case reports

Identification of Novel PGR-NR4A3 Fusion in Extraskeletal Myxoid Chondrosarcoma and Resultant Patient Benefit From Tamoxifen Therapy

H. Catherine Wilbur, MD[1,2]; Dan R. Robinson, PhD[3,4]; Yi-Mi Wu, PhD[3,4]; Chandan Kumar-Sinha, PhD[3,4]; Arul M. Chinnaiyan, MD, PhD[3,4]; and Rashmi Chugh, MD[1]

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Background

Extraskeletal myxoid chrondrosarcoma (EMC) is a rare soft-tissue sarcoma of uncertain histogenesis, typically affecting males to females in a 2:1 ratio with a median age of 50 years.[1,2] EMC most often presents as enlarging soft-tissue mass in the proximal extremities.[2] The tumor has local recurrence and metastasis rates between 29%-48% and 40%-50%,[2,3] respectively. Despite this, the survival rate at 10 years is 65%-70%,[4,5] which often belies the indolent nature of the disease even when metastatic.

Seventy percent of EMC are characterized by a chromosomal rearrangement involving the *NR4A3* gene on chromosome 9 with *EWSR1* on chromosome 22, t(9; 22).[6–8] Rearrangements of *NRA4A3* with *TAF15, TCF12, TFG,* and *HSPA8* have also been described and are associated with poorer outcomes.[9] Although the characteristic morphology of EMC consists of oval to short spindled cells arranged in cords or a reticular pattern, rare cases are hypercellular with a high degree of cytologic atypia, known as the cellular variant. This variant is frequently associated with non-*EWSR1-NR4A3* rearrangements.[1,9–11]

Treatment of localized EMC consists of wide-local excision with or without radiation.[5] Metastatic disease is typically multifocal, progressive, and incurable, and the most common cause of death is progressive lung involvement. We report a case of a young woman who was diagnosed in the setting of pregnancy with metastatic EMC with a novel translocation involving the progesterone receptor *(PGR)* and *NR4A3* identified using next-generation sequencing. The investigators obtained written informed consent from the patient to publish all information and images.

Case Presentation

A 35-year-old G1P0 woman at 20 weeks gestation presented with right groin pain. Ultrasound revealed a 4.7-cm mass that was aspirated and yielded thick bloody fluid, and the patient was diagnosed with a hematoma. Three weeks later, she presented with severe lower abdominal pain and underwent emergent surgery for presumed incarcerated hernia in the same area. Intraoperatively, there was an encapsulated mass that extended inferiorly toward the vagina and biopsy revealed the cellular variant of extraskeletal myxoid chondrosarcoma (EMC). Immunohistochemical evaluation negative for WT-1, CDX2, GATA3, CK-7, CK-20, MOC31, PAX-8, calretinin, LCA, pan-cytokeratin, and desmin with focal nonspecific reactivity for CD99. Fluorescent in situ hybridization was negative for *EWSR1* rearrangement.

Subsequent magnetic resonance imaging of the pelvis performed when patient was at 25 weeks gestation revealed two lesions with internal hemorrhage and enhancing irregular walls measuring 4 cm within the deep subcutaneous adipose tissue of the inguinal areas bilaterally. Surgery was recommended but deferred until pregnancy completion per patient preference, and tumors were followed with serial ultrasounds (Figs 1B and 1C). The patient underwent a planned cesarean section at 34 weeks gestation, which was complicated by cellulitis and wound dehiscence, further delaying resection of enlarging tumors.

Forty days after delivery, patient underwent wide excision with en bloc bilateral inguinal nodal dissection with negative margins. Pathology demonstrated EMC, cellular variant, grade 2/3 with masses measuring 10.5 cm and 8.2 cm (Fig 1D). Adjuvant radiation was recommended but delayed because of recurrent abscesses and cellulitis. Imaging performed 2 months postoperatively revealed recurrence of several soft-tissue masses within the midline abdominal subcutaneous tissue. Repeat wide surgical excision revealed adherent tumor along the right labia, which ruptured intraoperatively. Pathology was again consistent with cellular variant of EMC, measuring 7.3 cm with a positive lateral margin. Concurrently, tumor was submitted for next-generation sequencing as part of the Michigan Oncology Sequencing program (MI-ONCOSEQ). RNA sequencing was performed using a lab-developed, exome-capture RNA-sequence protocol.[12]

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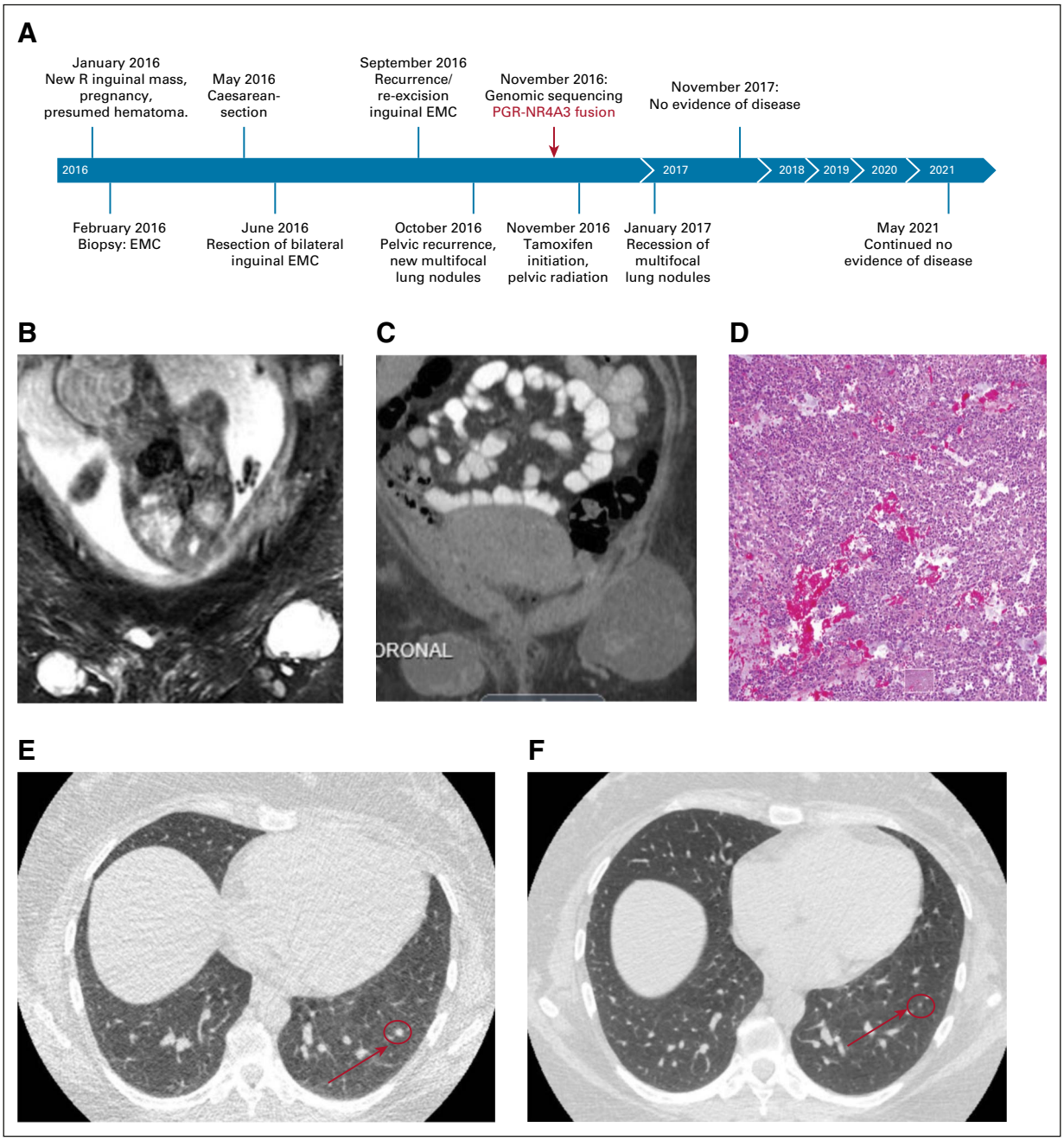

FIG 1. Clinical timeline and radiologic/pathologic correlation. (A) Summary of key clinical events. (B) MRI revealing rapid progression of inguinal masses during pregnancy. (C) Continued growth of extraskeletal myxoid chondrosarcoma after pregnancy. (D) Resection specimen revealing cellular variant of extraskeletal myxoid chondrosarcoma. (E) CT of the chest before initiation of tamoxifen highlighting new solid pulmonary nodules. (F) CT of the chest 4 months after initiation of tamoxifen showing decrease in size of example pulmonary nodule without evidence of new pulmonary nodules. CT, computed tomography; MRI, magnetic resonance imaging.

One month later, restaging computed tomography imaging revealed several new solid bilateral pulmonary nodules measuring up to 7 mm and a 1.8 cm nodule in the left mons pubis consistent with metastatic and locally recurrent disease. Given the rapid progression and presence of metastases, no further surgical intervention was recommended. Full timeline of patient course is depicted in Figure 1A.

The results of next-generation sequencing revealed gene fusion of progesterone receptor, *PGR* (exon2) to the 5′ untranslated region (UTR) of *NR4A3* (exon2) (Fig 2A). Outlier expression of *ESR1, PGR,* and *GREB1* was also noted (Fig 2B, Table 1). Given the gene fusion involving *PGR,* driven by estrogen, and outlier expression of *ESR1*, *PGR*, and *GREB1* further indicative of an activated estrogen-

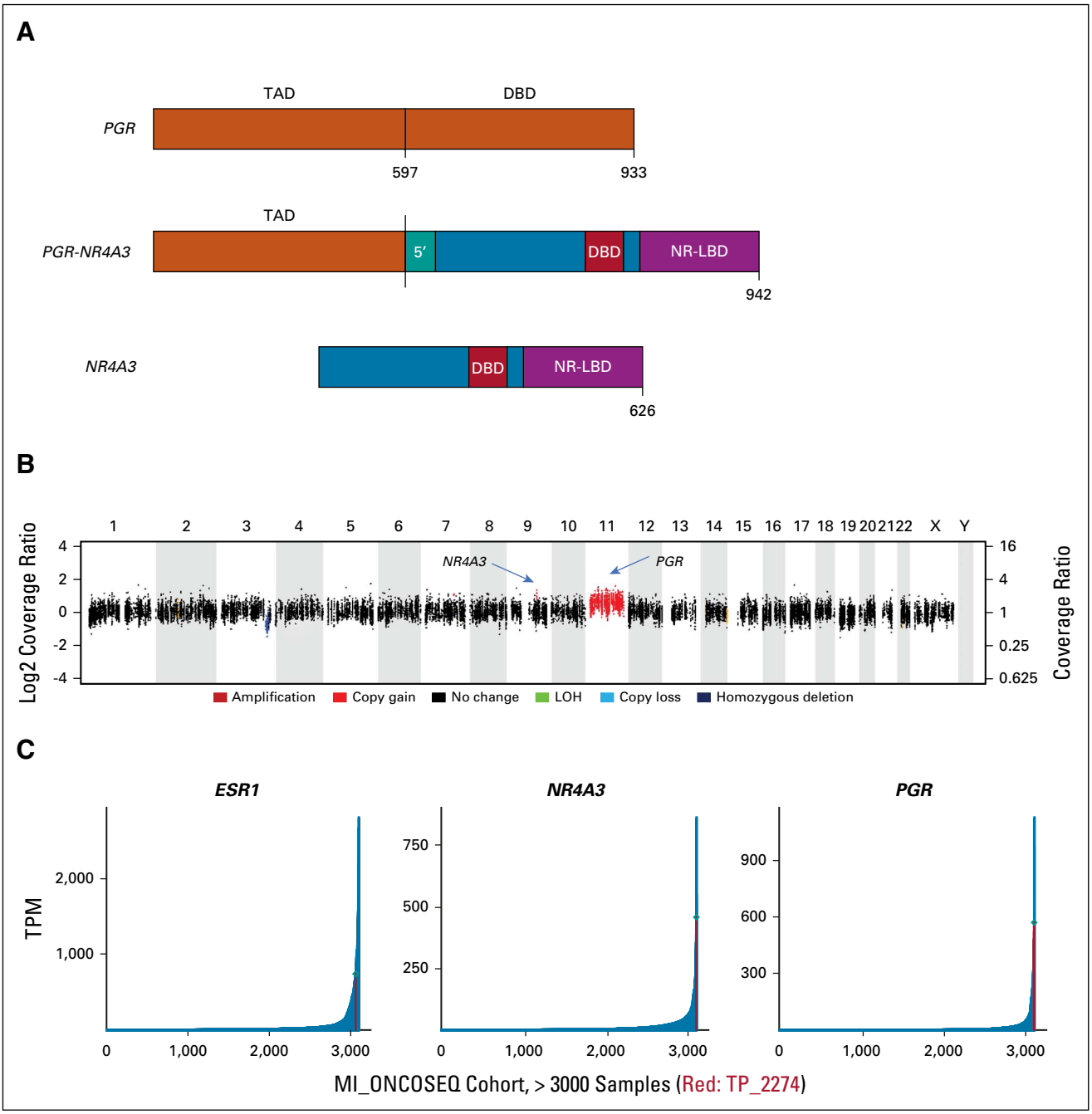

FIG 2. Genomic analysis. (A) PGR-NR4A3 gene fusion product. The TAD of *PGR* is fused to 5′UTR *NR4A3*. (B) Copy number profile on the basis of 1,500 exome capture sequencing, showing locations of *NR4A3* and *PGR*. (C) Gene expression of *ESR1, PGR,* and *NR4A3*. Shown is the percentile ranking of TPM of the respective genes with index patient (red) in relation to the expression levels of these genes among > 3,000 pan-cancer cases in the MI-ONCOSEQ cohort. ESR, estrogen receptor; LOH, loss of heterozygosity; MI-ONCOSEQ, Michigan Oncology Sequencing program; *NR4A3,* nuclear receptor subfamily 4 group A member 3; *PGR,* progesterone receptor; TAD, transcriptional activation domain; TPM, transcripts per million mapped reads.

signaling pathway, a multidisciplinary precision medicine tumor board recommended anti-estrogen therapy.[13–16] She began targeted therapy with tamoxifen, a selective estrogen receptor modulator. Since initiation of tamoxifen was over 5 years ago, she has had ongoing decrease in size of her pulmonary nodules and no evidence of disease progression despite intraoperative rupture and previously rapid, aggressive recurrences (Fig 1E).

Discussion and Conclusions

EMC is a rare soft-tissue sarcoma, representing approximately 3% of all soft-tissue sarcomas.[2] Although EMC

TABLE 1. Summary of OncoSeq Findings

Mutation Class	Gene/Aberration
Somatic point mutations (1)	No informative, actionable, recurrent mutations
Copy number aberrations	*NR4A3*, focal gain (four copies) Copy gain (three copies): chr11 (*PGR*, *AIP*)
Gene fusions	*PGR* (exon2)-*NR4A3* (exon2, 5′UTR)
Outlier gene expression	*NR4A3, PGR, ESR1*
Germline variants for disclosure	No cancer-associated pathogenic variants

typically does have an indolent growth rate, propensity for local recurrence and distant metastasis is high. Our patient's disease behaved aggressively with rapid local recurrence and metastases within only 4 months of primary resection, which is more typical in the cellular variant.[2]

No treatment is US Food and Drug Administration–approved specifically for metastatic EMC and typical cytotoxic agents for metastatic soft-tissue sarcoma, including anthracyclines, dacarbazine, gemcitabine, and docetaxel, yield little to no benefit.[5,17–19] In a small case series, partial response to sunitinib, a multitargeted receptor tyrosine kinase inhibitor (TKI), was confirmed in EMC patients with the hallmark *EWSR1-NR4A3* fusion only.[20] Subsequently, a phase II trial of 23 patients with EMC treated with another antiangiogenic TKI, pazopanib, resulted in a median progression-free survival of 19 months (95% CI, 11 to 27). Detectable tumor shrinkage occurred only in patients with an *EWSR1-NR4A3* translocation, further suggesting potential therapeutic relevance of translocation type.[13] Although these TKIs benefited a subset of patients with EMC, they did not have activity in non-*EWSR1–NR4A3* translocations.

Beyond the known translocations, there has been limited additional clinical information garnered from sequencing these tumors. In a previously reported case series, six patients with metastatic EMC were genomically profiled, all expressing the pathognomonic *EWSR1-NR4A3* translocation with disappointingly minimal other actionable changes.[21] There is no known direct inhibitor to the *EWSR1-NR4A3* fusion product; however, peroxisome proliferator activated receptor gamma is significantly overexpressed in fusion-positive tumors. Targeting peroxisome proliferator activated receptor gamma as a downstream effector has been suggested, but clinically, this has not proceeded further in development.[16,22,23]

In our patient, evaluation of her genomic profile yielded a profound result, an actionable translocation, which significantly altered the course of her rapidly progressive disease. To our knowledge, this is the first report of EMC involving the transcription factor *NR4A3* driven by a novel 5′ partner, *(PGR)*. *PGR* expression has been well-described within breast cancer literature, and it is established that estrogen drives *PGR* expression.[24,25] In a randomized control trial, tamoxifen, a selective estrogen receptor modulator, improved recurrence-free and overall survival in patients whose tumors had over 75% *PGR*-positive nuclei.[14] Tamoxifen is a first-line treatment for premenopausal women with hormone receptor–positive breast cancer.[26] Thus, given the gene fusion involving the pathognomonic transcription factor, *NR4A3*, and the novel 5′ partner of *PGR*, whose expression is regulated by estrogen[14–16,27]; anti-estrogen therapies were suggested for our premenopausal patient. Outlier expression of *ESR1, PGR*, and *GREB1* also supported the presence of activated estrogen-signaling pathway in the tumor[14,25] (Fig 2). Previously, Chiang et al reported four cases of uterine epithelioid leiomyosarcoma also with a *PGR-NR4A3* fusion. However, the clinical implications of anti-estrogen treatment were not mentioned.[28] In our patient, there was clear benefit to tamoxifen treatment even in the postpartum state when there would be an expected natural decline in estrogen and progesterone. Until initiation of tamoxifen, her tumor rapidly progressed.

In addition to providing critical information to influence clinical care, this case highlights the potential of genomic sequencing in tumors that are already typified by translocations. More than one third of sarcomas possess characteristic molecular alterations, and the number of newly identified translocations is growing.[29] The laboratory-developed RNA sequencing protocol used here captures all expressed genes in a given sample, which allows for unbiased detection of both known and novel gene fusions. To our knowledge, this fusion would have not been captured by existing commercial vendors which use panel-based approaches that do not include *PGR* or *N4A3*.[12]

Although some simple alterations are associated with therapeutic opportunities (eg, KIT tyrosine kinase mutation in gastrointestinal stromal tumor), most translocations are yet not targetable, with the exception of the collagen type I alpha1 *(COLIA1)* gene to the platelet-derived growth factor *(PDGF)* B-chain *(PDGFB)* gene, associated with dermatofibrosarcoma protuberans.[30] In both diseases, TKIs have had a profound clinical impact on outcomes of patients with both locally aggressive and metastatic disease by targeting the KIT and PDGFR tyrosine kinase receptors, respectively.[30,31]

The majority of translocations in sarcoma encode for transcription factors, which have proven challenging to target.[29] Despite years of intensive research, a successful therapy targeting translocation fusion products, such as the pathogenic *EWS-FLI1* in Ewing's sarcoma, remains elusive, although ongoing trials continue.[32,33] The utility of genomically sequencing sarcomas associated with translocations that are not currently targetable varies. In this patient, a novel translocation was identified and essentially proved to be highly clinically significant. As such, next-generation sequencing has the potential to add value to the individualized care of patients even when their tumor histology typically harbors a nontargetable translocation.[34] Sequencing should be considered in every patient and even more so in patients with an unusual clinical course for the histology.

AFFILIATIONS

[1]Department of Medicine, Division of Hematology and Oncology, and Rogel Cancer Center, University of Michigan School of Medicine, Ann Arbor, MI
[2]Department of Oncology, Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD
[3]Michigan Center for Translational Pathology, University of Michigan School of Medicine, Ann Arbor, MI
[4]Department of Pathology and Rogel Cancer Center, University of Michigan School of Medicine, Ann Arbor, MI

CORRESPONDING AUTHOR

Rashmi Chugh, MD, University of Michigan, C407 MIB SPC 5848, 1500 E. Medical Center Dr, Ann Arbor, MI 48109-5848; e-mail: rashmim@med.umich.edu.

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AUTHOR CONTRIBUTIONS

Conception and design: All authors
Financial support: Rashmi Chugh
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Collection and assembly of data: H. Catherine Wilbur, Dan R. Robinson, Yi-Mi Wu, Chandan Kumar-Sinha, Rashmi Chugh
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors
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Chandan Kumar-Sinha

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Arul M. Chinnaiyan

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