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REVIEW

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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[1] | Scott R. Lauer[2] | Rajiv M. Patel[3,4] | Claudia I. Vidal[5] | Konstantinos Linos[6]

[1]Department of Dermatology, University of Florida College of Medicine, Gainesville, Florida

[2]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska

[3]Michigan Medicine Department of Pathology, University of Michigan, Ann Arbor, Michigan

[4]Michigan Medicine Department of Dermatology, University of Michigan, Ann Arbor, Michigan

[5]Dermatology Center of Southern Indiana, Bloomington, Indiana

[6]Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH and Geisel School of Medicine, Hanover, New Hampshire

Correspondence
Konstantinos Linos, MD, Department of Pathology, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon NH 03766.
Email: konstantinos.linos@hitchcock.org

Abstract

Background: Secondary angiosarcoma (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 fluorescence in situ hybridization (FISH) for *MYC* amplification or immunohistochemistry (IHC) for MYC overexpression were included.

Results: Sixteen studies met inclusion criteria. Overall, 93% of cases evaluated by FISH and IHC were concordant. The sensitivity of FISH in primary AS was only 6.8%, and protein overexpression occurred without amplification in sun-damaged skin. FISH and IHC were over 78% sensitive in secondary AS but negative in over 98% 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.

KEYWORDS

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

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.

1 | INTRODUCTION

1.1 | Primary and secondary cutaneous angiosarcoma

Angiosarcoma (AS) accounts for 1% to 2% of all soft tissue sarcomas, and 60% of cases are cutaneous.[1] AS is a highly aggressive tumor, with 5-year survival ranging from 30% to 50%. 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] affecting 0.27% of 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] Because of 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 an extremity because of chronic lymphedema, most commonly following radical mastectomy for breast cancer.[8] Rarely, lymphedema-associated AS arises in the context of congenital lymphedema, postfilarial infection, lymph node dissection, or morbid obesity.[9]

1.2 | Atypical vascular lesions

Atypical vascular lesions (AVLs) show a spectrum of morphologic features, but the two 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 show 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 because of overlapping clinicopathologic features.[4,12] In limited or peripheral sampling, low-grade secondary AS can be indistinguishable from AVL.[12] Consequently, up to 50% of resection specimens for AVL contained secondary AS.[13] Importantly, upgrading to AS in resection specimens should be distinguished from true progression. Based on three retrospective series reflecting 71 patients with an

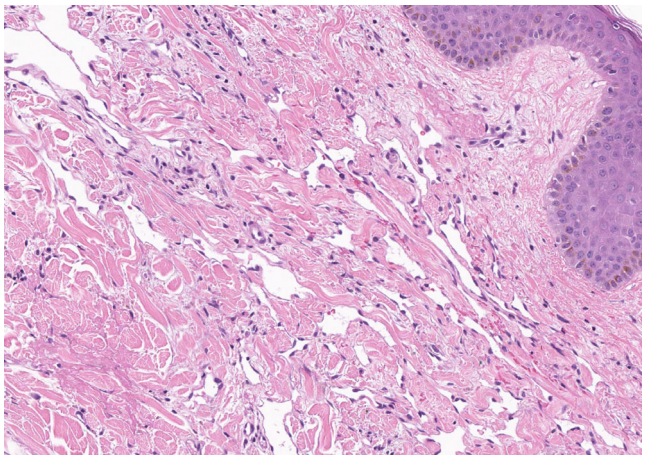

FIGURE 2 Dilated, ectatic vessels in lymphatic atypical vascular lesion (H&E, ×100 magnification)

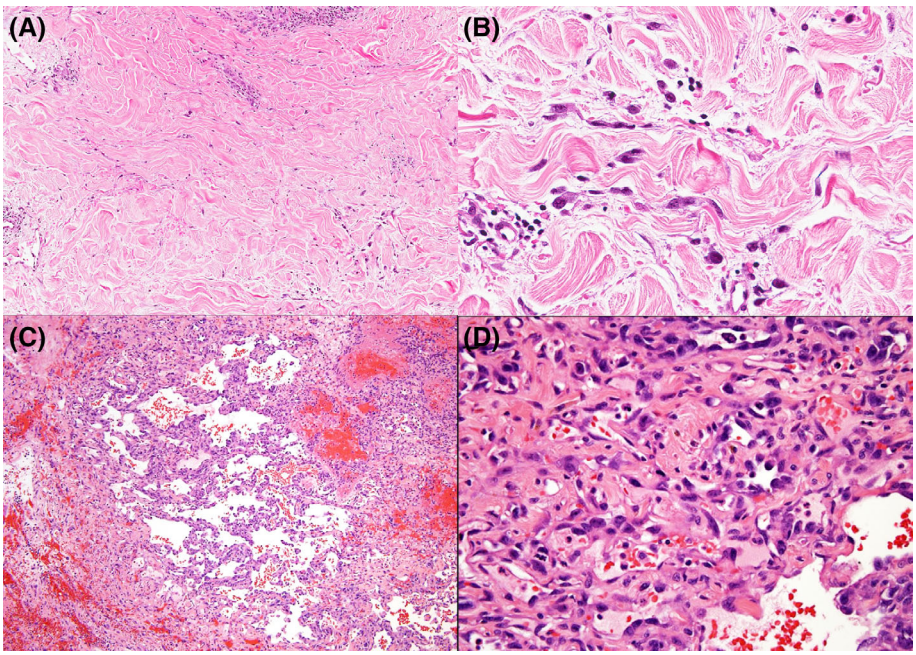

FIGURE 1 Subtle vasoformation (A, H&E, ×100 magnification) but prominent cytologic atypia (B, H&E, ×400 magnification) in postirradiation angiosarcoma. Subsequent resection shows vessels with multilayered endothelia, hemorrhage, and cytologic atypia (C, H&E, ×100 magnification and D, H&E, ×400 magnification)

average follow-up of 36 months (range 1-181), the rate of progression from AVL to PRAS is 7% (5/71 patients).[10,14,15]

1.3 | 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% 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.

2 | METHODS

PubMed was searched for relevant studies in the English language between 1967 and 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.

2.1 | 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, two studies[29,30] examined 100 nuclei, and four 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 three to eight copies of *MYC* and CEP8.[27] Tissue microarrays (TMAs) 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 non-informative 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 vs positive expression by IHC are lacking.[34] Three studies considered any nuclear reactivity as positive,[4,26,33] three studies set a threshold at >5% of cells,[25,27,29] and one study set a threshold at >10% 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 nonreactive neoplastic vessels in stained sections.[12]

Almost 93% (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 showed 100% concordance between *MYC* amplification and protein expression in AVLs, primary AS of breast, and secondary AS of breast. However, there was only 65% concordance between FISH and IHC in primary AS of nonbreast 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 show *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]

3 | *MYC* IN PRIMARY ANGIOSARCOMA

In the included studies, the overall sensitivity of FISH for *MYC* amplification in primary AS was 6.8%.[12,18,23,25,27,28,29,30,31,32] The overall sensitivity of IHC for MYC overexpression was 19%.[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]

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 cutaneous and non-cutaneous	23 cases, mainly from patients with PRAS Average H-Score of 206		22 Average H-score 10; negative, focal weak or moderate	Used H-score to quantify IHC positivity; *MYC* amplification not studied
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	

(Continues)

TABLE 1 (Continued)

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	
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

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

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 one 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 nonbreast sites including skin, *MYC* amplification and overexpression were present in 20% and 45% 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% of cases tested harbored high- or low-level *MYC* amplification. Nearly 24% of tumors expressed MYC by IHC, and the majority of IHC-positive cases showed strong reactivity. While MYC IHC shows 66% sensitivity and 70% 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]

4 | *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%,[12,18,23,26,28,29,30,31,32,33] while the overall sensitivity of IHC for MYC overexpression was 78.3%.[12,26,28,29,33] Overall, FISH for *MYC* amplification and IHC for MYC overexpression were negative in 100% [4,11,12,18,25,26,28] and 98.6% [4,12,25,26,28] of AVLs, respectively. Quantification of MYC nuclear reactivity in AVLs showed 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% of PRAS from patients with a history of breast cancer showed *MYC* amplification, only 25% 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% 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% of lesional cells may be observed in AVLs and is not diagnostic of PRAS.[34]

5 | *MYC* IN LYMPHEDEMA-ASSOCIATED ANGIOSARCOMA

The overall sensitivity of FISH for *MYC* amplification in lymphedema-associated angiosarcoma was 93.3%,[12,18,23,24,28,29,31,32] and the overall sensitivity of IHC for MYC overexpression was 75%.[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.

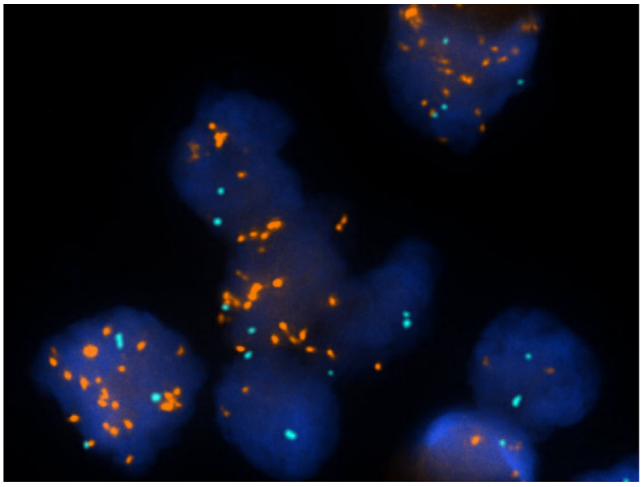

FIGURE 3 Fluorescence in situ hybridization demonstrating *MYC* amplification in postirradiation angiosarcoma. *MYC* (8q24) is labeled orange, while control centromere of chromosome 8 (CEP 8) is labeled aqua. Image courtesy of Michael Michal, MD, PhD

6 | 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]

7 | 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% (16) showed *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, five *PLCG1*-mutated secondary AS cases showed coexistent *MYC* amplifications. *KDR* mutations in two 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 eight cases of *MYC*-amplified secondary AS. This cluster contains

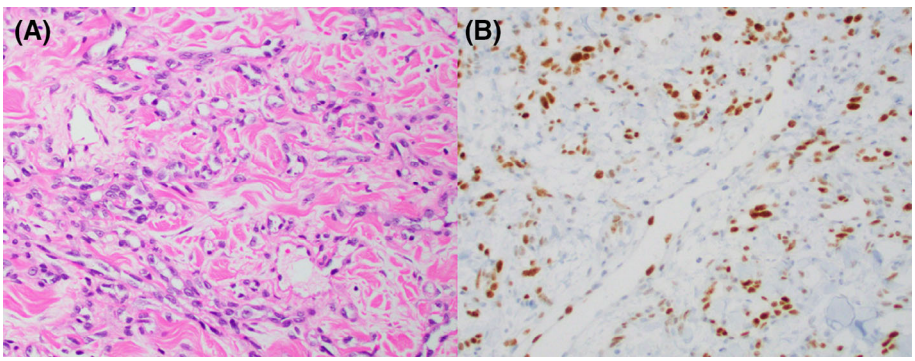

FIGURE 4 A, Compressed vascular spaces with subtle cytologic atypia in low-grade postirradiation angiosarcoma (H&E, ×200 magnification). B, Diffuse MYC expression by immunohistochemistry supports distinction from atypical vascular lesion (MYC, ×200 magnification)

TABLE 2 Sensitivity[a] 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 (%)	Positive cases	Negative cases	Sensitivity (%)
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

Abbreviations: AS: angiosarcoma; AVL: atypical vascular lesion; FISH: fluorescence in situ hybridization; IHC: immunohistochemistry; PRAS: postirradiation angiosarcoma.
[a]Includes both cutaneous and noncutaneous cases.

miRNAs that downregulate expression of *THBS1*. *THBS1* (15q14) encodes thrombospondin-1, a potent endogenous inhibitor of angiogenesis, and *MYC*-amplified AS showed 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]

8 | 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 to 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 toward 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 nonstatistically 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 vs noncutaneous 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) showed 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 toward 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 histopathology, 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 histopathology was significantly associated with increased MYC expression in primary AS but not in secondary AS.[34]

9 | 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% 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% 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.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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

DATA AVAILABILITY STATEMENT

No original data are submitted.

ORCID

Kiran Motaparthi https://orcid.org/0000-0003-0562-0826

Rajiv M. Patel https://orcid.org/0000-0002-1521-4947

Claudia I. Vidal https://orcid.org/0000-0003-2672-4974

Konstantinos Linos https://orcid.org/0000-0001-9462-652X

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