocated promoter region (TPR), general transcription factor 3C polypeptide 2 (GTF3C2), and dynactin (DCTN1)[40,44-46]. ALK gene fusions have been described in all the pathological entities along the spectrum of Spitz neoplasms. Both immunohistochemistry, with the use of D5F3 antibody clone and fluorescent *in situ* hybridization have been applied to identify ALK gene fusions in these neoplasms[47,48]. The detection of ALK protein by immunohistochemistry has the considerable advantage to allow a simultaneous morphological and molecular evaluation, highlighting the striking infiltrative growth pattern of ALK-fused tumors. Indeed, small clusters of ALK positive cells can be appreciated at considerable distance from the main tumour mass with a tendency to invade adnexal structures such as erector pili, eccrine ducts, and follicular epithelium[44]. Although the presence of ALK gene rearrangements has not traditionally been considered useful in discriminating benign from malignant lesions, in a recent series of 17 ALK-fused Spitz neoplasms, none met sufficient criteria for a diagnosis of melanoma. Similarly, in a series of 246 paediatric spitzoid neoplasms, ALK fusion was observed in 16.7% of cases, but none was characterized by morphological or biological features of melanoma neither associated to the disease recurrence[48]. Only in the series published by Wiesner et al[40] and by Yeh et al[44], 1 out of 14 (7.14%) and 4 out of 32 (12.5%) respectively, ALK-fused spitzoid neoplasms met the criteria for Spitz melanoma. However, ALK gene fusions in Spitz tumors have been associated with distinct clinical-pathological features[44,46]. They are more common in young patients (median, 12 years), but occurrence in older patients (64 years) has also been described[44,45,49]. Clinically, Spitz tumours harbouring ALK gene rearrangement generally appear as large tumours or polypoid nodules located on the lower extremities. Microscopically, a plexiform dermal growth with intersecting fascicles of large fusiform melanocytes is more commonly observed[44,45,49]. In addition, a wedge-shaped silhouette of the lesion and absence of pigment were found to be significantly associated to ALK expression in Spitz tumors[47].

ALK gene fusions have also been described also in non-spitzoid melanocytic neoplasms. Indeed, in a recently published original article, Perron et al[50] have proposed the new entity among melanocytic tumors harboring ALK gene fusions, named Melanocytic Myxoid Spindle Cell Tumor with ALK Rearrangement. It is characterized by a dermal melanocytic nevus with dermal expan-

sion of spindle-shaped amelanotic cells floating in a myxoid stroma. In the four cases reported by the authors, four different ALK fusion partners were identified, namely FBXO28, NPAS2, TPM3, PPFIBP1[50]. Despite the presence of a relatively strong ALK immunostaining in cellular blue naevus and deep penetrating naevus, significant ALK rearrangements were not found in these subtypes of melanocytic naevi[51].

A case of ALK-positive malignant melanoma with spitzoid features and a case of ALK-positive atypical Spitz tumor are showed in Figure 2 and Figure 3, respectively.

ALK Copy Gain

Besides rearrangements, ALK gene deregulation and subsequent protein constitutive activation could be observed because of other genetic alterations, such as copy gains, amplifications and point mutations[52]. In particular, ALK gene copy number gains and amplifications are commonly found in neuroblastoma and NSCLC, associated with a poor prognosis[52]. Recently, a case of spitzoid melanoma with ALK copy gain with ≥3 intact ALK signals in 36% of the tumour cells has been reported[53]. The tumour nodule was of 3 cm in size, histologically characterized by the typical growth pattern of ALK-rearranged spitzoid neoplasms, with intradermal fusiform amelanotic melanocytes arranged in intersecting fascicles. However, in contrast to ALK fused spitzoid tumors, with strong staining for ALK antibody, only weak-to-moderate, albeit diffuse, ALK immunostaining was observed in such cases. This could be easily explained considering that copy gains alone do not imply any modification in the transcriptional control of the ALK gene[19,53].

Mutations and ALK^{ATI}

In melanocytic tumours, ALK gene overexpression can be also related to genetic mutations other than structural alterations. Indeed, ALK

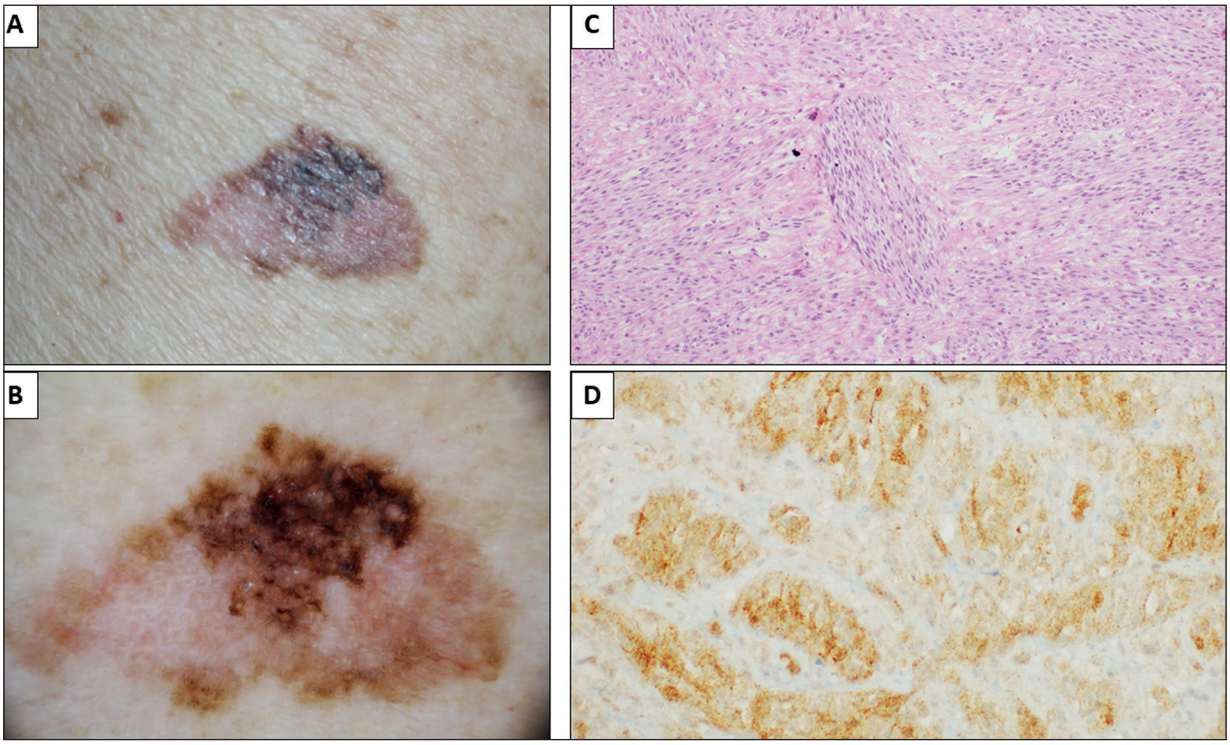

Figure 2. Immunohistochemical detection of ALK protein in a case of melanoma. A lesion located on the back of a 65 years old woman, diagnosed as melanoma with spitzoid morphological features. **A,** Clinically, the lesion appeared as a partially pigmented macule characterized by border irregularity and asymmetric distribution of pigment. **B,** In dermoscopy, atypical pigment network and regression structures were visible. **C,** Histological examination showed a proliferation of spindle-shaped cells, also organized in a fascicular pattern (H&E stain, original magnification 10x**). D,** Immunohistochemistry demonstrated the expression of ALK protein in the melanocytes (ALK immunostain, original magnification 20x). FISH demonstrated a wild-type ALK gene. Abbreviations: ALK: anaplastic lymphoma kinase; FISH: fluorescent in situ hybridization; Hematoxylin and Eosin.

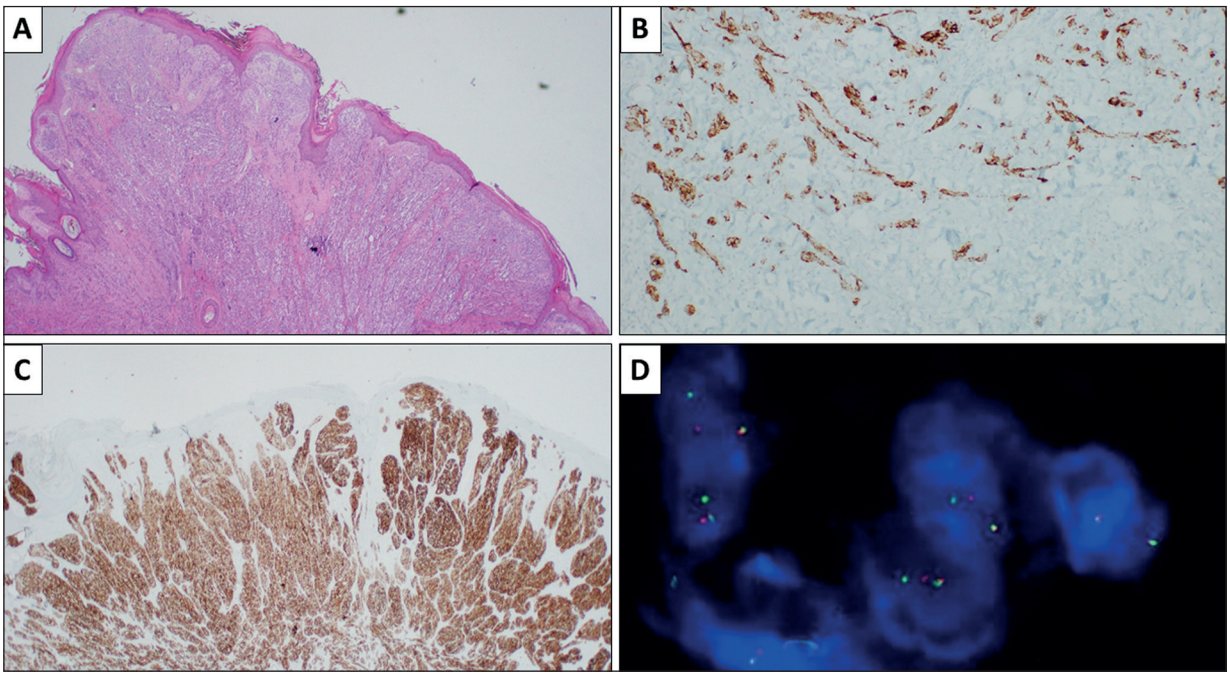

Figure 3. ALK-rearranged atypical Spitz tumor. A lesion located on the buttock of a 4 years old girl, diagnosed as atypical Spitz tumor. Breslow thickness was 5 millimeters and ulceration was absent. The patient was submitted to re-excision and sentinel lymph-node was removed. The histological examination of the lymph-node demonstrated the presence of small metastatic nodules in the sub-capsule lymph-node cortex. **A**, Histological examination showed an exophytic and asimmetrical melanocytic proliferation, with spindle-cell morphology (Hematoxylin and Eosin stain, original magnification 2.4x). **B**, Spindle cell morphology and plexiform pattern were evident also in the deeper component of the lesion, at the dermo-ipodermal junction (Hematoxylin and Eosin stain, original magnification 20x). **C**, The melanocytes strongly expressed the ALK protein (ALK immunostain, original magnification 20x). The immunostaining also highlighted the invasive pattern of growth at the bottom of the lesion. **D**, FISH demonstrated the rearrangement of ALK gene. Abbreviations: ALK: anaplastic lymphoma kinase.

missense mutations have been identified by NGS analysis on the tissue samples from a series of pan-negative melanomas, defined as melanomas lacking the usual recurrent mutations in genes such as BRAF, NRAS, KIT, GNAQ, and GNA11[54]. In this subset of tumors, 12 significantly mutated genes were recognised, including ALK, STK31, DGKI, RAC1, EPHA4, ADAMTS18, EPHA7, ERBB4, TAF1L, NF1, SYK and KDR[54]. In this series of genes harbouring point mutation, ALK represents the most frequently mutated gene[54]. Although the concomitant increase of protein activity in such cases was not investigated, at least one third of the identified ALK missense mutations predict a medium to high impact on protein function according to the Mutation Assessor[54].

A novel ALK gene isoform has been described in melanomas, named ALK wth Alternative Trascription Initiation (ALK^{ATI}), generated from an alternative transcription initiation site in ALK intron 19 (Figure 4)[55-56]. ALK^{ATI} transcript shows 3 different functional start codons, producing 3 different proteins, each maintaining intact intracellular tyrosine kinase domain, but lacking the extracellular and transmembrane domains of wild-type ALK[56]. *In vitro* experiments demonstrated the ALK^{ATI} ability to stimulate cell proliferation, independently from any growth factor[56]. In addition, its tumorigenic activity has also been documented *in vivo*[56]. ALK^{ATI} has been described in both primary and metastatic melanoma, indicating its early occurrence during oncogenesis. The two hitherto published studies on large series reported discording incidences for ALK^{ATI} expression in melanomas, 2% and 11% respectively, probably due to the different study design and applied methodologies[55,56]. In their study, Busam et al[55] assessed immunohistochemical ALK expression in a series of non-spitzoid melanomas and found variable ALK positivity in 7 out of 300 (2.3%) non-spitzoid primary melanomas and in 9 out of 303 (2.97%) non-spitzoid metastatic melanomas. Interestingly, all ALK-positive cases showed a predominant expression of the

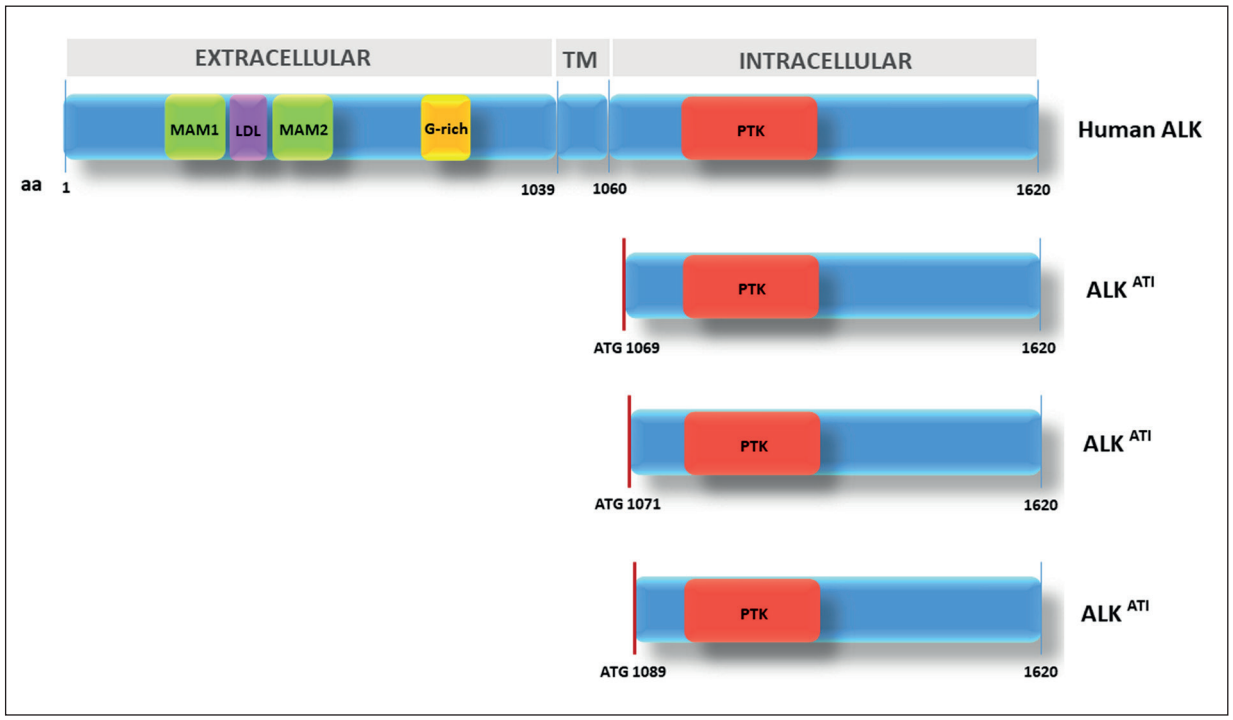

Figure 4. ALK^{ATI} isoforms expressed in melanoma. ALK^{ATI} isoforms may result from the existence of three translational start codons (ATG 1069, 1071, and 1089) in the ALK^{ATI} transcript. ALK^{ATI} transcript encodes three ALK proteins that conserve the tyrosine kinase domain, but lack the extracellular and transmembrane domains of wild-type ALK. *Abbreviations:* ALK: anaplastic lymphoma kinase; ATI: alternative transcription initiation; MAM: meprin, A5-protein, LDL: low-density lipoprotein receptor class A domain; G-rich: glycine-rich domain; TM: transmembrane domain; PTK: Protein kinase domain.

ALK^{ATI} isoform over wild-type ALK at Nanostring counter analysis, and in none of these cases ALK translocations were detected. However, in another published paper, co-existence of ALK translocation and ALK^{ATI} isoform was recently described in 1 out of 8 (12.5%) ALK^{ATI}-expressing melanomas[57]. In the study by Wiesner et al[56] the reported frequency of ALK^{ATI}-expressing melanomas was significantly higher (11%). As previously stated, this discrepancy is likely related to the first-line use of the transcriptome analysis (RNA-seq) to screen the population, a far more sensitive technology than ALK immunohistochemistry[55,56]. Interestingly, ALK^{ATI} expression in melanomas was found regardless of *BRAF*, *NRAS* and *KIT* status[55,56], differently from what is observed in ALK-translocated Spitz neoplasm[40,44]. Eventual correlation between ALK^{ATI} genetic status and morphological features has yet to be studied. It's actually known that ALK immunostaining showed a cytoplasmic/nuclear distribution in ALK^{ATI}-expressing tumors, differently from the membrane distribution observed in ALK-fused melanocytic tumours[55-56]. Moreover, histopathologic preliminary results by Busam et al[55] demonstrated that most ALK^{ATI} melanomas were morphologically characterized by nodular amelanotic epithelioid cells.

Treatment of ALK Deregulated Melanoma

Since ALK deregulation has been only recently described in melanomas, treatment response to ALK inhibitors has not yet been well characterized. The supposable role of ALK as a target for the therapy of advanced melanoma has been addressed by Wiesner et al[56]. They found that the activity of ALK^{ATI} isoform in melanoma cells could be blocked by three ALK inhibitors (crizotinib, ceritinib, and TAE-684)[56]. Moreover, they demonstrated *in vivo* that crizotinib treatment caused regression of ALK^{ATI}-driven NIH-3T3 tumours with mice experiments[56]. These results disagree with those of Couts et al[57] of 45 melanoma patients-derived xenograft models analysed for ALK mRNA and protein expression. Eleven out of 45 (24.4%) melanomas showed ALK expression, including 10 cases expressing wild-type ALK and/or ALK^{ATI} and 1 case expressing sever-

al *EML4-ALK* fusion variants[57]. While sensitivity to ALK inhibitors was demonstrated *in vitro* and *in vivo* in the mucosal melanoma with EML4-ALK fusion variants models, ALK^{ATI} melanoma models did not show any response[57]. Additionally, a mucosal melanoma patient with ALK^{ATI} was unsuccessfully treated with entrectinib, an ALK/ROS1/TRK inhibitor, on a phase I trial[57].

Conclusions

These data underline the emerging recognition of a role for ALK gene deregulation in the pathogenesis of melanocytic tumours. In this clinical setting, ALK deregulation has been demonstrated as the effect of different types of gene alterations. Thus, translocations have been described in acral and mucosal melanoma, as well as in the Spitz tumours spectrum, in association with specific histopathological features, while the common ALK^{ATI} isoform has been observed in nodular melanoma. Currently, the role of ALK deregulation in the differential diagnosis of melanocytic tumours is limited. In particular, in Spitz tumors a diagnostic role for ALK has not yet been acknowledged, notwithstanding the established association of ALK fusions with benign rather than malignant lesions. Further studies are required to better define the frequency of ALK-expressing melanoma and characterize distinctive morphological phenotypes comprised in this 'molecular' category.

The potential role of ALK as a therapeutic target in advanced melanoma should be more deeply investigated. Indeed, whilst previous works demonstrated that ALK^{ATI}-expressing cell lines could be inhibited *in vitro* by several ALK inhibitors, recent studies on patient-derived xenograft suggest that ALK inhibitors only show efficacy in melanomas carrying ALK translocation and not in ALK^{ATI} melanomas. Similarly, in a phase I clinical trial, the treatment of an ALK^{ATI} melanoma patient with entrectinib, a potent inhibitor of the tyrosine kinases ALK, TRKA/B/C and ROS1, did not show the awaited results.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding Sources

The Authors have no funding to declare.

References

1) McDermott U, Settleman J. Personalized cancer therapy with selective kinase inhibitors: an emerging paradigm in medical oncology. J Clin Oncol 2009; 27: 5650-5659.
2) Iwahara T, Fujimoto J, Wen D, Cupples R, Bucay N, Arakawa T, Mori S, Ratzkin B, Yamamoto T. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene 1997; 14: 439-449.
3) Morris SW, Kirstein MN, Valentine MB, Dittmer K, Shapiro DN, Look AT, Saltman DL. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 1995; 267: 316-317.
4) Shiota M, Fujimoto J, Semba T, Satoh H, Yamamoto T, Mori S. Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. Oncogene 1994; 9: 1567-1574.
5) Tsuyama N, Sakamoto K, Sakata S, Dobashi A, Takeuchi K. Anaplastic large cell lymphoma: pathology, genetics, and clinical aspects. J Clin Exp Hematop 2017; 57: 120-142.
6) Bridge JA, Kanamori M, Ma Z, Pickering D, Hill DA, Lydiatt W, Lui MY, Colleoni GW, Antonescu CR, Ladanyi M, Morris SW. Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor. Am J Pathol 2001; 159: 411-415.
7) Cao S, Nambudiri VE. Anaplastic lymphoma kinase in cutaneous malignancies. Cancers (Basel). 2017; 9. pii: E123.
8) Chen Y, Takita J, Choi YL, Kato M, Ohira M, Sanada M, Wang L, Soda M, Kikuchi A, Igarashi T, Nakagawara A, Hayashi Y, Mano H, Ogawa S. Oncogenic mutations of ALK kinase in neuroblastoma. Nature 2008; 455: 971-974.
9) Mossé YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, Laquaglia MJ, Sennett R, Lynch JE, Perri P, Laureys G, Speleman F, Kim C, Hou C, Hakonarson H, Torkamani A, Schork NJ, Brodeur GM, Tonini GP, Rappaport E, Devoto M, Maris JM. Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 2008; 455: 930-935.
10) Soda M, Takada S, Takeuchi K, Choi YL, Enomoto M, Ueno T, Haruta H, Hamada T, Yamashita Y, Ishikawa Y, Sugiyama Y, Mano H. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci U S A 2008; 105: 19893-19897.
11) Sukov WR, Hodge JC, Lohse CM, Akre MK, Leibovich BC, Thompson RH, Cheville JC. ALK alterations in adult renal cell carcinoma: frequency, clinicopathologic features and outcome in a large series of consecutively treated patients. Mod Pathol 2012; 25: 1516-1525.
12) Franco R, Nicoletti G, Lombardi A, Di Domenico M, Botti G, Zito Marino F, Caraglia M. Current treatment of cutaneous squamous cancer and mo-

lecular strategies for its sensitization to new target-based drugs. Expert Opin Biol Ther 2012; 13: 51-66.

13) Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Jänne PA, Costa DB, Varella-Garcia M, Kim WH, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW, Iafrate AJ. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010; 363: 1693-1703.

14) Mossé YP, Lim MS, Voss SD, Wilner K, Ruffner K, Laliberte J, Rolland D, Balis FM, Maris JM, Weigel BJ, Ingle AM, Ahern C, Adamson PC, Blaney SM. Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study. Lancet Oncol 2013; 14: 472-480.

15) Karachaliou N, Santarpia M, Gonzalez Cao M, Teixido C, Sosa AE, Berenguer J, Rodriguez Capote A, Altavilla G, Rosell R. Anaplastic lymphoma kinase inhibitors in phase I and phase II clinical trials for non-small cell lung cancer. Expert Opin Investig Drugs 2017; 26: 713-722.

16) Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, Choi HG, Kim J, Chiang D, Thomas R, Lee J, Richards WG, Sugarbaker DJ, Ducko C, Lindeman N, Marcoux JP, Engelman JA, Gray NS, Lee C, Meyerson M, Jänne PA. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008; 14: 4275-4283.

17) Li Y, Ye X, Liu J, Zha J, Pei L. Evaluation of EML4-ALK fusion proteins in non-small cell lung cancer using small molecule inhibitors. Neoplasia 2011; 13: 1-11.

18) Franco R, Rocco G, Marino FZ, Pirozzi G, Normanno N, Morabito A, Sperlongano P, Stiuso P, Luce A, Botti G, Caraglia M. Anaplastic lymphoma kinase: a glimmer of hope in lung cancer treatment? Expert Rev Anticancer Ther 2013; 13: 407-420.

19) Zito Marino F, Liguori G, Aquino G, La Mantia E, Bosari S, Ferrero S, Rosso L, Gaudioso G, De Rosa N, Scrima M, Martucci N, La Rocca A, Normanno N, Morabito A, Rocco G, Botti G, Franco R. Intratumor heterogeneity of ALK-rearrangements and homogeneity of EGFR-mutations in mixed lung adenocarcinoma. PLoS One 2015; 10: e0139264.

20) George RE, Sanda T, Hanna M, Fröhling S, Luther W 2nd, Zhang J, Ahn Y, Zhou W, London WB, McGrady P, Xue L, Zozulya S, Gregor VE, Webb TR, Gray NS, Gilliland DG, Diller L, Greulich H, Morris SW, Meyerson M, Look AT. Activating mutations in ALK provide a therapeutic target in neuroblastoma. Nature 2008; 455: 975-978.

21) Lin JJ, Riely GJ, Shaw AT. Targeting ALK: precision medicine takes on drug resistance. Cancer Discov 2017; 7: 137-155.

22) Amin SM, Haugh AM, Lee CY, Zhang B, Bubley JA, Merkel EA, Verzì AE, Gerami P. A comparison of morphologic and molecular features of BRAF, ALK, and NTRK1 fusion spitzoid neoplasms. Am J Surg Pathol 2017; 41: 491-498.

23) Lovly CM, Gupta A, Lipson D, Otto G, Brennan T, Chung CT, Borinstein SC, Ross JS, Stephens PJ, Miller VA, Coffin CM. Inflammatory myofibroblastic tumors harbor multiple potentially actionable kinase fusions. Cancer Discov 2014; 4: 889-895.

24) Li G, Dai WR, Shao FC. Effect of ALK-inhibitors in the treatment of non-small cell lung cancer: a systematic review and meta-analysis. Eur Rev Med Pharmacol Sci 2017; 21: 3496-3503.

25) Zito Marino F, Rossi G, Brunelli M, Malzone MG, Liguori G, Bogina G, Morabito A, Rocco G, Franco R, Botti G. Diagnosis of anaplastic lymphoma kinase rearrangement in cytological samples through a fluorescence in situ hybridization-based assay: cytological smears versus cell blocks: ALK rearrangement in cytological samples. Cancer Cytopathol 2017; 125: 303-312.

26) Armstrong F, Duplantier MM, Trempat P, Hieblot C, Lamant L, Espinos E, Racaud-Sultan C, Allouche M, Campo E, Delsol G, Touriol C. Differential effects of X-ALK fusion proteins on proliferation, transformation, and invasion properties of NIH3T3 cells. Oncogene 2004; 23: 6071-6082.

27) Lin JJ, Shaw AT. Differential sensitivity to crizotinib: does EML4-ALK fusion variant matter? J Clin Oncol 2016; 34: 3363-3365.

28) Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. Nat Rev Cancer 2008; 8: 11-23.

29) Zhang G, Scarborough H, Kim J, Rozhok AI, Chen YA, Zhang X, Song L, Bai Y, Fang B, Liu RZ, Koomen J, Tan AC, Degregori J, Haura EB. Coupling an EML4-ALK-centric interactome with RNA interference identifies sensitizers to ALK inhibitors. Sci Signal 2016; 9: rs12.

30) Janoueix-Lerosey I, Lequin D, Brugières L, Ribeiro A, de Pontual L, Combaret V, Raynal V, Puisieux A, Schleiermacher G, Pierron G, Valteau-Couanet D, Frebourg T, Michon J, Lyonnet S, Amiel J, Delattre O. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. Nature 2008; 455: 967-970.

31) Murugan AK, Xing M. Anaplastic thyroid cancers harbor novel oncogenic mutations of the ALK gene. Cancer Res 2011; 71: 4403-4411.

32) Wang YW, Tu PH, Lin KT, Lin SC, Ko JY, Jou YS. Identification of oncogenic point mutations and hyperphosphorylation of anaplastic lymphoma kinase in lung cancer. Neoplasia 2011; 13: 704-715.

33) Della Corte CM, Viscardi G, Di Liello R, Fasano M, Martinelli E, Troiani T, Ciardiello F, Morgillo F. Role and targeting of anaplastic lymphoma kinase in cancer. Mol Cancer 2018; 17: 30.

34) Zito Marino F, Bianco R, Accardo M, Ronchi A, Cozzolino I, Morgillo F, Rossi G, Franco R. Molecular heterogeneity in lung cancer: from mechanisms of origin to clinical implications. Int J Med Sci 2019; 16: 981-989.

35) Paolino G, Bekkenk MW, Didona D, Eibenschutz L, Richetta AG, Cantisani C, Viti G, Carbone A, Buccini P, De Simone P, Ferrari A, Scali E, Calvieri S, Silipo V, Cigna E, Viti GP, Bottoni U. Is the prognosis and course of acral melanoma related to site-specific clinicopathological features? Eur Rev Med Pharmacol Sci 2016; 20: 842-848.

36) Potrony M, Badenas C, Aguilera P, Puig-Butille JA, Carrera C, Malvehy J, Puig S. Update in genetic susceptibility in melanoma. Ann Transl Med 2015; 3: 210.

37) Niu HT, Zhou QM, Wang F, Shao Q, Guan YX, Wen XZ, Chen LZ, Feng QS, Li W, Zeng YX, Zhang XS. Identification of anaplastic lymphoma kinase break points and oncogenic mutation profiles in acral/mucosal melanomas. Pigment Cell Melanoma Res 2013; 26: 646-653.

38) Yeh I, Jorgenson E, Shen L, Xu M, North JP, Shain AH, Reuss D, Wu H, Robinson WA, Olshen A, von Deimling A, Kwok PY, Bastian BC, Asgari MM. Targeted genomic profiling of acral melanoma. J Natl Cancer Inst 2019; 111: 1068-1077.

39) Casso EM, Grin-Jorgensen CM, Grant-Kels JM. Spitz nevi. J Am Acad Dermatol 1992; 27: 901-913.

40) Wiesner T, He J, Yelensky R, Esteve-Puig R, Botton T, Yeh I, Lipson D, Otto G, Brennan K, Murali R, Garrido M, Miller VA, Ross JS, Berger MF, Sparatta A, Palmedo G, Cerroni L, Busam KJ, Kutzner H, Cronin MT, Stephens PJ, Bastian BC. Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. Nat Commun 2014; 5: 3116.

41) Harms KL, Lowe L, Fullen DR, Harms PW. Atypical Spitz tumors: a diagnostic challenge. Arch Pathol Lab Med 2015; 139: 1263-1270.

42) Dimonitsas E, Liakea A, Sakellariou S, Thymara I, Giannopoulos A, Stratigos A, Soura E, Saetta A, Korkolopoulou P. An update on molecular alterations in melanocytic tumors with emphasis on Spitzoid lesions. Ann Transl Med 2018; 6: 249.

43) Botton T, Yeh I, Nelson T, Vemula SS, Sparatta A, Garrido MC, Allegra M, Rocchi S, Bahadoran P, McCalmont TH, LeBoit PE, Burton EA, Bollag G, Ballotti R, Bastian BC. Recurrent BRAF kinase fusions in melanocytic tumors offer an opportunity for targeted therapy. Pigment Cell Melanoma Res 2013; 26: 845-851.

44) Yeh I, de la Fouchardiere A, Pissaloux D, Mully TW, Garrido MC, Vemula SS, Busam KJ, LeBoit PE, McCalmont TH, Bastian BC. Clinical, histopathologic, and genomic features of Spitz tumors with ALK fusions. Am J Surg Pathol 2015; 39: 581-591.

45) Busam KJ, Kutzner H, Cerroni L, Wiesner T. Clinical and pathologic findings of Spitz nevi and atypical Spitz tumors with ALK fusions. Am J Surg Pathol 2014; 38: 925-933.

46) Wu G, Barnhill RL, Lee S, Li Y, Shao Y, Easton J, Dalton J, Zhang J, Pappo A, Bahrami A. The landscape of fusion transcripts in spitzoid melanoma and biologically indeterminate spitzoid tumors by RNA sequencing. Mod Pathol 2016; 29: 359-369.

47) Kiuru M, Jungbluth A, Kutzner H, Wiesner T, Busam KJ. Spitz tumors: comparison of histological features in relationship to immunohistochemical staining for ALK and NTRK1. Int J Surg Pathol 2016; 24: 200-206.

48) Lee CY, Sholl LM, Zhang B, Merkel EA, Amin SM, Guitart J, Gerami P. Atypical Spitzoid neoplasms in childhood: a molecular and outcome study. Am J Dermatopathol 2017; 39: 181-186.

49) Amin HM, Lai R. Pathobiology of ALK+ anaplastic large-cell lymphoma. Blood 2007; 110: 2259-2267.

50) Perron E, Pissaloux D, Charon Barra C, Karanian M, Lamant L, Parfait S, Alberti L, de la Fouchardière A. Melanocytic Myxoid Spindle Cell Tumor with ALK rearrangement (MMySTAR): report of 4 cases of a nevus variant with potential diagnostic challenge. Am J Surg Pathol 2018; 42: 595-603.

51) Dunn ALJ, Gardner JM, Kaley JR, Bellamy W, Shalin SC. ALK Rearrangements are infrequent in cellular blue nevus and deep penetrating nevus. Am J Dermatopathol 2018; 40: 469-478.

52) Zito Marino F, Rocco G, Morabito A, Mignogna C, Intartaglia M, Liguori G, Botti G, Franco R. A new look at the ALK gene in cancer: copy number gain and amplification. Expert Rev Anticancer Ther 2016; 16: 493-502.

53) Farah M, Nagarajan P, Curry JL, Tang Z, Kim TB, Aung PP, Torres-Cabala CA, Eterovic AK, Wargo JA, Prieto VG, Tetzlaff MT. Spitzoid melanoma with histopathological features of ALK gene rearrangement exhibiting ALK copy number gain: a novel mechanism of ALK activation in spitzoid neoplasia. Br J Dermatol 2019; 180: 404-408.

54) Xia J, Jia P, Hutchinson KE, Dahlman KB, Johnson D, Sosman J, Pao W, Zhao Z. A meta-analysis of somatic mutations from next generation sequencing of 241 melanomas: a road map for the study of genes with potential clinical relevance. Mol Cancer Ther 2014; 13: 1918-1928.

55) Busam KJ, Vilain RE, Lum T, Busam JA, Hollmann TJ, Saw RP, Coit DC, Scolyer RA, Wiesner T. Primary and metastatic cutaneous melanomas express ALK through alternative transcriptional initiation. Am J Surg Pathol 2016; 40: 786-795.

56) Wiesner T, Lee W, Obenauf AC, Ran L, Murali R, Zhang QF, Wong EW, Hu W, Scott SN, Shah RH, Landa I, Button J, Lailler N, Sboner A, Gao D, Murphy DA, Cao Z, Shukla S, Hollmann TJ, Wang L, Borsu L, Merghoub T, Schwartz GK, Postow MA, Ariyan CE, Fagin JA, Zheng D, Ladanyi M, Busam KJ, Berger MF, Chen Y, Chi P. Alternative transcription initiation leads to expression of a novel ALK isoform in cancer. Nature 2015; 526: 453-457.

57) Couts KL, Bemis J, Turner JA, Bagby SM, Murphy D, Christiansen J, Hintzsche JD, Le A, Pitts TM, Wells K, Applegate A, Amato C, Multani P, Chow-Maneval E, Tentler JJ, Shellman YG, Rioth MJ, Tan AC, Gonzalez R, Medina T, Doebele RC, Robinson WA. ALK inhibitor response in melanomas expressing EML4-ALK fusions and alternate ALK isoforms. Mol Cancer Ther 2018; 17: 222-231.