EJC Skin Cancer 2 (2024) 100272

Contents lists available at ScienceDirect

EJC Skin Cancer

journal homepage: www.journals.elsevier.com/ejc-skin-cancer

Case Report

MBNL2-ALK: A novel ALK fusion transcript in a sinonasal melanoma

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ARTICLE INFO

Keywords:
Melanoma
ALK, MBNL2

A variety of *ALK* gene alterations has been described in oncology, including point mutations, deletions, amplifications and rearrangements. A wide spectrum of *ALK* fusions occurs in cancer, in which the kinase domain of ALK and the amino-terminal portion of various protein partners are fused [1]. Recently, a novel isoform of the anaplastic lymphoma kinase named ALK ATI was described in cutaneous melanoma. The novel ALK transcript initiates from a de novo alternative transcription initiation (ATI) site in *ALK* intron 19; is expressed in ~ 11 % of melanomas and sporadically in other human cancer types, but not in normal tissues [2].

We present a case of sinonasal melanoma (pigmented lesion, dimension: 35 mm, mitosis: 10/mm2, linfovascular invasion: present, pT3 CN0,M0, R1, stage II) in which NGS (Oncomine Dx Express test, ThermoFisher) lead us to identify ALK 5' versus 3' expression imbalance.

The patient, a 46 years old woman, came to our institution due to prolonged epistaxis and clinical and radiological examination revealed a mass in the nasal cavity that was biopsied. After the histological diagnosis of malignant melanoma, the patient underwent surgical removal of the lesion. Histological examination confirmed the diagnosis of malignant sinonasal melanoma. At the microscope, the neoplasm was composed of epithelioid cells with abundant cytoplasm, irregular nuclei and eosinophilic prominent nucleoli, arranged in a solid and nested pattern. Within the lesion there were deposits of melanotic pigment and areas of necrosis (Fig. 1). A minimal intraepithelial component was observed. Immunohistochemical analysis showed positivity for MART-1, HMB-45, SOX-10 and S-100 and negativity for epithelial and lymphoid markers performed (respectively, pan-cytokeratin AE1/AE3 cocktail and a panel composed of CD4, CD8, CD30, CD43).

Immunohistochemistry with anti ALK (D5F3, Ventana Medical Sysistem Inc) MoAb resulted strongly positive as well as fluorescence in situ hybridization (ALK breakapart probe, MetaSystems). Anchored Multiplex (AMP) PCR-based NGS (Archer™ FUSIONPlex™ Lung v2 panel, Integrated DNA Technologies) confirmed *ALK* imbalance and revealed a novel fusion gene comprising *MBNL2* and *ALK* genes. The novel fusion gene expressed two transcript isoforms: *MBNL2*(EX1)-*ALK* (EX18) and *MBNL2*(EX1)-*ALK*(EX17) with a depth of 5069 and of 3358 unique reads (Fig. 2).

The fusion gene *MBNL2::ALK* has never been reported in cancer. It appears that *MBNL2* ((muscleblind like splicing regulator 2) provides the 5' half of the fusion gene, thus comprising the promoter region driving expression of the fusion gene, and ALK provides the 3' half of *ALK*, including the catalytic domain and similar to most fusion genes involving *ALK*. Therefore, given the widespread expression of *MBNL2* gene in most tissues, the predicted in-frame fusions generated, the preservation of the 3' half of *ALK*, coding for the tyrosine kinase domain, as well as the significant ALK protein overexpression observed by immunohistochemistry, we can robustly suppose that this novel fusion gene provides ALK oncogenic constitutive activation.

While in most known *ALK* fusion genes the breakpoint is upstream of exon 20, in the present case *ALK* exons 17–18 are retained in the fusion gene, thus suggesting a more proximal breakpoint than usual. Notably, the *MBNL2::ALK* fusion gene detected is predicted to retain the transmembrane domain located upstream the catalytic domain. Indeed,

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https://doi.org/10.1016/j.ejcskn.2024.100272
Received 24 August 2024; Received in revised form 7 October 2024; Accepted 22 October 2024
Available online 24 October 2024

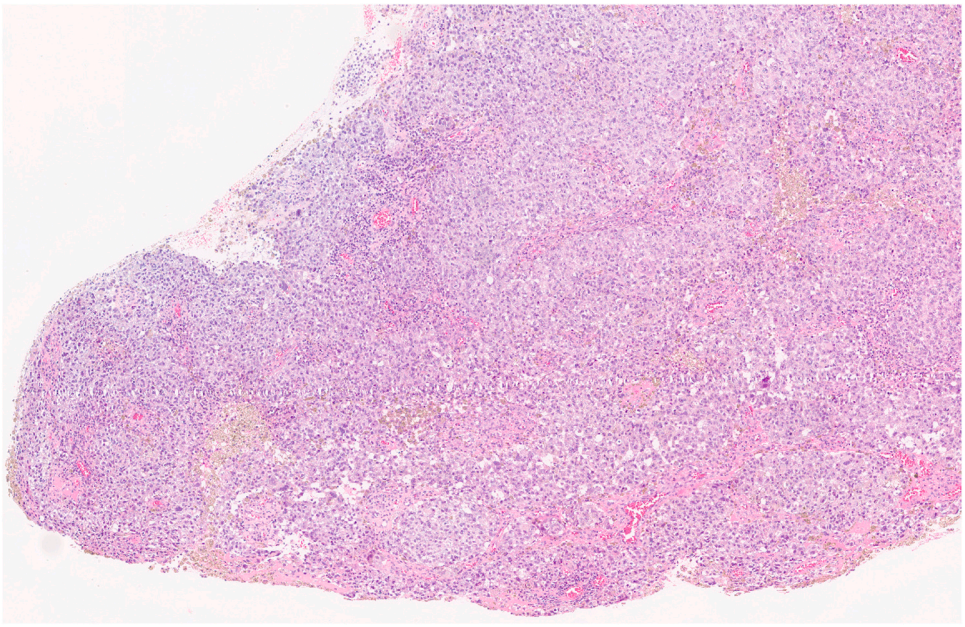

Fig. 1. Low power magnification hystological picture of the lesion (40x).

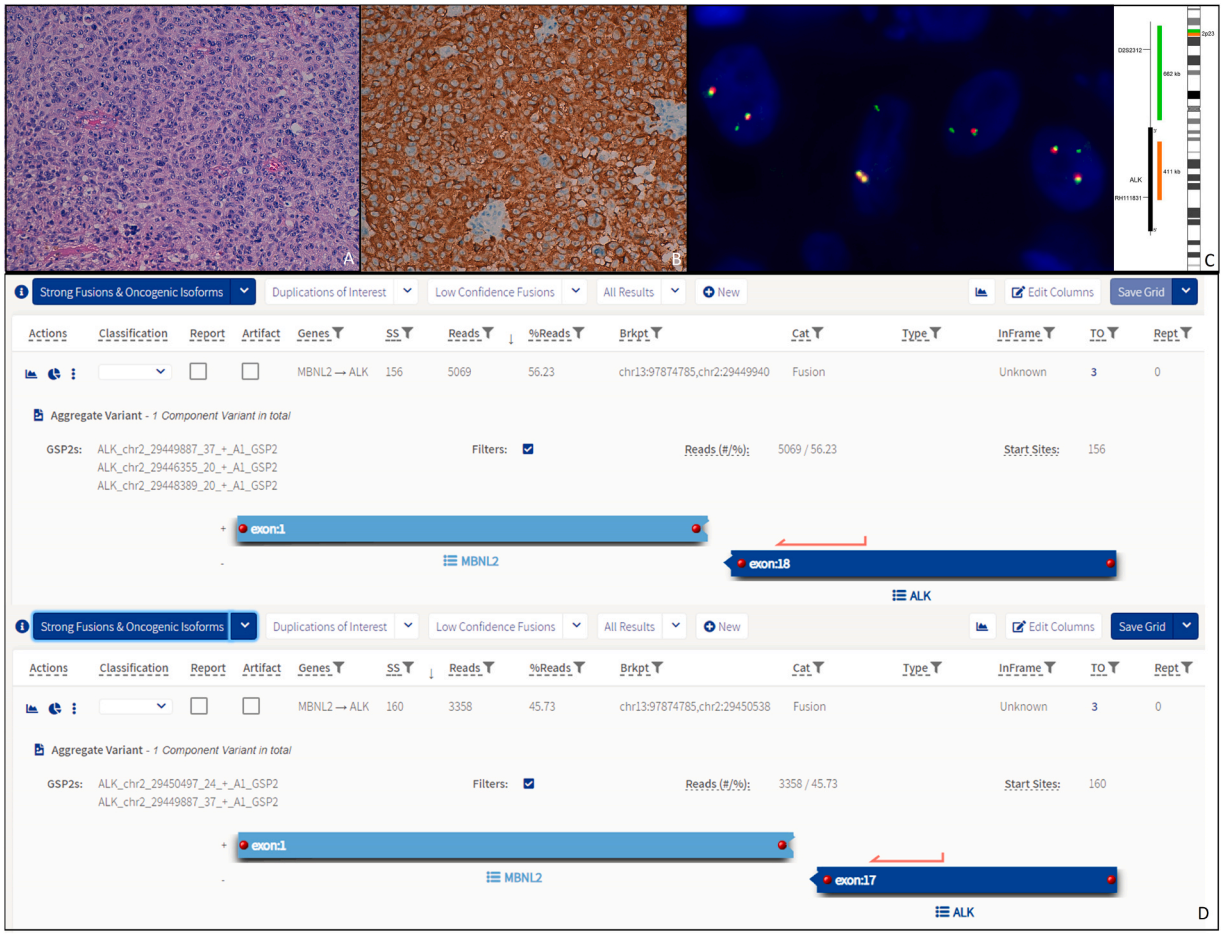

Fig. 2. A) Histopathological finding from hematoxylin–eosin staining (200x); B) Immunohistochemistry with anti ALK (D5F3, Ventana Medical Sysistem Inc); C) ALK FISH result with breakapart probe (MetaSystems) showing a single green signal (broken 3'-ALK as shown in the cartoon) in addition to fused signals; D) Anchored Multiplex (AMP) PCR-based NGS (Archer™ FUSIONPlex™ Lung v2 panel, Integrated DNA Technologies) shows a novel in-frame fusion involving *MBNL2* and *ALK* genes.

although uncommon, breakpoint location retaining ALK exons 17/18 has already been described in rare fusion genes with EML4, AGAP1, KANK2, ACTG2 [3].

MBNL2 is located on chromosome 13 and it is a member of the RNA binding protein MBNL family. It is supposed to play a tumor suppressive function in cancer and it is a central mediator of cancer cell responses to hypoxia; it has been described to be induced under hypoxic conditions and it has been involved in hypoxia adaptation by regulating the hypoxia-response genes, such as vascular endothelial growth factor A (*VEGFA)* [4]. Hoek KS. reported up-regulation of MBNL2 expression in melanoma [5].

The prognostic or predictive role of MBNL2::ALK translocation in our patient is unknown, but our findings suggest it represents a primary event.

After endoscopic resection in August 2023 the patient received adrotherapy and adjuvant immunotherapy with anti PD-1 planned for 1 year is ongoing. No relapse has occurred.

Cancer genome atlas defines four subtypes of melanoma based on the pattern of the most prevalent significantly mutated genes: mutant *BRAF*, mutant *RAS*, mutant *NF1*, and Triple wild type [6]. ALK expression is not evaluated routinely in melanoma and so far, no therapeutic targets are validated for *ALK* gene rearrangements in cutaneous neoplasm.

Considering the paucity of targets in melanoma, further studies are needed to understand *ALK*'s role in the biology of melanoma and its possible prognostic or predictive role. To note, no data are available about the effectiveness of immune-checkpoint inhibitors in case of *ALK* alterations and very few in vitro studies were performed using anti-ALK drugs [7].

This is the first report of a rare fusion in a mucosal melanoma. Although so far unique, our intriguing observation generates the hypothesis to look for ALK rearrangements in mucosal melanoma.

CRediT authorship contribution statement

Marzia Giagnacovo: Data curation. **Stefano Zannella:** Writing – review & editing. **Francesca Caspani:** Writing – review & editing. **Elena Bolzacchini:** Writing – original draft, Conceptualization. **Monica Giordano:** Supervision. **Daniela Furlan:** Writing – review & editing, Supervision, Data curation. **Piergiorgio Modena:** Writing – original draft, Data curation, Conceptualization. **Carlo Patriarca:** Supervision, Conceptualization. **Muhammad Adnan:** Data curation.

Patient consent

Patient's consent was achieved.

Funding Information

No fundings were received.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] B. Hallberg, R.H. Palmer, The role of the ALK receptor in cancer biology, Ann. Oncol. 27 (3) (2016) iii4–iii15, https://doi.org/10.1093/annonc/mdw301. PMID: 27573755.

[2] T. Wiesner, W. Lee, A.C. Obenauf, L. Ran, R. Murali, Q.F. Zhang, E.W. Wong, W. Hu, S.N. Scott, R.H. Shah, I. Landa, J. Button, N. Lailler, A. Sboner, D. Gao, D.A. Murphy, Z. Cao, S. Shukla, T.J. Hollmann, L. Wang, L. Borsu, T. Merghoub, G.K. Schwartz, M. A. Postow, C.E. Ariyan, J.A. Fagin, D. Zheng, M. Ladanyi, K.J. Busam, M.F. Berger, Y. Chen, P. Chi, Alternative transcription initiation leads to expression of a novel ALK isoform in cancer, Nature 526 (7573) (2015) 453–457, https://doi.org/10.1038/nature15258. PMID: 26444240; PMCID: PMC4807020.

[3] L.E. Davis, K.D. Nusser, J. Przybyl, J. Pittsenbarger, N.E. Hofmann, S. Varma, S. Vennam, M. Debiec-Rychter, M. van de Rijn, M.A. Davare, Discovery and characterization of recurrent, targetable ALK fusions in leiomyosarcoma, Mol. Cancer Res. 17 (3) (2019) 676–685, https://doi.org/10.1158/1541-7786.MCR-18-1075. Epub 2018 Dec 5.

[4] S. Fischer, A. Di Liddo, K. Taylor, J.S. Gerhardus, K. Sobczak, K. Zarnack, J. E. Weigand, Muscleblind-like 2 controls the hypoxia response of cancer cells, RNA 26 (5) (2020) 648–663, https://doi.org/10.1261/rna.073353.119. Epub 2020 Mar 3. PMID: 32127384; PMCID: PMC7161353.

[5] K.S. Hoek, DNA microarray analyses of melanoma gene expression: a decade in the mines, Pigment Cell Res. 20 (6) (2007) 466–484, https://doi.org/10.1111/j.1600-0749.2007.00412.x. PMID: 17935490.

[6] Cancer Genome Atlas Network, Genomic classification of cutaneous melanoma, Cell 161 (7) (2015) 1681–1696, https://doi.org/10.1016/j.cell.2015.05.044. PMID: 26091043; PMCID: PMC4580370.

[7] K.L. Couts, J. Bemis, J.A. Turner, S.M. Bagby, D. Murphy, J. Christiansen, J. D. Hintzsche, A. Le, T.M. Pitts, K. Wells, A. Applegate, C. Amato, P. Multani, E. Chow-Maneval, J.J. Tentler, Y.G. Shellman, M.J. Rioth, A.C. Tan, R. Gonzalez, T. Medina, R.C. Doebele, W.A. Robinson, ALK Inhibitor Response In Melanomas Expressing *EML4-ALK* fusions and alternate *ALK* isoforms, Mol. Cancer Ther. 17 (1) (2018) 222–231, https://doi.org/10.1158/1535-7163.MCT-17-0472. Epub 2017 Oct 20. PMID: 29054983; PMCID: PMC5752582.