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CLINICAL TRIAL

Angiosarcoma and atypical vascular lesions of the breast: diagnostic and prognostic role of *MYC* gene amplification and protein expression

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Abstract *MYC* amplification has been reported as a prominent feature of secondary angiosarcomas (SAS). The differential diagnosis between atypical vascular lesion (AVL) and low-grade angiosarcoma (AS) can be occasionally very difficult or even impossible, and *MYC* amplification status has been pointed as an important diagnostic tool to distinguish cutaneous vascular lesions of the breast. We assessed *MYC* amplification and protein expression status by fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC), respectively, in 49 patients diagnosed with breast AS, and 30 patients diagnosed with post-radiation AVL of the breast. Clinical and pathological features, and follow-up data were collected, and survival analyses were performed. Among 37 patients with SAS, twenty patients had tumors with high-level *MYC* amplification and protein overexpression (54 %). None of primary angiosarcomas (PAS) or AVL cases showed *MYC* amplification or protein expression. Concordance between *MYC* amplification (FISH) and protein expression (IHC) was 100 % in AVL, PAS, and SAS. Survival analysis of the SAS patients demonstrates that those with *MYC* amplification had a significantly worse overall survival compared to cases without MYC amplification ($P = 0.035$). There was a non-significant trend toward a poor disease-free survival between cases with and without *MYC* amplification ($P = 0.155$). Our findings show that *MYC* amplification is a highly specific but poorly sensitive marker for SAS and, therefore, a negative result does not exclude the diagnosis of angiosarcoma. *MYC* amplification was associated with adverse prognosis, suggesting a prognostic role of *MYC* amplification status on SAS of the breast.

Keywords Angiosarcoma · Post-irradiation · MYC · Cutaneous · FISH · Atypical vascular lesion

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Introduction

Secondary angiosarcoma (SAS) is an aggressive tumor that arises in the setting of previous irradiation field or chronic lymphedema (Stewart–Treves syndrome) and is associated with poor prognosis [1–3]. Atypical vascular lesions (AVL) of the breast, however, are a benign radiation-associated vascular proliferation that shows morphological similarities with low-grade secondary angiosarcomas [4–9]. The differential diagnosis between AVL and low-grade angiosarcoma (AS) can be occasionally very difficult or even impossible, mostly in small-sized skin biopsies, due to the histologic overlap characteristics between low-grade AS and AVL [5, 10]. Some studies reported patients with AVL who later developed AS, suggesting that AVL may be a precursor to or an incipient angiosarcoma [6, 7, 11].

Some studies have shown that *c-myc* amplification is a recurrent genetic alteration in secondary angiosarcomas, but not in primary angiosarcomas and AVL, suggesting distinct pathogenetic mechanisms between them [12–15]. However, recent studies have shown primary angiosarcomas with *MYC* amplification, raising the possibility of *MYC* participation in its pathogenesis [10, 12, 16]. *MYC* is a proto-oncogene that codes for a transcription factor involved in the regulation of cellular proliferation, cell growth, and apoptosis [4, 12]. The most common mechanisms by which *MYC* activation occurs in tumors are gene amplification and gene rearrangement [4, 10]. The deregulation of *c-myc* has been associated with human cancers and has also been implicated in angiogenesis [17]. MYC protein expression promotes cell proliferation through inappropriate entry to S-phase from G1 phase following ionizing radiation, resulting in its function as an oncogene [18].

As accurate morphological diagnosis between low-grade AS and AVL can be difficult and the utility of immunohistochemical (IHC) stains for this particular diagnostic dilemma has not been established [11], new methods to distinguish these two biologic entities are a welcome tool in the clinical setting.

In this study, we aim to evaluate the diagnostic utility of *MYC* amplification and overexpression in the scenario of AVL and AS to further assess whether *MYC* amplification is implicated in prognosis.

Materials and methods

We searched the database from the Department of Anatomic Pathology of the European Institute of Oncology (IEO) Milan, Italy, and Portuguese Institute of Oncology (IPO), Lisbon, Portugal, and retrieved all female patients with the diagnosis of either angiosarcoma (primary and secondary) or AVL of the breast from 1999 to 2014. Post-radiation AVL and angiosarcoma arising at sites other than the breast and cases without representative tumor blocks were not included in this study. The medical records were reviewed and hematoxylin- and eosin-stained slides were re-examined by three of the authors (CFG, SA, and HG) to confirm the diagnosis. Histopathological diagnostic criteria proposed by Fineberg and Rosen [19] were used in the pathological review. The histological grade of breast angiosarcomas was determined as previously described by Donell et al. [20] modified by Schnitt and Collins [21]. Suitable blocks were chosen to obtain additional sections for *MYC* immunostainings and interphase FISH analysis.

For IHC analysis, standard whole sections were immunostained for *MYC* with a rabbit monoclonal anti-*c-MYC* antibody (Y69, 1:50, Epitomics [Cat no. 1472-1], Burlingame, CA) using heat-induced epitope retrieval and an automated immunostainer (Ventana, Oro Valley, AZ, USA). Appropriate positive and negative controls were used. Only nuclear reactivity was considered positive. Immunostained sections were then examined by routine light microscopy. The cases were scored as 'negative' (< 5 % positive cells), '1+' (5–25 % positive cells), '2+' (26–50 % positive cells), or '3+' (≥51 % positive cells), as previously proposed by Shon et al. [12].

Interphase FISH was performed using commercially available FISH probe for *MYC* (8q24), and a probe designed to detect CEP8 (Abbot Molecular, Des Plines, IL, USA). All tissue sections were pretreated, digested, and washed as recommended by the probes supplier. A minimum of 50 non-overlapping intact interphase nuclei were assessed for the presence of amplification and were analyzed by two observers blinded to the original diagnosis. The hybridized slides were reviewed and the ratio of *MYC* (red) and CEP8 (green) signals was calculated. MYC/CEP8 ratio of 2.0 or higher defined *MYC* amplification.

When comparing characteristics between primary, secondary angiosarcomas with *MYC* amplification and secondary angiosarcomas without *MYC* amplification, for categorical variables, we used the Fisher's exact test or the Chi-square test. For continuous variables, the non-parametric median two-sample test was used. Disease-free survival (DFS) was calculated from the date of diagnosis of angiosarcoma to any local, regional, distant relapse or death from any cause, whichever occurred first, or to last visit date in case of no events. Overall survival (OS) was defined as the time interval from date of diagnosis of angiosarcoma to death from any cause or to last date of follow-up. DFS and OS were calculated with the Kaplan–Meier method and compared across different subgroups by means of the Log-rank test or Log-rank test for trend, as appropriate. Multivariable Cox regression models were used to adjust the effect of the different types of angiosarcoma on survival. Variables that were significant in the univariate analysis were tested in the multivariable models and only significant or borderline significant ($P < 0.10$) variables in the multivariable models were included in the final model. Hazard ratios (HR) and 95 % confidence intervals (CI) were reported. All analyses were carried out with the Statistical Analysis System (SAS) software (SAS Institute, Cary, NC) and the R (http://cran.r-project.org/) software. All the reported *P* values were two sided.

Informed consent was obtained from all patients and/or guardians and institutional review board approvals were obtained for all parts of the study.

Results

Clinicopathological features

Forty-nine patients were diagnosed with breast angiosarcoma. Of these 49 patients diagnosed with breast

angiosarcoma, thirty-seven patients had a previous history of breast carcinoma treated with radiation therapy (Fig. 1d), and twelve patients had a diagnosis of primary (sporadic) angiosarcoma. All patients with primary AS presented with palpable mass, without any skin changes. Patients with secondary AS presented with skin changes (rash and ecchymosis) with concomitant ulceration and/or bruising. Clinicopathological features from 28 cases of AS included in the present series were previously described [3]. Thirty patients were diagnosed with post-radiation AVL of the breast (Fig. 1a), but one patient was excluded from this study because there was no more representative tumor block available for *MYC* analysis. Clinicopathological details of these patients with AVL were published previously [11]. The main method used for the pathological diagnosis of AS was core biopsy (92 % of cases), and for

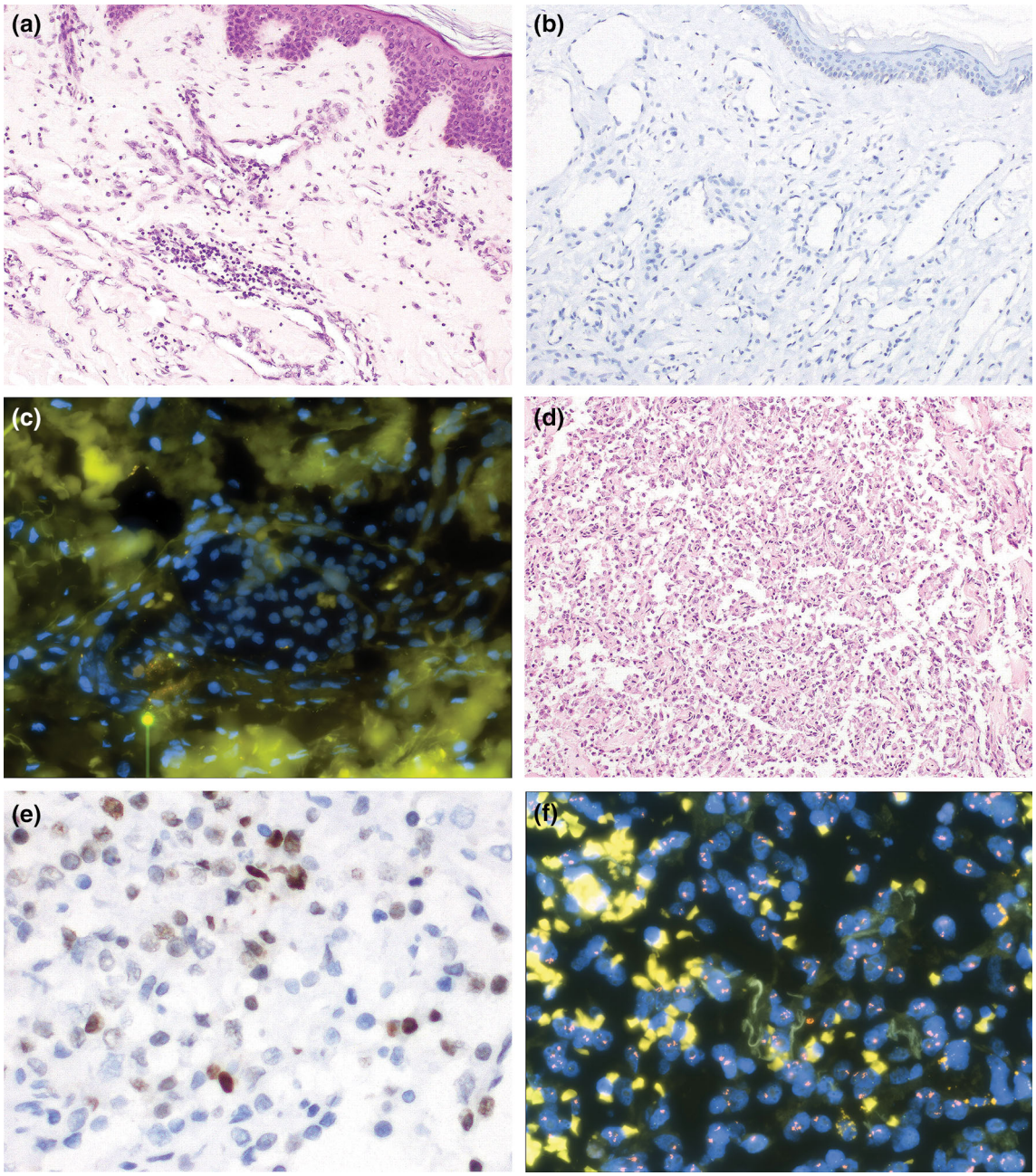

Fig. 1 **a** AVL of the breast skin showing circumscribed proliferation of vessels in the upper dermis, mild endothelial atypia, and associated lymphocytic infiltration. Hematoxylin and eosin, ×200. **b** In a MYC-immunostained section of the same AVL, there was no nuclear staining in endothelial cells. **c** FISH for *MYC* amplification showing no MYC amplification in AVL. **d** Secondary AS with epithelioid morphology showing proliferation of atypical vessels. Hematoxylin and eosin, ×200. **e** Secondary AS showing nuclear MYC protein expression in proliferative tumor cells.**f** FISH analysis showed high-level *MYC* gene amplification in the secondary AS

diagnosis of AVL was punch biopsy (50 % of cases), followed by excisional biopsy. The clinicopathological characteristics of the three study groups are summarized in Tables 1, 2, and 3.

Immunohistochemical data

Immunohistochemical stains for MYC protein were performed on all cases of AVL and AS. None of the 29 cases of AVL displayed nuclear immunoreactivity for *MYC* (Table 1, Fig. 1b). Twenty specimens of secondary angiosarcoma (54 %) stained positive for *c-myc* (20/37 cases = 54 %), with strong positive staining ('3+' or >51 % positive) observed in 10 cases (10/20 cases = 50 %) (Table 3; Fig. 1e). None of the primary AS cases showed MYC protein expression (Table 2).

Fluorescence in situ hybridization (FISH)

None of the 29 cases of AVL (Fig. 1c) or PAS showed *MYC* amplification (Tables 1, 2). Twenty out of 37 cases (54 %) of secondary AS showed *MYC* amplification using interphase FISH analysis (Table 3; Fig. 1f). Of 3 patients with secondary angiosarcoma with epithelioid features, 2 cases showed *MYC* amplification, and MYC protein overexpression.

Concordance between FISH and IHC

Concordance between protein expression (IHC) and *MYC* amplification was 100 % in AVL and primary and secondary AS. The two cases of AVL that progressed to AS (Cases 22 and 23, Table 1) showed no *MYC* amplification

Table 1 Clinicopathological, FISH, and IHC data for atypical vascular lesion

#	Age	Latency interval (months)*	Location	Follow-up (months)	FISH	IHC
1	67	117	Breast skin	Alive NED (16)	NAMP	–
2	47	57	Breast skin	Dead of metastatic CRC (25)	NAMP	–
3	67	1	Axillary skin	Alive NED (25)	NAMP	–
4	43	19	Breast skin	Alive NED (93)	NAMP	–
5	54	31	Breast skin	Dead of metastatic BC (24)	NAMP	–
6	60	26	Breast skin	Alive NED (31)	NAMP	–
7	59	42	Breast skin	Alive NED (90)	NAMP	–
8	57	49	Breast skin	Alive NED (93)	NAMP	–
9	37	28	Breast skin	Alive, with recurrent AVL (26)	NAMP	–
10	47	124	Breast skin	Alive NED (27)	NAMP	–
11	81	124	Breast skin	Alive NED (36)	NAMP	–
12	67	48	Not available	Dead of cardiomyopathy (54)	NAMP	–
13	65	33	Breast skin	Alive NED (61)	NAMP	–
14	62	80	Breast skin	Alive NED (109)	NAMP	–
15	67	83	Breast skin	Alive NED (72)	NAMP	–
16	68	93	Breast skin	Alive NED (38)	NAMP	–
17	55	77	Breast skin	Alive NED (60)	NAMP	–
18	54	37	Breast skin	Alive NED (8)	NAMP	–
19	75	17	Breast skin	Alive NED (41)	NAMP	–
20	43	56	Breast skin	Lost to follow-up	NAMP	–
21	51	79	Breast skin	Dead of metastatic BC (18)	NAMP	–
22	**62**	**54**	**Breast skin**	**Alive, progression to AS (97)**	**NAMP**	**–**
23	**65**	**40**	**Breast skin**	**Dead, progression to AS (107)**	**NAMP**	**–**
24	75	34	Breast skin	Alive NED (5)	NAMP	–
25	73	146	Axillary skin	Alive NED (6)	NAMP	–
26	44	72	Breast skin	Alive NED (12)	NAMP	–
27	50	25	Breast skin	Alive NED (2)	NAMP	–
28	68	41	Breast skin	Alive NED (3)	NAMP	–
29	50	10	Breast skin	Alive NED (2)	NAMP	–

NED no evidence of disease (AVL); *BC* breast cancer; *CRC* colorectal cancer; *AS* angiosarcoma; *NAMP* no amplification

* Latency interval between radiotherapy and development of AVL

Table 2 Clinicopathological, FISH, an IHC data for primary angiosarcomas (AS)

#	Age	Location	Tumor size (cm)	Tumor grade	FISH	IHC	Follow-up (months)
1	61	Breast	1.0	Intermediate	NAMP	–	Lost to follow-up
2	55	Breast	2.1	Low	NAMP	–	Alive NED (133)
3	38	Breast	2.2	Intermediate	NAMP	–	Alive NED (164)
4	34	Breast	3.0	Intermediate	NAMP	–	Alive NED (149)
5	69	Breast	3.0	High	NAMP	–	Dead (53)
6	42	Breast	3.0	Low	NAMP	–	Alive LR (104)
7	42	Breast	10.0	High	NAMP	–	Alive NED (58)
8	36	Breast	8.0	High	NAMP	–	Dead MT (12)
9	30	Breast	15.0	Intermediate	NAMP	–	Dead MT (30)
10	45	Breast	14.0	Intermediate	NAMP	–	Dead (42)
11	77	Breast	10.0	High	NAMP	–	Dead LR (14)
12	59	Breast	10.0	High	NAMP	–	Alive NED (28)

NAMP no amplification; *NED* no evidence of disease (AS); *LR* local recurrence; *MT* metastasis

or protein overexpression both at diagnosis of AVL, as at the time of diagnosis of AS (Cases 4 and 11, Tables 3, 4).

MYC amplification/protein overexpression as a prognostic factor

Clinical follow-up data of patients with diagnosis of breast angiosarcoma were available for 48 of the 49 patients (median 32 months, range from 1 to 163 months).

There was no correlation between histological tumor grade and *MYC* amplification ($P = 0.365$). No significant association of tumor size and *MYC* amplification was found ($P = 0.289$).

When comparing DFS between primary AS, secondary AS without *MYC* amplification and secondary AS with *MYC* amplification, there were statistically significant differences at both univariate and multivariable analyses ($P = 0.033$, Fig. 2; Table 5; $P = 0.016$; Table 6). When comparing OS between primary AS, secondary AS without *MYC* amplification and secondary AS positive for *MYC* amplification, there were statistically significant differences only at multivariable analyses ($P = 0.084$, Fig. 3; Table 5; $P = 0.012$; Table 6). When limiting the survival analysis to the secondary AS patients, we observed that cases with *MYC* amplification had a significantly worse OS compared to cases without *MYC* amplification (size and grade-adjusted HR: 3.47 (1.09–11.1)). There was a non-significant trend toward a poor DFS between cases with and without *MYC* amplification (size and grade = adjusted HR: 1.89 (0.78–4.55), Table 7).

Other disease parameters (tumor size and tumor grade) were also independently predictive of worse outcome in both primary and secondary angiosarcomas. Patients that presented tumor size >5 cm had a short-term DFS ($P = 0.054$) and poor OS ($P = 0.020$) when compared with cases showing tumor size ≤5 cm in multivariable analysis (Table 6). High-grade tumors were associated with worse DFS and OS ($P = 0.056$ and $P = 0.018$, respectively; Table 6) when compared with low-grade tumors.

MYC amplification, however, had no significant association with a shorter latency period (time from radiation therapy to the diagnosis of secondary angiosarcoma) in cases with versus in cases without *MYC* amplification ($P = 0.161$).

Discussion

MYC amplification has been described in different solid tumors. *MYC* high-level amplification has been reported as a prominent feature of radiation-induced angiosarcomas and is also prevalent in other radiation-induced sarcomas, suggesting a strong association between irradiation and *MYC* gene amplification [22]. The perspective of *MYC* as an important anticancer target determines the importance to understand the specific role of *MYC* in different subsets of sarcomas [23, 24].

Our study evaluated the largest series of *MYC* amplification in the largest series of primary and secondary angiosarcomas and AVL of the breast, and it is the unique study that explored exclusively vascular lesions occurring within the breast. In our study, *MYC* amplification was detected in 54 % of secondary breast angiosarcomas. Two other studies found 55 and 67 % of *MYC* amplification in secondary angiosarcomas, respectively [14, 16]. We did not find *MYC* amplification or protein overexpression in any case of AVL or primary angiosarcoma of the breast, and our results confirm previous findings of other series [5, 10, 13–15].

Table 3 Clinicopathological, FISH, and immunohistochemical (IHC) data for secondary angiosarcomas (SAS)

#	Age	Location	Latency interval (months)*	Tumor size (cm)	Tumor grade	FISH	IHC	Follow-up (months)
1	69	Breast skin	18	1.4	Low	NAMP	–	Alive NED (159)
2	66	Breast skin	70	1.2	High	NAMP	–	Dead LR(48)
3	78	Breast skin	192	7.0	High	NAMP	–	Dead MT(9)
4	**64**	**Breast skin**	**79**	**5.5**	**High**	**NAMP**	**–**	**Alive NED (122)**
5	50	Breast skin	118	6.0	High	AMP	+	Dead (20)
6	48	Breast skin	105	0.7	Low	NAMP	–	Dead LR (31)
7	63	Breast skin	82	2.0	High	AMP	++	Dead LR (24)
8	81	Breast skin	66	2.0	High	AMP	+++	Dead LR (63)
9	43	Breast skin	283	9.5	Intermediate	NAMP	–	Alive LR (86)
10	80	Breast skin	48	6.0	Low	NAMP	–	Alive LR (81)
11	**73**	**Breast skin**	**134**	**8.0**	**High/Epithelioid**	**NAMP**	**–**	**Dead CR (42)**
12	46	Breast skin	111	2.5	Low	NAMP	–	Alive NED (71)
13	62	Breast skin	41	11.0	High/Epithelioid	AMP	+++	Dead MT (12)
14	66	Breast skin	104	NA	High	AMP	+	Dead MT (2)
15	77	Breast skin	120	4.2	High	AMP	+	Dead MT (19)
16	70	Breast skin	60	3.0	High	AMP	+++	Dead LR (13)
17	69	Breast skin	97	2.0	Low	AMP	+++	Alive NED (52)
18	37	Breast skin	51	5.5	High	NAMP	–	Alive LR (65)
19	88	Breast skin	113	2.0	High	AMP	+	Dead LR (25)
20	73	Breast skin	118	8.9	High/Epithelioid	AMP	+	Dead LR (8)
21	60	Breast skin	90	3.5	Low	AMP	+	Alive LR (34)
22	40	Breast skin	82	3.0	High	AMP	+++	Alive LR (22)
23	66	Breast skin	71	4.0	High	AMP	+++	Dead MT (25)
24	71	Breast skin	53	5.5	Low	AMP	+	Alive LR (40)
25	70	Breast skin	146	2.5	High	AMP	+	Alive MT (32)
26	81	Breast skin	60	14.0	Low	AMP	+++	Alive NED (66)
27	56	Breast skin	118	20.0	Intermediate	NAMP	–	Dead of disease (35)
28	77	Breast skin	71	10.0	High	NAMP	–	Dead of disease (37)
29	77	Breast skin	135	6.0	Intermediate	AMP	++	Dead of disease (8)
30	67	Breast skin	138	17.0	High	NAMP	–	Dead of disease (1)
31	64	Breast skin	331	7.0	Intermediate	NAMP	–	Dead of disease (4)
32	79	Breast skin	93	4.5	Low	NAMP	–	Alive NED (21)
33	66	Breast skin	75	12.0	High	AMP	+++	Dead of disease (6)
34	84	Breast skin	106	5.0	Low	AMP	+++	Alive NED (12)
35	72	Breast skin	93	7.0	Intermediate	AMP	+++	Dead of disease (17)
36	79	Breast skin	87	3.0	Intermediate	NAMP	–	Dead of disease (133)
37	69	Breast skin	261	14.0	Low	NAMP	–	Alive NED (2)

NA not available; *NAMP* no amplification; *AMP* amplification; *NED* no evidence of disease (AS); *LR* local recurrence; *MT* metastasis; *CR* contralateral recurrence

* Latency interval between radiotherapy and development of AS. Cases 4 and 11 (highlighted) had previous diagnosis of AVL that progressed to AS

Some studies, however, have found that a high level of amplification of *MYC* on chromosome 8q24-21 is present in 100 % of the patients with post-radiation angiosarcoma and lymphedema-associated angiosarcoma, but not in AVL and primary angiosarcoma [5, 10, 13, 15], suggesting *MYC* analysis as a crucial diagnostic tool in the setting of vascular lesions. All these studies, however, involved small case series. They also studied cases of angiosarcoma from non-mammary sites and lymphedema-associated angiosarcoma all together, and this fact may represent a selection

Table 4 Population characteristics

Variables	Classification	PAS no. (col %)	SAS *MYC AMP negative* no. (col %)	SAS *MYC AMP positive* no. (col %)	*P* value	*P* value[a]
All patients		12 (100.0)	17 (100.0)	20 (100.0)		
Age (years)	Median (range)	44 (30–77)	67 (37–80)	70 (40–88)	0.027	0.278
	≤60	9 (75.0)	5 (29.4)	3 (15.0)	0.020	0.637
	61–70	2 (16.7)	6 (35.3)	8 (40.0)		
	>70	1 (8.3)	6 (35.3)	9 (45.0)		
Diameter (cm)	Median (range)	5.5 (1.0–15.0)	6.0 (0.7–20.0)	4.2 (2.0–14.0)	0.460	0.210
	≤2	1 (8.3)	3 (17.7)	3 (15.8)	0.524	0.289
	2.1–5	5 (41.7)	3 (17.7)	8 (42.1)		
	>5	6 (50.0)	11 (64.7)	8 (42.1)		
	Missing	0 (-)	0 (-)	1 (-)		
Grade	1	2 (16.7)	6 (35.3)	5 (25.0)	0.254	0.365
	2	5 (41.7)	4 (23.5)	2 (10.0)		
	3	5 (41.7)	7 (41.2)	13 (65.0)		
Time from primary radiotherapy (years)	Median (range)	–	9.3 (1.5–27.7)	7.2 (3.4–12.2)	–	0.260
	<7	–	5 (29.4)	8 (40.0)	–	0.161
	7–10	–	5 (29.4)	9 (45.0)		
	>10	–	7 (41.2)	3 (15.0)		

PAS primary angiosarcoma; *SAS* secondary angiosarcoma; *MYC AMP*, *MYC* amplification

[a] Among secondary angiosarcomas only. Percentage calculations did not include missing values

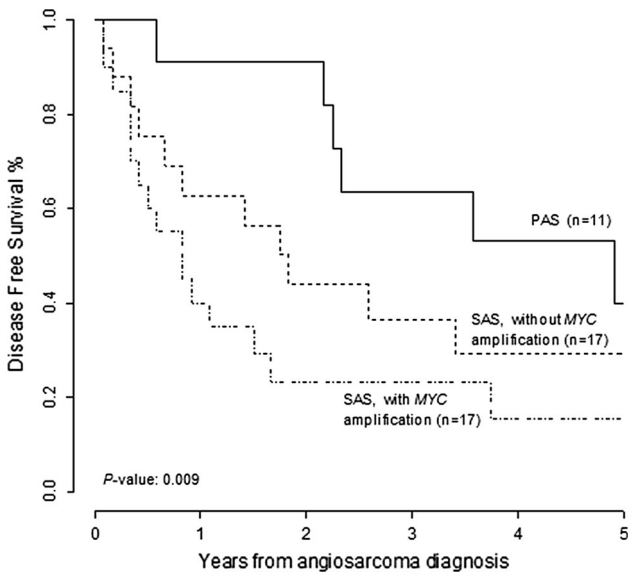

Fig. 2 Disease-free survival according to type of angiosarcoma and MYC amplification

bias. Kacker et al. found 58 % frequency of *MYC* high-level amplifications in their series of radiation-induced angiosarcomas, including sarcomas of the breast but they also included AS of other organs. When only AS of the breast was counted, the frequency of *MYC* high-level amplifications was 86 % [22].

Based on our findings, the diagnostic usefulness of interphase FISH and *MYC* immunohistochemistry in distinguishing low-grade secondary angiosarcoma from AVL is limited due to the low sensitivity of these assays. Indeed, a negative result does not exclude the diagnosis of angiosarcoma.

In our series, none of the 12 primary angiosarcoma of the breast showed *MYC* amplification. Recent studies, however, have shown a small subset of primary angiosarcoma that also presents *MYC* amplification, suggesting that genomic amplification of *MYC* is not restricted to secondary AS, as previously recognized [10, 12, 16]. Shon et al. detected *MYC* abnormalities in a small number of primary cutaneous angiosarcomas, but they included male and female patients with angiosarcomas from non-mammary skin [12]. Italiano et al. found *MYC* amplification in three out of the six primary cases of angiosarcoma (2 out of the 3 cases occurring within the breast) [16]. It seems that the absence of high-level gene amplifications does not exclude a possible role of *MYC* in the pathogenesis of primary angiosarcomas. We can also hypothesize that *MYC* amplification is not a specific genomic aberration induced by ionizing radiation in secondary angiosarcomas, as *MYC* amplification has also been shown even in lymphedema-associated angiosarcomas [10, 13–15].

Two patients of our series with initial diagnosis of AVL showed progression to high-grade cutaneous angiosarcoma

Table 5 Univariate survival analysis

Variables	Classification	At risk no.	DFS: events (5-year survival)	*P* value	OS: events (5-year survival)	*P* value
Age (years)	≤60	17	11 (33.6)	0.158	6 (60.1)	0.035
	61–70	15	10 (24.1)		10 (23.0)	
	>70	16	13 (17.1)		11 (38.4)	
Diameter (cm)	≤5	22	13 (40.5)	0.044	10 (56.0)	0.045
	>5	25	20 (14.7)		16 (30.7)	
Grade	1	13	5 (54.0)	0.006	1 (90.0)	<0.001
	2	10	8 (30.0)		7 (40.0)	
	3	25	21 (8.8)		19 (20.5)	
Time from primary radiotherapy (years)	<7	13	10 (23.1)	0.445	7 (52.8)	0.092
	7–10	14	10 (30.6)		8 (36.5)	
	>10	10	8 (11.3)		7 (22.5)	
Angiosarcoma group	PAS	11	6 (39.8)	0.033	5 (49.1)	0.084
	SAS *MYC AMP neg*	17	12 (29.3)		9 (47.9)	
	SAS *MYC AMP pos*	20	16 (15.6)		13 (35.9)	

PAS primary angiosarcoma; *SAS* secondary angiosarcoma; *MYC AMP*, *MYC* amplification; *neg* negative for MYC amplification; *pos* positive for MYC amplification

Table 6 Multivariable analysis in all patients

Variables	Comparison	DFS HR (95 % CI)	*P* value	OS HR (95 % CI)	*P* value
Diameter	>5 versus ≤5	2.15 (0.99–4.69)	0.054	3.15 (1.20–8.26)	0.020
Grade	2 versus 1	2.46 (0.76–7.97)	0.056	15.9 (1.79–141)	0.018
	3 versus 1	3.42 (1.25–9.35)		19.6 (2.50–154)	
Angiosarcoma group	SAS *MYC AMP neg* versus PAS	2.31 (0.86–6.24)	0.016	1.44 (0.47–4.43)	0.012
	SAS *MYC AMP pos* versus PAS	4.56 (1.62–12.8)		5.69 (1.67–19.4)	

SAS secondary angiosarcoma; *MYC AMP neg*, negative for *MYC* amplification; *MYC AMP pos*, positive for *MYC* amplification; *PAS* primary angiosarcoma. One patient had no follow-up data

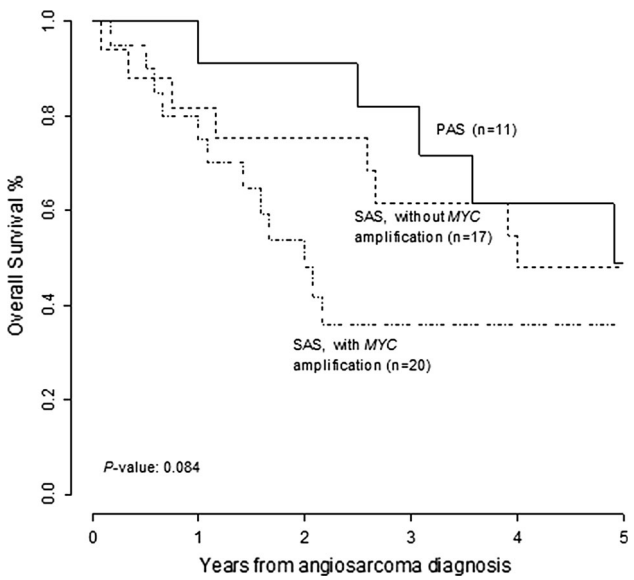

Fig. 3 Overall survival according to type of angiosarcoma and MYC amplification

(Cases 22 and 23, Table 1). Patient of case 22 developed a high-grade breast AS 19 months after diagnosis of AVL in the exact same location of previous punch biopsy. Patient of case 23 developed a bilateral high-grade epithelioid AS 89 months after the initial diagnosis of AVL in the previous punch biopsy site. Details of these cases were previously described [11]. Both at diagnosis of AVL, as at the time of diagnosis of AS, no *MYC* amplification and protein overexpression were observed. Santi et al. found a common mutational pathway (mutational inactivation of *TP53* gene) among AVL and AS, suggesting that they are biologically related entities and could represent the extremes of a morphological continuum [25]. Most studies that explored *MYC* amplification status cast doubt on this hypothesis, since, to date, no case of *MYC* amplification in AVL has been identified [13, 15]. However, based on our findings, the hypothesis that AVL may represent a precursor lesion should not be discarded based only on *MYC* amplification status, since not all cases of post-radiation angiosarcoma shows *MYC* amplification.

Table 7 Multivariable analysis in secondary angiosarcoma patients (SAS)

Variables	Comparison	DFS HR (95 % CI)	*P* value	OS HR (95 % CI)	*P* value
Diameter	>5 versus ≤5	2.14 (0.89–5.21)	0.090	2.92 (0.99–8.68)	0.053
Grade	2 versus 1	2.67 (0.72–9.91)	0.055	13.6 (1.46–125)	0.030
	3 versus 1	3.90 (1.29–11.8)		16.7 (2.09–133)	
MYC AMP	*Pos* versus *Neg*	1.89 (0.78–4.55)	0.155	3.47 (1.09–11.1)	0.035

MYC AMP Pos, positive for *MYC* amplification; MYC AMP *Neg*, negative for MYC amplification

We also found excellent FISH and IHC concordance in primary and secondary angiosarcomas, and AVL of the breast, confirming previous studies [5, 13]. Different from our results, Ginter et al. found a poor concordance (65 %) between IHC and FISH for *MYC* in AS of non-mammary sites [10]. Shon et al. reported MYC protein overexpression in cases lacking gene amplification in primary cutaneous angiosarcomas, suggesting other mechanisms of *MYC* activation, but they also included cases of AS from other organs [12]. Therefore, we can conclude that *MYC* amplification and protein overexpression in angiosarcomas are highly specific but low sensitive marker for the diagnosis of angiosarcomas of the breast and other non-mammary sites.

Our findings confirm the fact that secondary tumors have a worse prognosis when compared with primary disease, as we previously reported in a series addressing a smaller number of cases [3, 26]. We also found a significant association between *MYC* amplification and poor prognosis in secondary angiosarcomas of the breast. Previous studies have shown an association between gene amplification and/or protein overexpression of *MYC* and advanced stage in a variety of non-angiosarcoma human malignancies [12, 27], but none of the available studies that explored the prognostic role of *MYC* gene in angiosarcomas was able to find any association between *MYC* amplification and clinical prognosis or tumor grade [12, 14, 16, 22].

Our study is the first one to demonstrate a consistent association between *MYC* amplification status and clinical outcome on secondary angiosarcoma. We did not find any association between *MYC* amplification and tumor size, tumor grade or shorter latency period from radiation therapy, and diagnosis of AS. To date, only the study of Kacker et al. found a non-significant statistical trend toward a shorter latency between primary tumor and sarcoma in cases with *MYC* amplification ($P = 0.2$).

Data from current literature and our results demonstrate that the genetic and molecular aberrations involved in AS tumorigenesis remain poorly understood. The genetic heterogeneity of these tumors has been observed by other authors, and new potential genomic events have been investigated. Guo et al. identified *FLT4* gene coamplification with *MYC* in 25 % of secondary angiosarcomas, but none of AVL and primary AS showed this abnormality [15]. Italiano et al. observed that the NOTCH pathway effector gene *MAML1* (5q35.3) is amplified and overexpressed in 18 % of secondary angiosarcomas, in all these cases, coamplified with *FLT4*. They did not find any difference in clinical or pathologic characteristics between AS with and without 5q35 amplification [16].

Radiation-induced AS seems to be genetically different from primary angiosarcomas and AVL, but there is a clear evidence of genetic heterogeneity even among secondary cases. Therefore, further studies are necessary to identify the oncogenic trigger events of this subset of tumors.

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Conflict of interest The authors have declared no conflicts of interest.

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