Motaparthi Kiran (Orcid ID: 0000-0003-0562-0826)
PATEL Rajiv (Orcid ID: 0000-0002-1521-4947)
Vidal Claudia (Orcid ID: 0000-0003-2672-4974)
Linos Konstantinos (Orcid ID: 0000-0001-9462-652X)

MYC gene amplification by fluorescence in situ hybridization and MYC protein expression by immunohistochemistry in the diagnosis of cutaneous angiosarcoma: systematic review and appropriate use criteria

Kiran Motaparthi MD (1), Scott R. Lauer MD (2), Rajiv M. Patel MD (3), Claudia I. Vidal MD PhD, (4) Konstantinos Linos MD (5)

1) Department of Dermatology, University of Florida College of Medicine, Gainesville, FL
2) Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE
3) Michigan Medicine Departments of Pathology and Dermatology, University of Michigan, Ann Arbor, MI
4) Dermatology Center of Southern Indiana, Bloomington, IN
5) Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH and Geisel School of Medicine, Hanover, NH

Corresponding author:
Konstantinos Linos, MD
Assistant Professor of Pathology and Laboratory Medicine
Department of Pathology
Dartmouth-Hitchcock Medical Center
One Medical Center Drive
Lebanon NH 03766Office: Borwell, Level 4
Phone: 6036507211
Email: Konstantinos.Linos@hitchcock.org

Conflicts of Interest:
The authors have no conflicts of interest to disclose.

Funding:
The authors have no funding to report.

Word count:
Abstract: 200
Main text (excluding abstract and acknowledgments): 2688
References: 36
Tables: 2
Figures: 4

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cup.13912

Key Words: angiosarcoma, MYC, fluorescence in situ hybridization, immunohistochemistry, appropriate use criteria, atypical vascular lesions

Acknowledgments:
The authors would like to thank the American Society of Dermatopathology and the other members of the Appropriate Use Committee for their ongoing support and commitment to the advancement in dermatopathology.

Abstract

Background: Secondary AS most commonly follows breast cancer and includes postirradiation AS (PRAS) and lymphedema-associated AS. The frequent amplification of *MYC* (8q24.21) in secondary AS and the rising incidence of PRAS and atypical vascular lesions (AVLs) have prompted interest in the diagnostic and prognostic utility of *MYC* in AS.

Methods: Retrospective series with ≥ 2 cases of cutaneous AS and describing the use of FISH for *MYC* amplification or IHC for MYC overexpression were included.

Results: Sixteen studies met inclusion criteria. Overall, 93 percent of cases evaluated by FISH and IHC were concordant. The sensitivity of FISH in primary AS was only 6.8 percent, and protein overexpression occurred without amplification in sun-damaged skin. FISH and IHC were over 78 percent sensitive in secondary AS, but negative in over 98 percent of AVLs. *MYC* amplification and *FLT4* coamplification were associated with shorter overall survival in secondary AS.

Conclusion: FISH for *MYC* amplification and IHC for MYC overexpression are useful in distinguishing PRAS from AVLs and may also have prognostic value in secondary AS. In

contrast, these methods have little diagnostic or prognostic value in primary AS and should not be used to distinguish primary AS from benign vascular neoplasms.

Introduction

Primary and secondary cutaneous angiosarcoma

Angiosarcoma (AS) accounts for 1-2 percent of all soft tissue sarcomas, and 60 percent of cases are cutaneous.[1] AS is a highly aggressive tumor, with 5-year survival ranging from 30-50 percent. Predictors of poor prognosis include age > 70 years, tumor size > 5 cm, location on the scalp or face, resection with positive margins, and advanced stage.[2] Primary or idiopathic cutaneous AS most frequently occurs on the sun-damaged skin of the scalp or face and affects elderly men more often than women. In contrast, secondary cutaneous AS includes postirradiation AS (PRAS) and lymphedema-associated AS.[3] While secondary AS may arise in any anatomic location, it most commonly occurs on the chest of patients with a history of radiation therapy for breast cancer.[4] PRAS of the breast (Figure 1) is the most common radiation-induced sarcoma in women,[5] with an incidence of 0.27 percent among women treated for breast cancer.[6] Radiotherapy is associated with a 16-fold increased relative risk of AS compared to patients without preceding radiotherapy.[5] Due to the paradigm shift favoring breast-conserving surgery and radiotherapy for stage I and II breast cancer,[7] the incidence of PRAS is increasing.[5] Stewart-Treves syndrome describes AS of the extremity due to chronic lymphedema, most commonly following radical mastectomy for breast cancer.[8] Rarely, lymphedema-associated AS

arises in the context of congenital lymphedema, post-filarial infection, lymph node dissection, and morbid obesity.[9]

Atypical vascular lesions

Atypical vascular lesions (AVLs) demonstrate a spectrum of morphologic features, but the 2 most frequently observed subtypes are the lymphatic subtype and the vascular subtype. Lymphatic AVLs present with dilated, ectatic vessels simulating lymphangioma (Figure 2). Vascular AVLs demonstrate compressed vascular spaces with hobnail endothelial cells.[10] AVLs can occasionally demonstrate histopathologic features of low-grade secondary AS, including cytologic atypia, prominent nucleoli, mitotic activity, poor circumscription, or extension to the subcutis.[4,11] AVL and low-grade PRAS both occur in middle-aged to elderly women following a variable latency.[11] Thus, the differential diagnosis between AVL and low-grade secondary AS can be challenging due to overlapping clinicopathologic features.[4,12] In limited or peripheral sampling, low-grade secondary AS can be indistinguishable from AVL.[12] Consequently, up to 50 percent of resection specimens for AVL contained secondary AS.[13] Importantly, upgrading to AS in resection specimens should be distinguished from true progression. Based on 3 retrospective series reflecting 71 patients with an average follow-up of 36 months (range 1-181), the rate of progression from AVL to PRAS is 7 percent (5/71 patients).[10,14,15]

The pathogenic role of MYC

Myc is a family of protooncogenes that includes c-myc (*MYC* or *MYCC*), l-myc (*MYCL*), and n-myc (*MYCN*). *MYC* (8q24.21) encodes a basic helix-loop-helix and leucine zipper transcription factor that promotes cellular proliferation and differentiation, apoptosis, invasion, and metastasis.[16] *MYC* also has effects on cell cycle dysregulation.[17] MYC protein expression includes both MYC-I and MYC-II isoforms.[18] MYC-II specifically promotes proliferation and transformation of cells in *in vitro* models.[19] MYC-II, but not MYC-I, heterodimerizes with MAX.[18] The MYC/MAX complex then binds DNA to activate transcription.[20] Normally, *MYC* promotes angiogenesis by activating vascular endothelial growth factor (VEGF).[21] MYC overexpression can occur via amplification, translocation, transcriptional activation, or polysomy.[17] Overexpression and subsequent VEGF activation occur in multiple malignancies.[21]

Angiosarcoma accounts for 40 percent of all radiation-induced sarcomas, and *MYC* amplification is unique in PRAS compared to other radiation-induced sarcomas.[18] Following ionizing radiation, MYC protein expression promotes proliferation via inappropriate entry to S-phase from G1 phase.[22] In 2010, array-comparative genomic hybridization (aCGH) identified high-level amplification of 8q24.21 in more than half of secondary AS,[23] leading to subsequent studies of AS which utilized fluorescence in situ hybridization (FISH) for *MYC* amplification and immunohistochemistry (IHC) as a surrogate of MYC overexpression.

Methods

PubMed was searched for relevant studies in the English language between 1967-2018. Retrospective series with ≥ 2 cases of cutaneous AS and describing the use of IHC and/or FISH for *MYC* were included. Reviews, case reports, series with < 2 cases of cutaneous AS, non-English publications, publications indexed after 2018, and studies without IHC or FISH for *MYC* were excluded. Ultimately, 16 studies met inclusion criteria; these studies are summarized in Table 1.

Fluorescence in situ hybridization and immunohistochemistry for *MYC*

When specified, the number of nonoverlapping intact interphase nuclei examined was variable among included studies. Five studies[24,25,26,27,28] examined 50 or fewer nuclei, 2 studies[29,30] examined 100 nuclei, and 4 studies[18,23,31,32] examined at least 200 nuclei per case. The majority of studies defined *MYC* amplification as a ratio of *MYC*/CEP8 ≥ 2.[4,11,12,23,25,26,27,30,33] Six studies also defined amplification based on clustering of signals, usually > 8 or 10.[23,24,26,28,29,31] Low-level *MYC* amplification was based on a *MYC*/CEP8 ratio ≥ 2,[27] while high-level amplification was variably described as > 9 copies per nucleus,[24] > 21 copies,[27] or ≥ 9-10 signals.[23]

One caveat of FISH interpretation is polysomy, a proportional gain of signals such that the ratio of *MYC*/CEP8 is still < 2. Polysomy is characterized by 3-8 copies of *MYC* and CEP8.[27] Tissue microarrays (TMA) may be less informative than whole sections in the performance of FISH for *MYC* amplification.[29] Lastly, if biopsies with features suspicious for low-grade secondary AS are

noninformative or negative by FISH, a repeat study in an excision specimen may be considered to exclude false-negative results.[4]

Well-defined criteria for interpretation of negative versus positive expression by IHC are lacking.[34] Three studies considered any nuclear reactivity as positive,[4,26,33] 3 studies set a threshold at > 5 percent of cells,[25,27,29] and 1 study set a threshold at > 10 percent of cells.[12] Udager et al. quantified expression as the product of percent of cells with nuclear reactivity (0-100) and strength of expression (0-3).[34] The most commonly used dilution for anti-MYC was 1:50,[4,12,25,29,33] but some authors[26,27,28] used a dilution of 1:100 while Udager et al. used a dilution of 1:25.[34]

MYC expression by IHC can occur in lymphangiomas, benign or reactive endothelia, lymphocytes, and granulation tissue.[12,34] Nonspecific focal or heterogeneous staining in partial biopsies can potentially lead to overdiagnosis of AS.[12] False-negative results are also possible, as benign vessels can be mistaken for non-reactive neoplastic vessels in stained sections.[12]

Almost 93 percent (234/252) of cases evaluated both by FISH for *MYC* amplification and by IHC for MYC overexpression were concordant.[12,18,25,26,27,28,29] Of note, the majority of studies evaluated primary and secondary AS, including but not limited to cutaneous cases, as well as AVLs. Ginter et al. demonstrated 100 percent concordance between *MYC* amplification and

protein expression in AVLs, primary AS of breast, and secondary AS of breast. However, there was only 65 percent concordance between FISH and IHC in primary AS of non-breast sites including skin.[12] In primary cutaneous AS from sun-damaged skin, amplification was observed in both IHC-positive and IHC-negative cases. Most IHC-positive cases failed to demonstrate *MYC* amplification: MYC overexpression reflected mechanisms of activation other than amplification, such as polysomy.[27] Therefore, IHC attempted in isolation for primary cutaneous AS should be interpreted with caution. Epigenetic alterations - including transcriptional, translational, and posttranslational modification - can result in MYC protein expression without *MYC* amplification, resulting in discordance between FISH and IHC results.[12]

MYC in primary angiosarcoma

In the included studies, the overall sensitivity of FISH for *MYC* amplification in primary AS was 6.8 percent.[12,18,23,25,27,28,29,30,31,32] The overall sensitivity of IHC for MYC overexpression was 19 percent.[12,25,27,28,29] In a series of 38 cases of primary AS, including 16 primary cutaneous AS, mean quantified expression of MYC was significantly lower when compared to a series of secondary AS largely composed of PRAS.[34]

Primary AS of the breast is very rare and arises from the breast parenchyma before invading the skin.[35] *MYC* amplification is uncommon and was observed in only 1 case of primary AS of the breast in the studies reviewed here.[12,25,30] Ginter et al. highlighted the influence of anatomic site

on *MYC* amplification and expression in primary AS. In 17 primary AS of the breast, *MYC* amplification and overexpression were absent. In contrast, in 20 non-breast sites including skin, *MYC* amplification and overexpression were present in 20 percent and 45 percent of primary AS cases, respectively.[12]

In a series of 38 primary AS mainly composed of tumors from sun-damaged skin, largely from the head and neck of elderly patients, only 17 percent of cases tested harbored high- or low-level *MYC* amplification. Nearly 24 percent of tumors expressed MYC by IHC, and the majority of IHC-positive cases demonstrated strong reactivity. While MYC IHC demonstrates 66 percent sensitivity and 70 percent specificity for *MYC* amplification in primary AS from sun-damaged skin, MYC IHC also detects chromosome 8 polysomy, and there is a low frequency of amplification or overexpression in this context. Therefore, FISH for *MYC* amplification and IHC for MYC overexpression are unlikely to have value in discriminating primary cutaneous AS of sun-damaged skin from benign vascular neoplasms.[27]

MYC in postirradiation angiosarcoma and atypical vascular lesions

In the included studies, the overall sensitivity of FISH for *MYC* amplification in PRAS (Figure 3) was 78.8 percent,[12,18,23,26,28,29,30,31,32,33] while the overall sensitivity of IHC for MYC overexpression was 78.3 percent.[12,26,28,29,33] Overall, FISH for *MYC* amplification and IHC for MYC overexpression were negative in 100 percent [4,11,12,18,25,26,28] and 98.6 percent [4,12,25,26,28] of

AVLs, respectively. Quantification of MYC nuclear reactivity in AVLs demonstrated significantly lower mean expression compared to secondary AS including PRAS.[34]

Huang et al. compared *MYC* amplification in PRAS based on antecedent history of breast cancer. While 90 percent of PRAS from patients with a history of breast cancer demonstrated *MYC* amplification, only 25 percent of PRAS from patients without antecedent breast cancer were *MYC*-amplified.[31] Despite indistinguishable morphologies, MYC amplification is distinctly uncommon in primary AS of the breast but present in up to 100 percent of PRAS of breast in some series. This finding suggests a distinct pathogenesis underlying PRAS of the breast.[30]

While morphology is sufficient to distinguish AVL from high-grade AS, IHC can support the differentiation of AVL from low-grade secondary AS including PRAS (Figure 4). MYC IHC has high sensitivity and specificity for PRAS compared to AVLs, suggesting utility in subtle examples of PRAS.[34] For example, PRAS may simulate chronic radiation dermatitis, with inapparent or subtle vasoformation at low power, scattered neoplastic cells resembling radiation fibroblasts that form wavy or linear arrangements, and hemorrhage associated with a fibrotic stroma. In a series of PRAS with radiation dermatitis-like features, 10 of 10 cases evaluated were positive for MYC IHC.[33] MYC IHC can also highlight subtle AVL-like areas at the periphery of PRAS suggesting a utility for mapping in resections.[28] Importantly, negative MYC IHC does not

exclude the diagnosis of PRAS when morphology is diagnostic, and focal weak MYC expression in less than 5 percent of lesional cells may be observed in AVLs and is not diagnostic of PRAS.[34]

MYC in lymphedema-associated angiosarcoma

The overall sensitivity of FISH for *MYC* amplification in lymphedema-associated angiosarcoma was 93.3 percent,[12,18,23,24,28,29,31,32] and the overall sensitivity of IHC for MYC overexpression was 75 percent.[12,24, 28,29] *MYC* amplification is relatively specific for secondary AS, but cannot be used to distinguish PRAS from lymphedema-associated AS.[29] Table 2 summarizes the sensitivities of FISH for *MYC* amplification and IHC for MYC overexpression in primary AS, PRAS, and lymphedema-associated AS.

Alternative methods to evaluate *MYC* in angiosarcoma

Dual-color dual-hapten *in situ* hybridization (DISH) enables rapid calculation of gene copy numbers with bright field microscopy. Additionally, DISH permits assessment of morphology and copy number simultaneously as well as indefinite archiving. DISH was equally sensitive compared to FISH for identifying *MYC* amplification in secondary AS of the breast and equally specific in differentiating from AVLs.[11]

Similar to IHC, western blot can also detect MYC protein expression, including both MYC-I and MYC-II isoforms and MAX protein. While MAX protein is detected in all AS and MYC-I is

variably detected in primary and secondary AS, MYC-II is only expressed in secondary AS. *MYC* amplification correlates tightly with expression of MYC-II and heterodimerization of MYC-II with MAX in secondary AS.[18]

Coexisting genetic abnormalities associated with *MYC* amplification and MYC expression

FLT4 (5q35.3) encodes VEGFR3, a tyrosine kinase receptor that regulates endothelial cell growth and angiogenesis.[23] Among 81 *MYC*-amplified secondary AS, 19.8 percent (16) demonstrated *FLT4* coamplification. The majority of these coamplified cases represented PRAS and lymphedema-associated AS of the breast.[18,26,31,32] In the included studies, all but 1 *FLT4*-amplified AS occurred in the context of secondary disease and MYC amplification.[31] Strong and diffuse FLT4 expression correlated with *FLT4* amplification.[26]

KDR (4q12), also known as *VEGFR2*, is a tyrosine kinase receptor that regulates angiogenesis. While KDR protein is overexpressed in almost all AS, activating mutations are uncommon. *PLCG1* (20q12) encodes a tyrosine kinase signal transducer that shares a common signaling pathway with KDR.[36] In a large series, 5 *PLCG1*-mutated secondary AS cases demonstrated coexistent *MYC* amplifications. *KDR* mutations in 2 PRAS following breast cancer also harbored *MYC* amplification.[31]

Based on expression profiles, miRNAS from the mir-17-92 cluster (13q31.3) were strongly upregulated without genomic changes in 8 cases of *MYC*-amplified secondary AS. This cluster contains miRNAs that downregulate expression of *THBS1*. *THBS1* (15q14) encodes thrombospondin-1, a potent endogenous inhibitor of angiogenesis, and *MYC*-amplified AS demonstrated significantly decreased *THBS1* mRNA expression.[32]

PROX1 is a homeobox gene with a central role in the differentiation of lymphatic vessels and endothelial cells. Compared to MYC IHC in PRAS, Prox-1 expression is less sensitive and specific, demonstrating frequent expression in AVLs.[28]

Prognostic implications of *MYC* amplification or MYC overexpression

Neither *MYC* amplification nor MYC overexpression correlated with survival in primary cutaneous AS from sun-damaged skin in cohort of 34 patients with a median follow-up of 2.7 years (range 2 months-19 years).[27] In a series of primary AS with median follow-up 27.3 months (range, 0.3-234), *MYC* amplification was associated with statistically insignificant trends towards poorer disease-free and overall survival.[31] In a cohort of 28 patients with primary AS with a mean follow-up of 34 months, quantified MYC expression by IHC was not significantly associated with death, but there was a non-statistically significant trend toward decreased disease-specific survival with high expression. Of note, this trend was attenuated following

adjustment for histologic grade but was made significant after adjustment for location (cutaneous versus non-cutaneous disease).[34]

In 11 patients with secondary AS and mean follow-up of 42.2 months, neither *MYC* amplification nor MYC overexpression was associated with survival.[29] In contrast, a series of 37 secondary AS from the breast with average follow-up of 32 months (range, 1-163) demonstrated significantly poorer overall survival in patients with *MYC*-amplified tumors. There was also a statistically insignificant trend toward poorer disease-specific survival with *MYC* amplification.[25] Similarly, in a study of secondary AS with median follow-up 27.3 months (range, 0.3-234), *MYC* amplification was associated with statistically insignificant trends towards poorer disease-free and overall survival. However, *FLT4* coamplification was significantly associated with shorter overall survival.[31] There was no significant association between shorter latency to development of secondary AS and *MYC* amplification.[25]

MYC amplification status did not correlate with tumor size or histologic grade – including high-grade histology, anaplasia, or degree of vascular differentiation – in primary or secondary AS.[23,25,32] Additionally, *MYC* amplification had no impact on proliferation index measured by Ki-67 IHC or on apoptosis measured by terminal deoxynucleotidyl transferase dUTP nick-end labeling.[23] High-grade histology was significantly associated with increased MYC expression in primary AS but not in secondary AS.[34]

Conclusion

Although standardized methods for FISH for *MYC* amplification and well-defined criteria for IHC for MYC overexpression are lacking, these methods are concordant in 93 percent of primary AS, secondary AS, and AVLs.[12,18,25,26,27,28,29] *MYC* amplification and MYC expression are infrequent in primary AS and are distinctly rare in primary AS of the breast. FISH and IHC should be interpreted with caution in primary AS of non-breast anatomic sites, particularly in sun-damaged skin, where these tests have little diagnostic value.[12,18,23,25,27,28,29,30,31,32] Additionally, these methods do not provide significant prognostication in primary AS.[27,31,34]

MYC amplification and MYC expression are identified in over 78 percent of PRAS but are absent in nearly all AVLs, providing diagnostic resolution in a potentially challenging and increasingly frequent clinical scenario.[4,11,12,18,23,25,26,28,29,30,31,32,33] The sensitivities of these methods in lymphedema-associated AS are also high but are based on smaller series.[12,18,23,24,28,29,31,32] In contrast to primary AS, *MYC* amplification and *FLT4* coamplification in secondary AS may predict significantly shorter overall survival.[25,31] *FLT4* coamplifications,[18,26,31,32] *KDR* and *PLCG1* mutations,[31,36] and mir-17-92 cluster upregulation followed by *THBS1* expression downregulation[32] are strongly associated with *MYC* amplification in secondary AS.

References

1. Rouhani P, Fletcher CD, Devesa SS, Toro JR. Cutaneous soft tissue sarcoma incidence patterns in the U.S. : an analysis of 12,114 cases. *Cancer.* 2008;113(3):616-627.
2. Sinnamon AJ, Neuwirth MG, McMillan MT, et al. A prognostic model for resectable soft tissue and cutaneous angiosarcoma. *J Surg Oncol.* 2016;114(5):557-563.
3. Billings SD, McKenney JK, Folpe AL, Hardacre MC, Weiss SW. Cutaneous angiosarcoma following breast-conserving surgery and radiation: an analysis of 27 cases. *Am J Surg Pathol.* 2004;28(6):781-788.
4. Fernandez AP, Sun Y, Tubbs RR, Goldblum JR, Billings SD. FISH for MYC amplification and anti-MYC immunohistochemistry: useful diagnostic tools in the assessment of secondary angiosarcoma and atypical vascular proliferations. *J Cutan Pathol.* 2012;39(2):234-242.
5. Huang J, Mackillop WJ. Increased risk of soft tissue sarcoma after radiotherapy in women with breast carcinoma. *Cancer.* 2001;92(1):172-180.
6. Kiss K, Andreasen S, Moller Talman ML, Daugaard S, Mentzel T. The incidence of radiotherapy-induced angiosarcoma of the skin after treatment for breast cancer in Denmark: a population-based study. *Mod Pathol*. 2017;132A:517.

7. Pass H, Vicini FA, Kestin LL, et al. Changes in management techniques and patterns of disease recurrence over time in patients with breast carcinoma treated with breast-conserving therapy at a single institution. *Cancer*. 2004;101(4):713-720.

8. Grobmyer SR, Daly JM, Glotzbach RE, Grobmyer AJ. Role of surgery in the management of postmastectomy extremity angiosarcoma (Stewart-Treves syndrome). *J Surg Oncol.* 2000;73(3):182-188.

9. Shon W, Ida CM, Boland-Froemming JM, Rose PS, Folpe A. Cutaneous angiosarcoma arising in massive localized lymphedema of the morbidly obese: a report of five cases and review of the literature. *J Cutan Pathol.* 2011;38(7):560-564.

10. Patton KT, Deyrup AT, Weiss SW. Atypical vascular lesions after surgery and radiation of the breast: a clinicopathologic study of 32 cases analyzing histologic heterogeneity and association with angiosarcoma. *Am J Surg Pathol.* 2008;32(6):943-950.

11. Ko JS, Billings SD, Lanigan CP, Buehler D, Fernandez AP, Tubbs RR. Fully automated dual-color dual-hapten silver in situ hybridization staining for MYC amplification: a diagnostic tool for discriminating secondary angiosarcoma. *J Cutan Pathol.* 2014;41(3):286-292.

12. Ginter PS, Mosquera JM, MacDonald TY, D'Alfonso TM, Rubin MA, Shin SJ. Diagnostic utility of MYC amplification and anti-MYC immunohistochemistry in atypical vascular lesions, primary or radiation-induced mammary angiosarcomas, and primary angiosarcomas of other sites. *Hum Pathol.* 2014;45(4):709-716.

13. Mattoch IW, Robbins JB, Kempson RL, Kohler S. Post-radiotherapy vascular proliferations in mammary skin: a clinicopathologic study of 11 cases. *J Am Acad Dermatol.* 2007;57(1):126-133.

14. Brenn T, Fletcher CD. Radiation-associated cutaneous atypical vascular lesions and angiosarcoma: clinicopathologic analysis of 42 cases. *Am J Surg Pathol.* 2005;29(8):983-996.

15. Fraga-Guedes C, Gobbi H, Mastropasqua MG, et al. Clinicopathological and immunohistochemical study of 30 cases of post-radiation atypical vascular lesion of the breast. *Breast Cancer Res Treat.* 2014;146(2):347-354.

16. Brooks TA, Hurley LH. The role of supercoiling in transcriptional control of MYC and its importance in molecular therapeutics. *Nat Rev Cancer.* 2009;9(12):849-861.

17. Dang CV. MYC on the path to cancer. *Cell.* 2012;149(1):22-35.

18. Guo T, Zhang L, Chang NE, Singer S, Maki RG, Antonescu CR. Consistent MYC and FLT4 gene amplification in radiation-induced angiosarcoma but not in other radiation-associated atypical vascular lesions. *Genes Chromosomes Cancer.* 2011;50(1):25-33.

19. Hann SR, Dixit M, Sears RC, Sealy L. The alternatively initiated c-Myc proteins differentially regulate transcription through a noncanonical DNA-binding site. *Genes Dev.* 1994;8(20):2441-2452.

20. Kretzner L, Blackwood EM, Eisenman RN. Myc and Max proteins possess distinct transcriptional activities. *Nature.* 1992;359(6394):426-429.

21. Baudino TA, McKay C, Pendeville-Samain H, et al. c-Myc is essential for vasculogenesis and angiogenesis during development and tumor progression. *Genes Dev.* 2002;16(19):2530-2543.

22. Sheen JH, Dickson RB. Overexpression of c-Myc alters G(1)/S arrest following ionizing radiation. *Mol Cell Biol.* 2002;22(6):1819-1833.

23. Manner J, Radlwimmer B, Hohenberger P, et al. MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema. *Am J Pathol.* 2010;176(1):34-39.

24. Harker D, Jennings M, McDonough P, et al. MYC amplification in angiosarcomas arising in the setting of chronic lymphedema of morbid obesity. *J Cutan Pathol.* 2017;44(1):15-19.

25. Fraga-Guedes C, André S, Mastropasqua MG, et al. Angiosarcoma and atypical vascular lesions of the breast: diagnostic and prognostic role of MYC gene amplification and protein expression. *Breast Cancer Res Treat.* 2015;151(1):131-140.

26. Cornejo KM, Deng A, Wu H, et al. The utility of MYC and FLT4 in the diagnosis and treatment of postradiation atypical vascular lesion and angiosarcoma of the breast. *Hum Pathol.* 2015;46(6):868-875.

27. Shon W, Sukov WR, Jenkins SM, Folpe AL. MYC amplification and overexpression in primary cutaneous angiosarcoma: a fluorescence in-situ hybridization and immunohistochemical study. *Mod Pathol.* 2014;27(4):509-515.

28. Mentzel T, Schildhaus HU, Palmedo G, Büttner R, Kutzner H. Postradiation cutaneous angiosarcoma after treatment of breast carcinoma is characterized by MYC amplification in contrast to atypical vascular lesions after radiotherapy and control cases: clinicopathological, immunohistochemical and molecular analysis of 66 cases. *Mod Pathol.* 2012;25(1):75-85.

29. Requena C, Rubio L, Lavernia J, et al. Immunohistochemical and Fluorescence In Situ Hybridization Analysis of MYC in a Series of 17 Cutaneous Angiosarcomas: A Single-Center Study. *Am J Dermatopathol.* 2018;40(5):349-354.

30. Laé M, Lebel A, Hamel-Viard F, et al. Can c-myc amplification reliably discriminate postradiation from primary angiosarcoma of the breast? *Cancer Radiother.* 2015;19(3):168-174.

31. Huang SC, Zhang L, Sung YS, et al. Recurrent CIC Gene Abnormalities in Angiosarcomas: A Molecular Study of 120 Cases With Concurrent Investigation of PLCG1, KDR, MYC, and FLT4 Gene Alterations. *Am J Surg Pathol.* 2016;40(5):645-655.

32. Italiano A, Thomas R, Breen M, et al. The miR-17-92 cluster and its target THBS1 are differentially expressed in angiosarcomas dependent on MYC amplification. *Genes Chromosomes Cancer.* 2012;51(6):569-578.

33. Daniels BH, Ko JS, Rowe JJ, Downs-Kelly E, Billings SD. Radiation-associated angiosarcoma in the setting of breast cancer mimicking radiation dermatitis: A diagnostic pitfall. *J Cutan Pathol.* 2017;44(5):456-461.

34. Udager AM, Ishikawa MK, Lucas DR, McHugh JB, Patel RM. MYC immunohistochemistry in angiosarcoma and atypical vascular lesions: practical considerations based on a single institutional experience. *Pathology.* 2016;48(7):697-704.

35. Adem C, Reynolds C, Ingle JN, Nascimento AG. Primary breast sarcoma: clinicopathologic series from the Mayo Clinic and review of the literature. *Br J Cancer.* 2004;91(2):237-241.

36. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. *Genes Cancer.* 2011;2(12):1097-1105.

Abbreviations

AS angiosarcoma

FISH	fluorescence in situ hybridization
IHC	immunohistochemistry
PRAS	postirradiation angiosarcoma
AVL	atypical vascular lesion
VEGF	vascular endothelial growth factor
DISH	dual-color dual-hapten in situ hybridization
TMA	tissue microarray
aCGH	array-comparative genomic hybridization

Terminology

Primary AS: Idiopathic or *de novo* AS

Secondary AS: postirradiation and lymphedema-associated AS

High-level amplification: > 21 copies of *MYC*

Low-level amplification: ratio of *MYC*/CEP8 greater than or equal to 2

Informative: sufficient materials for evaluation by IHC or FISH

Latency: time between radiation treatment and diagnosis of PRAS or AVL

Signal: ratio of gene copies of gene to copies of centromere

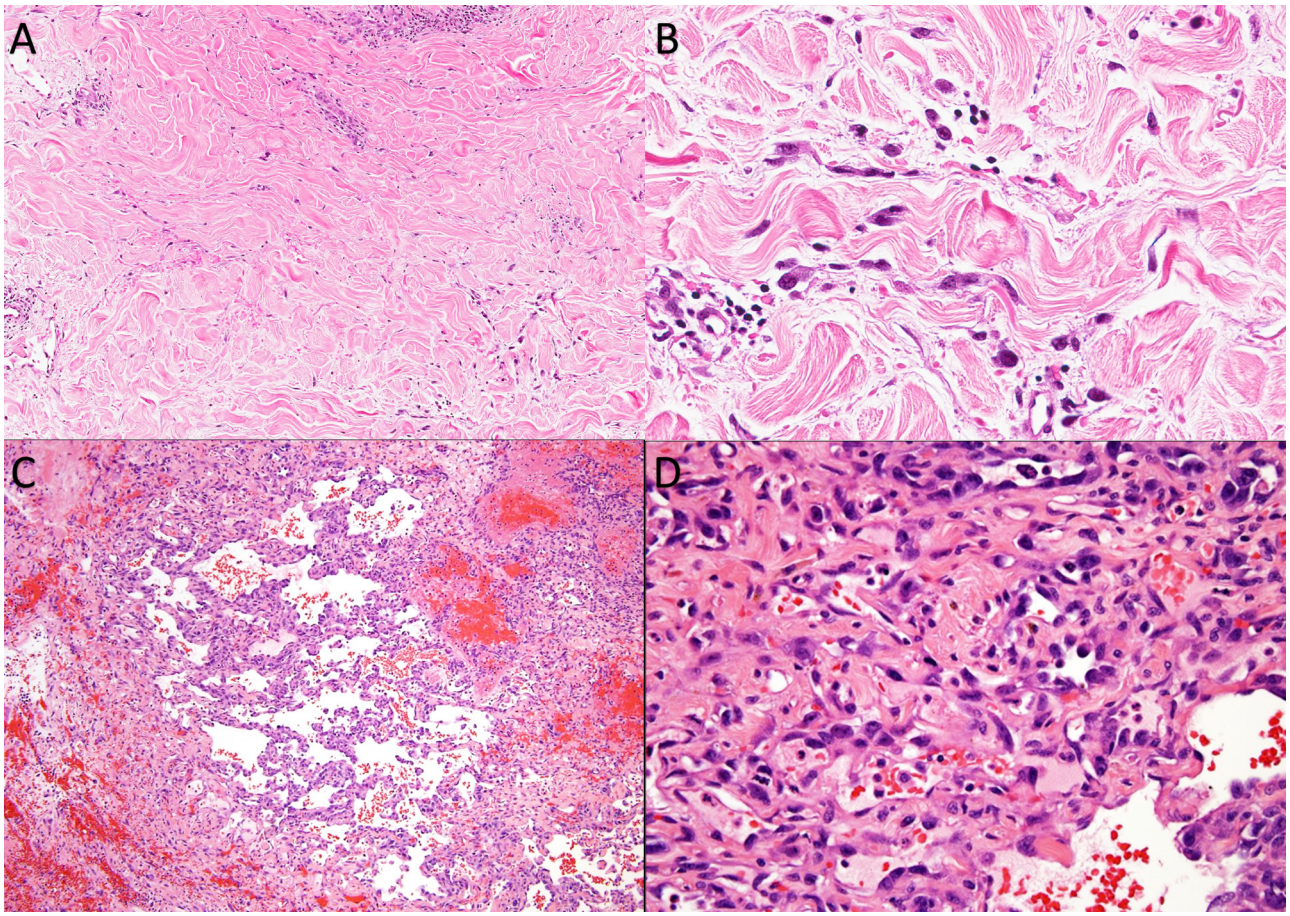
Figure legend

Figure 1: Subtle vasoformation (A: H&E, 100x magnification) but prominent cytologic atypia (B: H&E, 400x magnification) in postirradiation angiosarcoma. Subsequent resection demonstrates vessels with multilayered endothelia, hemorrhage, and cytologic atypia (C: H&E, 100x magnification and D: H&E, 400x magnification).

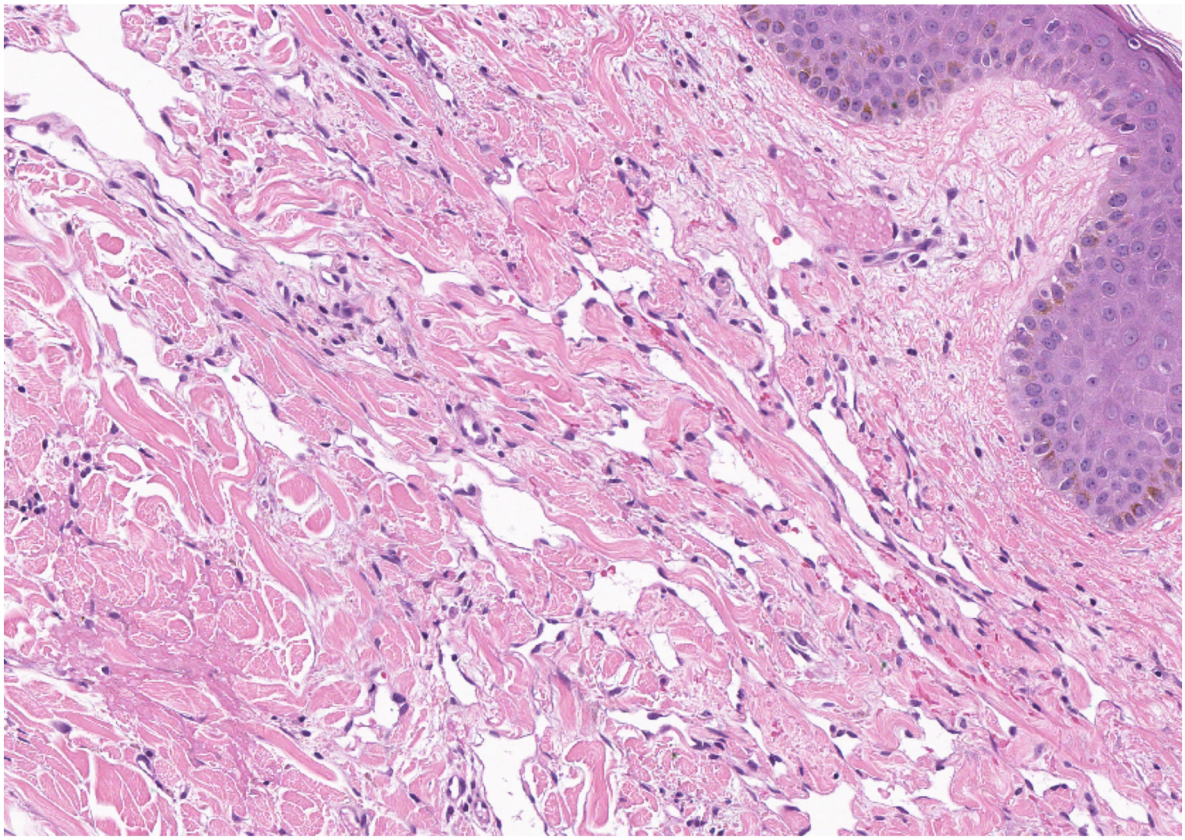
Figure 2: Dilated, ectatic vessels in lymphatic atypical vascular lesion (H&E, 100x magnification).

Figure 3: Fluorescence *in situ* hybridization demonstrating *MYC* amplification in postirradiation angiosarcoma. *MYC* (8q24) is labeled orange, while control centromere of chromosome 8 (CEP 8) is labeled aqua. Image courtesy of Michael Michal, MD, PhD.

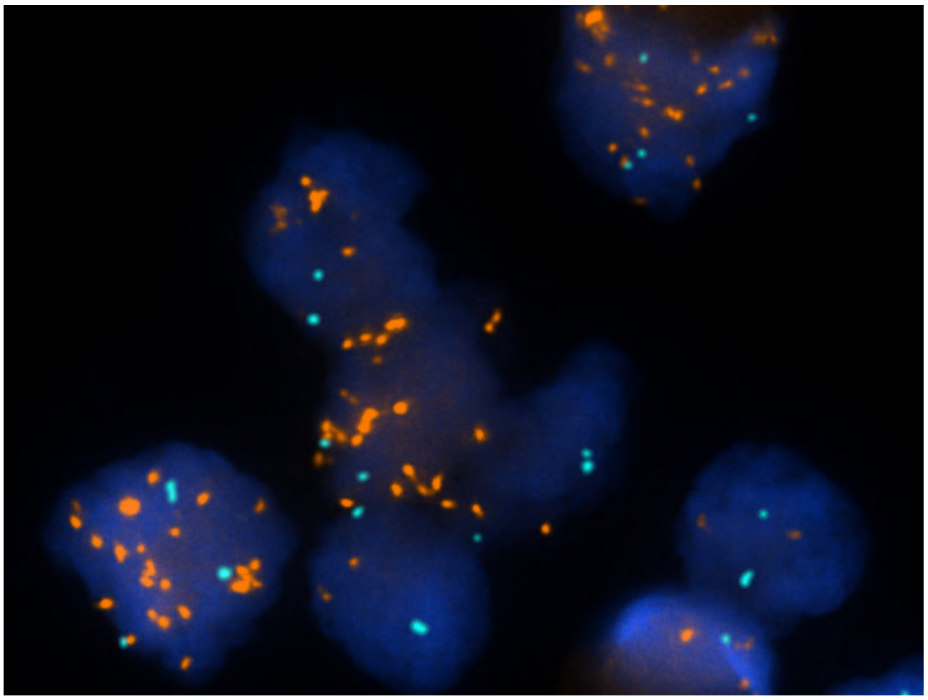
Figure 4: Compressed vascular spaces with subtle cytologic atypia in low-grade postirradiation angiosarcoma (A: H&E, 200x magnification). Diffuse MYC expression by immunohistochemistry supports distinction from atypical vascular lesion (B: MYC, 200x magnification).



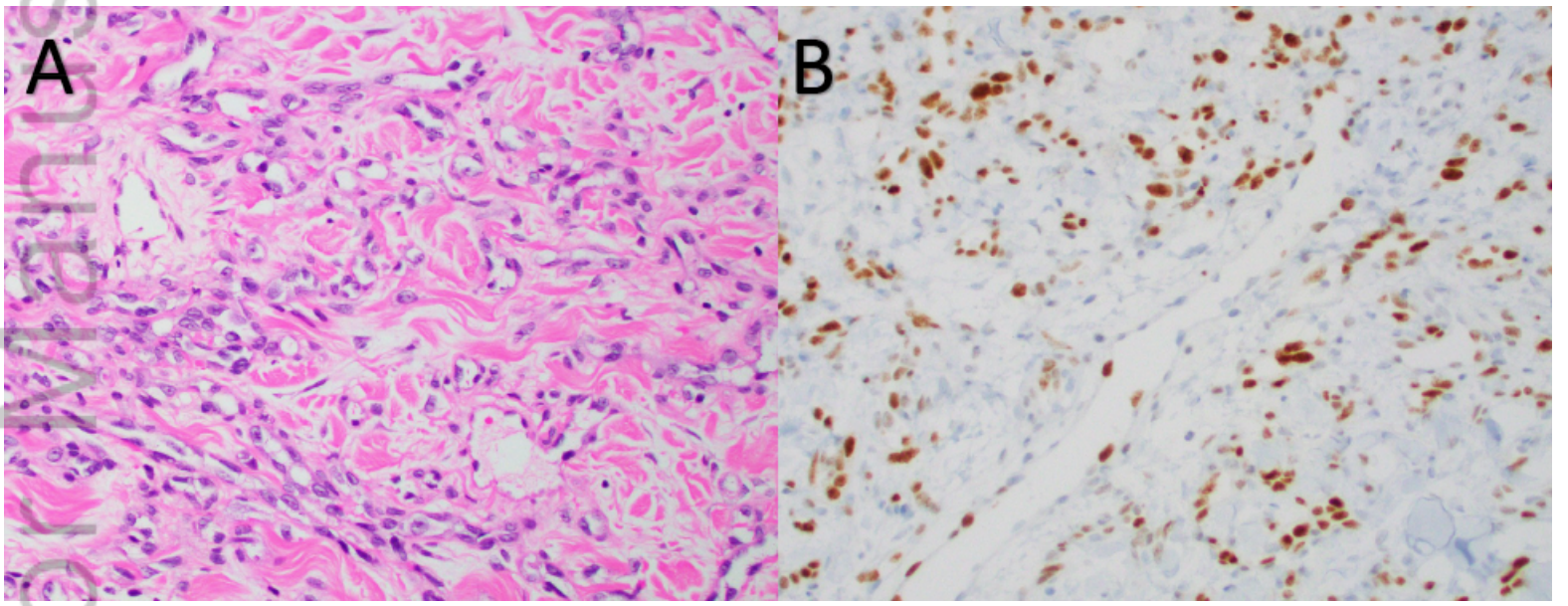
CUP_13912_Figure1.tif



CUP_13912_Figure2.tif



CUP_13912_Figure3.tif



CUP_13912_Figure4.tif

Table 1: MYC amplification and MYC overexpression in primary AS, PRAS, lymphedema-associated AS, and AVLs

Author and year	*MYC* amplification by FISH				MYC overexpression by IHC				Notes
	Primary AS	PRAS	Lymphedema-associated AS	AVLs	Primary AS	PRAS	Lymphedema-associated AS	AVLs	
Requena et al. 2018	0/6	5/10	1/1	N/A	1/6	6/10	1/1	N/A	Used TMA
Daniels et al. 2017	N/A	2/2	N/A	N/A	N/A	10/10	N/A	N/A	Radiation dermatitis-like features in PRAS
Harker et al. 2017	N/A	N/A	2/2	N/A	N/A	N/A	1/1	N/A	Lymphedema due to morbid obesity
Huang et al. 2016	5/69	History of breast cancer – 28/31 No history of breast cancer – 2/8	4/4	N/A	N/A	N/A	N/A	N/A	
Udager et al. 2016	N/A	N/A	N/A	N/A	16 cutaneous; 38 total Average H-score 54: No difference between	23 cases, mainly from patients with PRAS Average H-Score of 206		22 Average H-score 10; negative, focal	Used H-score to quantify IHC positivity; *MYC* amplification not studied

					cutaneous and non-cutaneous			weak or moderate	
Cornejo et al. 2015	N/A	14/17	N/A	0/18	N/A	14/17	N/A	0/18	
Fraga-Guedes et al. 2015	0/12	20/37 with high-level amplification		0/29	0/12	20/37 positive; 10/37 strong (3+) expression		0/29	
Lae et al. 2015	1/15	32/32	N/A	N/A	N/A	N/A	N/A	N/A	MYC overexpression not studied
Ginter et al. 2014	Non-breast including skin: 2/20 Breast: 0/17	8/8	1/1	0/7	Non-breast including skin: 9/20 Breast: 0/17	8/8	1/1	0/7	TMA and whole sections for FISH and IHC
Ko et al. 2014	N/A	11/13 by FISH 13/13 by DISH		0/5 by FISH 0/5 by DISH	N/A	N/A		N/A	Compared DISH to FISH for *MYC* amplification; MYC overexpression not studied
Shon et al. 2014	4/23	N/A	N/A	N/A	9/38	N/A	N/A	N/A	Primary AS from sun-damaged skin only

Fernandez et al. 2012	N/A	8/8		0/4	N/A	9/9		0/4	
Mentzel et al. 2012	0/7	25/25	0/1	0/16	0/7	24/25	0/1	1/16	
Italiano et al. 2012	3/6	6/10	2/2	N/A	N/A	N/A	N/A	N/A	MYC overexpression by IHC not studied
Guo et al. 2011	0/18	21/22	2/2	0/12	0/12 expressed MYC-II	8/8 expressed MYC-II	N/A	N/A	MYC-II expression evaluated by WB
Manner et al. 2010	0/28	16/31	2/2	N/A	N/A	N/A	N/A	N/A	MYC overexpression by IHC not studied

AS: angiosarcoma; PRAS: postirradiation angiosarcoma; AVL: atypical vascular lesion; FISH: fluorescence in situ hybridization; IHC: immunohistochemistry; TMA: tissue microarray; DISH: dual-color dual-hapten in situ hybridization; WB: western blot

Table 2: Sensitivity* of FISH for *MYC* amplification and IHC for MYC overexpression in primary AS, secondary AS, PRAS, and lymphedema-associated AS

	***MYC* amplification by FISH**			**MYC overexpression by IHC**		
	Positive cases	Negative cases	Sensitivity (percent)	Positive cases	Negative cases	Sensitivity (percent)
Primary AS	15	206	6.8	19	81	19
Secondary AS	212	57	78.8	94	26	78.3
PRAS	159	37	81.1	62	8	88.6
Lymphedema-associated AS	14	1	93.3	3	1	75
AVLs	0	91	N/A	1	73	N/A

AS: angiosarcoma; PRAS: postirradiation angiosarcoma; AVL: atypical vascular lesion; FISH: fluorescence in situ hybridization; IHC: immunohistochemistry

*Includes both cutaneous and non-cutaneous cases