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

H. Catherine Wilbur, MD1,2; Dan R. Robinson, PhD3,4; Yi-Mi Wu, PhD3,4; Chandan Kumar-Sinha, PhD3,4; Arul M. Chinnaiyan, MD, PhD3,4; and Rashmi Chugh, MD1

JCO Precis Oncol 6:e2200039. © 2022 by American Society of Clinical Oncology

Background

Extraskeletal myxoid chondrosarcoma (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. EMC most often presents as an enlarging soft-tissue mass in the proximal extremities. The tumor has local recurrence and metastasis rates between 29%-48% and 40%-50%, respectively. Despite this, the survival rate at 10 years is 65%-70%, 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). Rearrangements of NR4A3 with TAF15, TCF12, TFG, and HSPA8 have also been described and are associated with poorer outcomes. 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.

Treatment of localized EMC consists of wide-local excision with or without radiation. 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 1Band 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.
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-
signaling pathway, a multidisciplinary precision medicine tumor board recommended anti-estrogen therapy.\textsuperscript{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.\textsuperscript{2} Although EMC
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.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 estrogen14-16,27; 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 tumor14,25 (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 not have been captured by existing commercial vendors which use panel-based approaches that do not include PGR or NR4A3.12

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 (COL1A1) gene to the platelet-derived growth factor (PDGF) B-chain (PDGFRB) gene, associated with dermatofibrosarcoma protubersans.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 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.

| 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 |

Case Report

TABLE 1. Summary of OncoSeq Findings
**REFERENCES**

1. Aigner T, Oliveira AM, Nascimento AG. Extraskeletal myxoid chondrosarcomas do not show a chondrocytic phenotype. Mod Pathol 17:214-221, 2004
2. Lucas DR, Stenman G. Extraskeletal myxoid chondrosarcoma, in Fletcher CDM, Bridge JA, Hogendoorn PCW, et al (eds): World Health Organization (WHO) Classification of Tumors of Soft Tissue and Bone: Pathology And Genetics, Volume 5 (ed 2). Lyon, France, IARC Press, 2013. pp P223-P224
3. Chiousole B, Le Cesne A, Rastrelli M, et al: Extraskeletal myxoid chondrosarcoma: Clinical and molecular characteristics and outcomes of patients treated at two institutions. Front Oncol 10:828, 2020
4. Meis-Kindblom JM, Bergh P, Gunterberg B, Kindblom LG: Extraskeletal myxoid chondrosarcoma: A reappraisal of its morphologic spectrum and prognostic factors based on 117 cases. Am J Surg Pathol 23:636-650, 1999
5. Drilon AD, Popat S, Bhuchar G, et al: Extraskeletal myxoid chondrosarcoma: A retrospective review from 2 referral centers emphasizing long-term outcomes with surgery and chemotherapy. Cancer 113:3364-3371, 2008
6. Stenman G, Andersson H, Mandahl N, et al: Translocation t(9;22)(q22;q12) is a primary cytogenetic abnormality in extraskeletal myxoid chondrosarcoma. Int J Cancer 62:398-402, 1995
7. Hirabayashi Y, Ishida T, Yoshida MA, et al: Translocation (9;22)(q22;q12A) recurrent chromosome abnormality in extraskeletal myxoid chondrosarcoma. Cancer Genet Cytogenet 81:33-37, 1995
8. Benini S, Cocchi S, Gamberi G, et al: Diagnostic utility of molecular investigation in extraskeletal myxoid chondrosarcoma. J Mol Diagn 16:314-323, 2014
9. Agaram NP, Zhang L, Sung YS, et al: Extraskeletal myxoid chondrosarcoma with non-EWSR1-NR4A3 variant fusions correlate with rhabdoid phenotype and high-grade morphology. Hum Pathol 45:1084-1091, 2014
10. Enzinger FM, Shiraki M: Extraskeletal myxoid chondrosarcoma. An analysis of 34 cases. Hum Pathol 3:421-436, 1972
11. Shao R, Lai IW, Wang L, et al: Clinicopathologic and radiologic features of extraskeletal myxoid chondrosarcoma: A retrospective study of 40 Chinese cases with literature review. Ann Diagn Pathol 23:14-20, 2016
12. Cieslak M, Chugh R, Wu YM, et al: The use of exome capture RNA-seq for highly degraded RNA with application to clinical cancer sequencing. Genome Res 25:1372-1381, 2015
13. Stacchiotti S, Ferrari S, Redondo A, et al: Pazopanib for treatment of advanced extraskeletal myxoid chondrosarcoma: A multicentre, single-arm, phase 2 trial. Lancet Oncol 20:1252-1262, 2019
14. Stendahl M, Ryden L, Nordenskjold B, et al: High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients. Clin Cancer Res 12:4614-4618, 2006
15. Beard JA, Tenga A, Chen T: The interplay of NR4A receptors and the oncogene-tumor suppressor networks in cancer. Cell Signal 27:257-266, 2015
16. Filion C, Motol T, Olseni AB, et al: The EWSR1/NR4A3 fusion protein of extraskeletal myxoid chondrosarcoma activates the PPARG nuclear receptor gene. J Pathol 217:83-93, 2009
17. Patel SR, Burgess MA, Papadopoulos NE, et al: Extraskeletal myxoid chondrosarcoma. Long-term experience with chemotherapy. Am J Clin Oncol 18:161-163, 1995
18. McGrory JE, Rock MG, Nascimento AG, Oliveira AM: Extraskeletal myxoid chondrosarcoma. Clin Orthop Relat Res:185-190, 2001
19. Stacchiotti S, Dagrada GP, Sanfilippo R, et al: Anthracycline-based chemotherapy in extraskeletal myxoid chondrosarcoma: A retrospective study. Clin Sarcoma Res 3:16, 2013
20. Stacchiotti S, Pantaleo MA, Astolfi A, et al: Activity of sunitinib in extraskeletal myxoid chondrosarcoma. Eur J Cancer 50:1657-1664, 2014
21. Davis EJ, Wu YM, Robinson D, et al: Next generation sequencing of extraskeletal myxoid chondrosarcoma. Oncotarget 8:21770-21777, 2017
22. Burton JD, Goldenberg DM, Blumenthal RD: Potential of peroxisome proliferator-activated receptor gamma agonist compounds as therapeutic agents for a wide range of cancer types. PPAR Res 2008:494161, 2008
23. Pishvaian MJ, Cotaria I, Wagner AJ, et al: Final reporting of a phase I clinical trial of the oral PPAR-gamma agonist, CS-7017, in patients with advanced malignancies. J Clin Oncol 28:2526-2526, 2010 (suppl 15; abstr 2526)
24. Horwitz KB, Koseki Y, McGuire WL: Estrogen control of progesterone receptor in human breast cancer: Role of estradiol and antiestrogen. Endocrinology 103:1742-1751, 1978
25. Carroll JS, Meyer CA, Song J, et al: Genome-wide analysis of estrogen receptor binding sites. Nat Genet 38:1289-1297, 2006
26. Shagufa Ahmad: Tamoxifen a pioneering drug: An update on the therapeutic potential of tamoxifen derivatives. Eur J Med Chem 143:515-531, 2018
27. Labelle Y, Bussieres J, Courjal F, Goldring MB: The EWS/TEC fusion protein encoded by the t(9;22) chromosomal translocation in human chondrosarcomas is a highly potent transcriptional activator. Oncogene 18:3303-3308, 1999
28. Chiang S, Samore W, Zhang L, et al: PGR gene fusions identify a molecular subset of uterine epithelioid leiomyosarcoma with rhabdoid features. Am J Surg Pathol 43:810-818, 2019
29. Helman LJ, Meltzer P: Mechanisms of sarcoma development. Nat Rev Cancer 3:685-694, 2003
30. Shimizu A, O’Brien KP, Sjöblom T, et al: The dermatofibrosarcoma protubers-associated collagen type I alpha1/platelet-derived growth factor (PDGF) B-chain fusion gene generates a transforming protein that is processed to functional PDGF-BB. Cancer Res 59:3719-3723, 1999
31. von Mehren M, Joensuu H: Gastrointestinal stromal tumors. J Clin Oncol 36:136-143, 2018
32. Cidre-Aranaz F, Alonso J: EWS/FLI1 target genes and therapeutic opportunities in Ewing sarcoma. Front Oncol 5:162, 2015
33. Brown RE, Bunyanek J, Katz AM, et al: Alveolar rhabdomyosarcoma. Morphoproteomics and personalized tumor graft testing further define the biology of PAX3-FKHR(FOXO1) subtype and provide targeted therapeutic options. Oncotarget 7:46263-46272, 2016
34. Pestana RC, Groisberg R, Roszik J, Subbiah V: Precision oncology in sarcomas: Divide and conquer. JCO Precis Oncol 1:16, 2019