ONCOLOGY: BRIEF REPORT

PAX3-NCOA1 alveolar rhabdomyosarcoma of the tongue: A rare entity with challenging diagnosis and management

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Abstract

Alveolar rhabdomyosarcoma (ARMS) is associated with PAX3/PAX7-FOXO1 fusion, which confers specific clinic and biologic characteristics with inferior outcomes. A minority of tumors still histologically classified as "true" ARMS lack the canonical PAX-FOXO1 fusion but have new molecular alterations. We present the first case of PAX3-NCOA1 ARMS with clinical data and follow-up in a two-year-old girl with ARMS of the tongue and nodal extension, treated with chemotherapy, hemi glossectomy, lymph node dissection, and brachytherapy to conserve oral function and limit long-term sequelae. Given the rarity of such variant fusion in ARMS, international collaboration is required to evaluate its prognostic value.

KEYWORDS
alveolar rhabdomyosarcoma, brachitherapy, children, PAX3-NCOA1, surgery

1 | INTRODUCTION

Rhabdomyosarcomas (RMS) are the largest subset of soft-tissue pediatric sarcomas and are highly heterogeneous. The two main histological patterns recognized include embryonal rhabdomyosarcoma (ERMS) and alveolar rhabdomyosarcoma (ARMS). Approximately 80% of patients with ARMS present with the fusion genes paired box 3 (*PAX3*)-forkhead box protein O1 (*FOXO1*) or paired box 7 (*PAX7*)-*FOXO1* as a consequence of the reciprocal chromosomal translocations t(2;13)(q35;q14) or t(1;13)(p36;q14). Recent data have suggested that the *PAX3/7-FOXO1* fusion genes have prognostic significance,[1,2] and the modern Nord American and European RMS trials stratify therapy on *FOXO1*-fusion status. However, approximately 10%-20% of the ARMS do not display the canonical *PAX-FOXO1* fusion and new molecular rearrangement involving *PAX3-NCOA1* or *NCOA2* genes have been

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Abbreviations: ARMS, alveolar rhabdomyosarcoma; ERMS, embryonal rhabdomyosarcoma; GR, Gustave Roussy; IMRT, intensity-modulated radiation therapy; IVA, ifosfamide, vincristine, actinomycin; N1, nodal involvement; RMS, rhabdomyosarcoma.

identified.[3,4] Fewer than 10 cases of ARMS with *PAX3-NCOA1* or *PAX3-NCOA2* fusion have been reported so far, and the prognostic value of such variant fusions in ARMS is still unknown.

We therefore report a two-year-old girl with aggressive *PAX3-NCOA1* ARMS of the tongue and mouth floor and large nodal extension, successfully treated with chemotherapy, hemiglossectomy and lymph node dissection, and brachytherapy.

2 | CASE DESCRIPTION

A two-year-old girl was addressed to our Department (GR, Villejuif, France) for RMS of the tongue. The patient had no previous medical history, and the parents were not consanguineous. Parents first observed swelling on the ventral surface of the tongue when the child was seven months old. No imaging was performed, and the child had primary resection in Algeria at 16 months but the lesion rapidly recurred.

Magnetic resonance imaging confirmed the presence of an infiltrative lesion originating from the left hemi-tongue, $46 \times 20 \times 18$ mm (Figure 1A). After pathology review in GR, histological analysis confirmed ARMS diagnosis with round and spindle cells desmin-positive and ~100% cells expressing myogenin and MyoD1 (Figure 1B). The FISH showed gain of *FOXO1* (2-8 copies), but the absence of rearrangement/amplification with *PAX7* or *PAX3*. RNA sequencing using Archer FusionPlex Sarcoma 50 genes Panel on Formalin-Fixed Paraffin-Embedded tissue reported the presence of the transcript *PAX3-NCOA1* (Figure 1C-D). Additional workup imaging showed enlarged and necrotic bilateral cervical lymph nodes, the larger one measuring $27 \times 24 \times 19$ mm in the submandibular region (Figure 1A), and the presence of three equivocal pulmonary micronodules (max size 3.5 mm; 2.5 and 1.5 mm for the others). The patient was initially considered with metastatic disease and treated in Algeria with 6 cycles of "6 drug" regimen (IVA plus carboplatin, epirubicin, and etoposide) with 67% tumor volume reduction. The revision of pulmonary images in GR raised a doubt concerning the neoplastic nature of the micronodules that were not considered as expression of disseminated disease and the child was subsequently treated as N1M0 ARMS, very-high-risk group (H) according to EpSSG Protocol, based on the pathology without considering the fusion status.

After seven courses of chemotherapy, surgery was performed consisting of left hemiglossectomy extended to the floor of the mouth and reconstruction with the apposition of a latissimus flap, along with modified radical cervical lymph node dissection at left side and partial dissection at right side. The postoperative period was uneventful, and chemotherapy was restarted at day 18. The child started to have oral liquid and solid alimentation by day 17 and had almost normal elocution by day 34. Histological analysis showed the presence of about 50% of viable cells with in one area on the mouth central floor, clear margins ≤2 mm. All the 44 lymph nodes analyzed were negative for viable RMS cells.

Adjuvant chemotherapy was continued with IVA regimen for a total of 10 courses. Because of alveolar histology and clear margins ≤2 mm, additional local therapy was considered. New surgery would have

been associated with severe expected oral dysfunction. Brachytherapy was chosen among all radiotherapy technics, because of the best therapeutic ratio in terms of tumor dose and normal tissue sparing, with the possibility to minimize healthy tissues such as the mandible, teeth, and salivary glands. An interstitial brachytherapy procedure was then delivered at a dose of 60.06 Gy in 143 continuous pulses of 0.42 Gy per pulse (Figure 2).

Grade I mucositis was observed after brachytherapy for about 16 days. Because of young age, large cervical lymph node dissection, and absence of viable cells on lymph nodes examination, no additional radiotherapy was delivered on cervical lymph nodes. The treatment was completed by six months of vinorelbine and low-dose cyclophosphamide maintenance chemotherapy.[5] Twenty months after diagnosis, the child is in continuous complete remission off-therapy with no apparent complications, normal oral intakes, swallowing, and phonation. Longer follow-up is required to confirm disease control and assess specific long-term sequelae.

3 | DISCUSSION

Diagnosis of RMS has evolved in the last decades, integrating all parameters such as morphology and immunohistochemistry phenotype, but also molecular biology. FOXO1 fusion-positive ARMS accounts for ~80-90% of ARMS, with PAX3 and PAX7 fusions representing 60% and 20% of all ARMS cases, respectively. The majority of FOXO1 fusion-negative ARMS tumors are biologically and clinically distinct from ARMS FOXO1 fusion-positive tumors, with more favorable outcomes similar to those of ERMS.[6,7] The identification of PAX-FOXO1 fusion genes is thus mandatory to stratify the patients for therapy.[2]

Failure to identify the canonical fusion transcript PAX/FOXO1 has permitted the so-called fusion-negative RMS to emerge, grouping histological recognized ERMS but also some truly morphologically and phenotypically ARMS. Variant gene fusions have been reported in these ARMS such as PAX3-AFX, PAX3-FOXO4, PAX3-NCOA1, and PAX3-NCOA2.[7,8] PAX3-NCOA1 fusion can act as transcription activator on PAX3-regulated genes with a translocation-specific expression profile similar to canonical PAX3/FOXO1 fusion, suggesting similar clinical behavior.[4] So far, only six cases have been reported of PAX3-NCOA RMS, and to our knowledge, our case is the first with biological and clinical data.[3,4]

Concerning the nodal involvement, EBRT without node dissection would be standard of care. Considering the young age of the patient and the negative histology after surgery, an alternative approach was chosen in this specific case to avoid external RT.

Brachytherapy was chosen in addition to hemiglossectomy to achieve local control, instead of second-look mutilating surgery or external radiotherapy techniques. The use of brachytherapy is remarkable in this case for the young age of the patient, the difficulty related to the particular site to treat and the small volume to irradiate. Brachytherapy has advantages over other techniques in terms of short duration of the treatment and the potential reduction of long-term sequelae in comparison with external beam radiotherapy, such
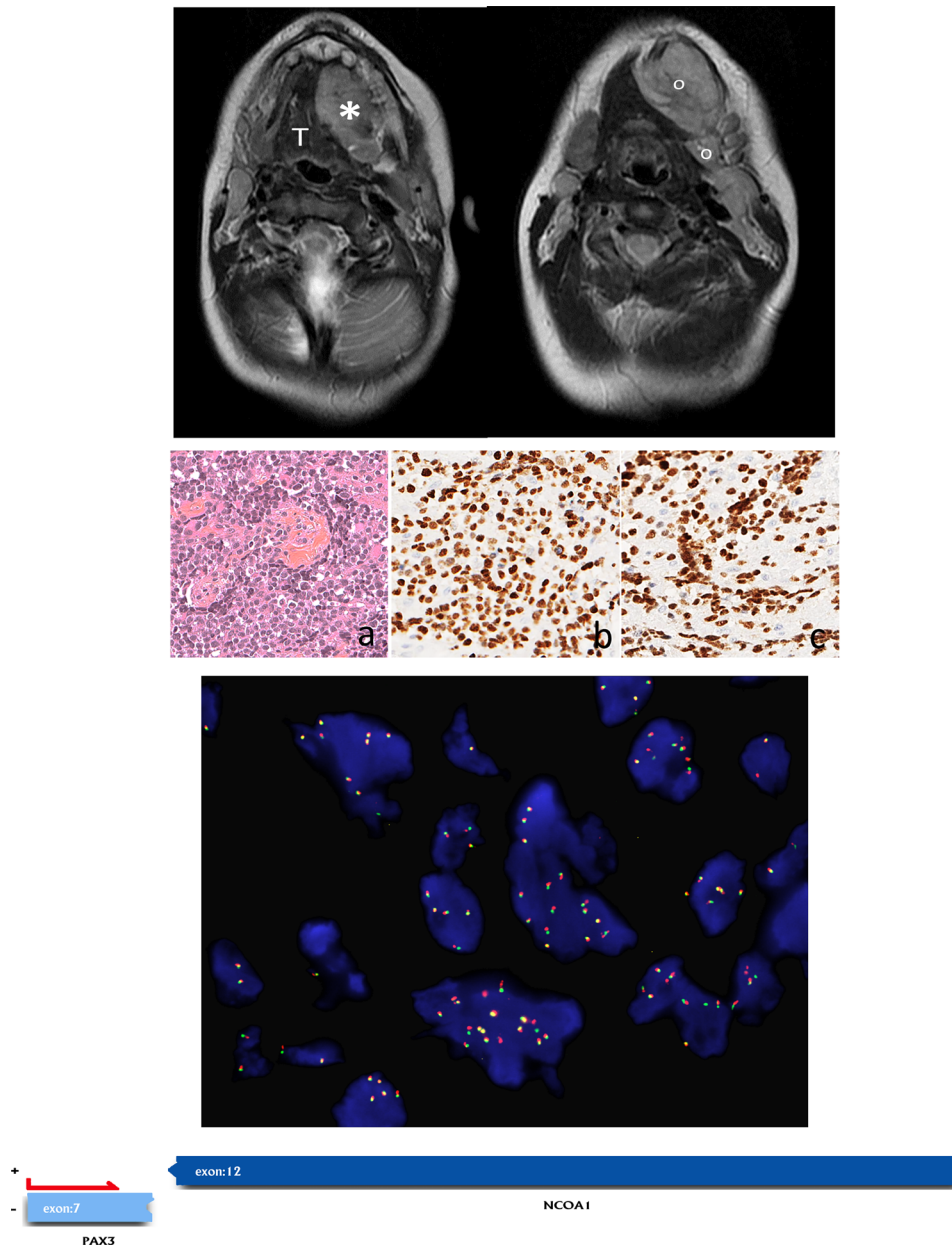
FIGURE 1 Imaging and biopathology. (A) Magnetic resonance imaging (on the left T: tongue; * primary tumor; ** lymph nodes). (B) Histological and immunohistochemical features. The tumor is made predominantly of small, round, poorly differentiated cells, with little intervening connective stroma (a), strongly expressing myogenin (b), and myo-D1 (c). a, Hematoxylin-eosin-saffron, original magnification : a, ×180; b and c, immunoperoxidase with nuclear counterstaining, original magnifications, x200. (C) FOXO1 break-apart FISH probe from Zytovision was hybridized on FFPE tissue slides: no FOXO1 rearrangement is detected as indicated by the presence of only fused yellow signals (1 orange + 1 green). The 3'FOXO1 is labeled with a green fluorochrome (ZyGreen), and the 5'FOXO1 is labeled with an orange fluorochrome (ZyOrange). A rearranged FOXO1 should lead to split signals and separate green, and orange signals are expected. If FOXO1 is not involved in a translocation, the gene remains unsplit leading to yellow signals in tumor cells. Of note, extra copies of FOXO1 are observed, probably the result of polysomy. (D) RNA-seq was performed using the ARCHER comprehensive sarcoma FUSIONPLEX from FFPE curls. Schematic depiction of the PAX3-NCOA1 fusion: exon 7 of PAX3 is fused to exon 12 of NCOA1

as facial asymmetry, poor dentition, and salivary dysfunction. Such postoperative procedure requires a close discussion with surgeons to properly cover the target volume, according to pathological findings. Proton therapy has been proposed as alternative treatment to brachytherapy as well, since it is known to guarantee the same target coverage as intensity-modulated radiotherapy, but with significant sparing of critical structures, by reducing normal tissue doses for head and neck RMS.[9] Dosimetric comparisons conducted in adults however show the superiority of brachytherapy in terms of dose distribution and minimization of the integral dose to the patient. This multimodal
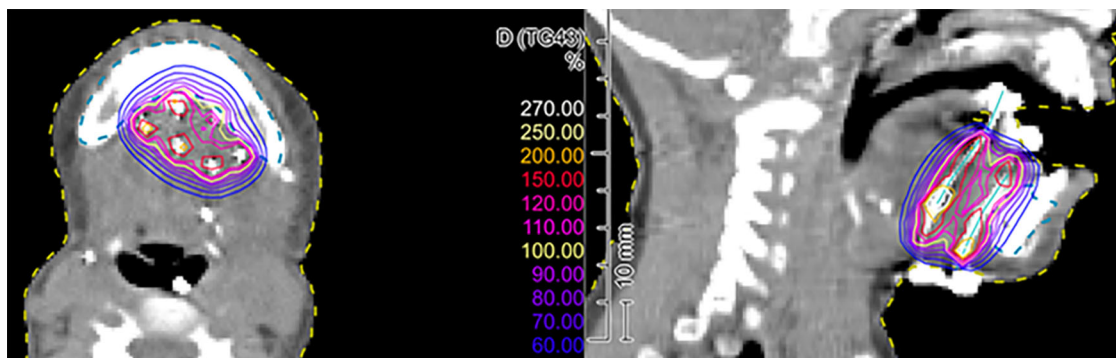
FIGURE 2 Brachytherapy. Dose distribution of the brachytherapy implant. The volume encompassed by the 60 Gy isoline receives at least 60 Gy. This dose is prescribed according to the Paris system rules, which means that the dose is prescribed on the 85% isodose. The volume encompassed by the purple isoline receives at least 54 Gy. The blue isoline is the 30 Gy isodose. The green line is the 12 Gy isodose. The dose to the parotids glands is minimal, and optimal sparing of bone structure through dwell times optimization could be achieved (sagittal view). The prescription of 60 Gy in 143 pulses allowed delivering at least 50 Gy to the tumor bed plus a safety margin, taking into account the rapid dose fall off and potential uncertainties in target volume definition in the postoperative context. According to the linear quadratic model, the dose of 60 Gy corresponds to an equivalent dose per 2 Gy fractions of 59.1 Gy_{EQD2} for tumor and 57.9 Gy for normal tissue involved in late damages (with alpha/beta value of 3 Gy and 10 Gy, respectively, and half-time repair of 1.5 h for both)

approach may therefore optimize functional outcome, while minimizing the risk of second cancer which may be a significant concern in the youngest patients treated with ionizing radiation.[10,11]

It is also important to underline that the previous European protocol has taken into account histology and not fusion status for risk group stratification. Instead, future European and current North American protocols use fusion status to stratify patients into risk groups. With current protocols, a patient with ARMS considered fusion-negative would have been treated with a less intensive treatment, although the prognostic value of some new fusions, e.g., PAX3-NCOA1, is unknown.

In conclusion, this is the first case of infant with ARMS and variant fusion *PAX3-NCOA1*, advanced tongue disease, and nodal extension at diagnosis, treated with a combination of chemotherapy (including maintenance therapy), surgery, and brachytherapy to limit long-term sequelae. Given the rarity of this variant fusion in ARMS, international collaboration is required to evaluate its prognostic value, in order to identify and clarify alternative molecular rearrangements with possibly different clinical behavior and prognosis.

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