TUMOR SUPPRESSOR GENE TP53 MUTATIONS IN ATYPICAL VASCULAR LESIONS OF THE BREAST SKIN FOLLOWING RADIOTHERAPY

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Keywords:	Atypical vascular lesions, Angiosarcoma, Radiation therapy, TP53,

For Peer Review

**TUMOUR SUPPRESSOR GENE *TP53* MUTATIONS IN ATYPICAL VASCULAR
LESIONS OF BREAST SKIN FOLLOWING RADIOTHERAPY**

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RUNNING TITLE: *TP53* in post-radiation vascular lesions

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KEY WORDS: Atypical vascular lesions, angiosarcoma, radiation therapy, *TP53*;

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ABSTRACT

▲ Atypical vascular lesions (AVL) occurring at the site of radiotherapy represent an uncommon but well-documented complication in the setting of breast-conserving therapy for breast carcinoma. Although the biological behaviour of AVL has been regarded as benign, it has been suggested that AVL may represent a precursor of angiosarcoma. A better understanding of the biology of AVL is essential in order to assess appropriate patient management.

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▲ The purpose of the present study was to investigate alterations of tumour suppressor gene *TP53* in a series of radiation-induced AVL and angiosarcomas (AS). Direct sequencing analysis of the *TP53* gene showed the presence of at least one variation in 10 of 12 (83.3%) AVL and in 7 of 8 (87.5%) AS. The most common alteration in both categories was the P72R polymorphism in exon 4. One angiosarcoma sample carried a pathogenetically relevant disruptive mutation c.592delG, a frameshift deletion in exon 6, causing a premature stop codon.

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▲ The presence of *TP53* alterations suggests that its mutational inactivation may be implicated in the pathogenesis of radiation-associated vascular proliferations. The common mutational pathway suggested by our data supports the hypothesis that AVL and AS are biologically related entities, most likely representing the edges of a morphological continuum.

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INTRODUCTION

Atypical vascular lesions (AVL) of the skin are a well-known complication occurring after radiation in the setting of breast-conserving therapy for breast carcinoma. Originally described by Fineberg and Rosen in 1994¹, these lesions are characterised by an anastomosing pattern of vascular channels lined by hyperchromatic endothelial cells and occasional dissection of dermal collagen, as observed in well-differentiated angiosarcomas (AS)¹.

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Despite morphological overlap with AS, the clinical behaviour of AVL was initially considered benign, as these lesions did not appear to represent a risk factor for the development of angiosarcoma, being able neither to recur nor to develop metastasis, and being safely cured by local excision^{1, 2}. In subsequent years, however, the indolent nature of AVL has been questioned, given that areas histologically indistinguishable from AVL were observed within AS^{3, 4, 5}. In addition, patients with AVL who later developed AS have been occasionally reported^{6, 3, 5}, raising the possibility that in some cases AVL may indeed represent a morphological precursor of AS. Recent studies have also emphasised the difficult histological interpretation of some cases due to inadequate tissue biopsies or sampling bias⁴. It has, therefore, been recommended that patients diagnosed with AVL undergo complete excision with careful specimen sampling and close follow up⁷.

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Little is known concerning the pathogenesis of AVL on irradiated skin. Some authors believe that AVL result from permanent dilatation of lymphatic capillaries associated with disruption of the flow of lymphatic fluid⁸, while others consider them to be a reactive proliferation secondary to vascular damage due to both surgery and radiation^{1, 2, 9}. Knowledge of the genetic changes, including *TP53* gene alterations, of the wide spectrum of radiation-induced vascular proliferations of breast skin, may be

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helpful in clarifying the pathogenesis, the biological significance (reactive vs. neoplastic process) of AVL and their relationship with AS.

Radiation causes DNA double-strand breaks, which may result in deletions and rearrangements and changes in gene expression. A potential target for ionising radiation is the tumour suppressor gene *TP53*, the most frequently mutated gene in a wide variety of human cancers. *TP53* deficient mice developed spontaneous AS, suggesting that the loss of p53 function plays a significant role in tumorigenesis of AS¹⁰. Little information exists on *TP53* gene mutation in human liver and cutaneous AS^{11, 12, 13}. The frequency of *TP53* mutations in AS differs by site, which might be due to different aetiologies¹³. Patients with angiosarcoma who had received radiotherapy for cavernous haemangioma or malignant lymphoma revealed *TP53* mutations, one of which was localised to a dipyrimidine site¹³. Although the exact role of *TP53* gene abnormalities in human AS tumorigenesis remains unclear, it has been underlined that a mutated form of the *TP53* gene induces the expression of mRNA encoding vascular endothelial growth factor (VEGF)¹⁴, a specific mitogen for endothelial cells *in vitro* and angiogenic factor *in vivo*¹⁵. Thus, *TP53* gene alterations, inducing over-expression of VEGF, may be involved in the development of AS, at least in some cases. VEGF and its receptor (*flt*) mRNA have been found to be co-expressed in tumour cells of angiosarcoma, suggesting some paracrine or autocrine control mechanisms of proliferation¹⁶.

The purpose of the present study was to investigate the presence of p53 alterations at the level of either protein expression or gene mutations in a series of AVL in comparison with post-radiation cutaneous AS, with the two-fold aim of verifying whether p53 is implicated in the pathogenesis of radiation-induced vascular

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proliferations, and of collecting evidence in support of a molecular link between AVL and AS.

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MATERIALS AND METHODS

Selection of cases

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Twenty patients classified as either radiation-associated cutaneous AVL (12 cases)

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or AS (8 cases), were identified from the files of the Division of Pathological Anatomy, Department of Critical Care Medicine and Surgery, University of Florence, Italy; the Department of Pathology and Oncology, University of Siena, Italy; and the Department of Pathology, University of Modena and Reggio Emilia, Modena, Italy.

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We employed the criteria of Fineberg and Rosen in distinguishing AVL from AS¹.

Clinical data were obtained from laboratory request forms, pathology reports, or referring dermatologists. The following data were recorded, when available, for each patient: age, sex, primary disease, time latency interval elapsed between radiotherapy and diagnosis of vascular lesion, clinical appearance, location and follow-up.

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Immunohistochemistry

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Immunohistochemical studies were performed both in AVL and in AS using the monoclonal antibody mouse anti-human p53 (clone DO-7, Ventana Medical Systems, Inc. Tucson, AZ; prediluted). Immunohistochemistry was performed on paraffin sections (5-µm thickness) of formalin-fixed tissues, using a Ventana BenchMark® XT automated staining system (Ventana Medical Systems). The sections were treated with an antigen-retrieval procedure for 30 minutes in Cell Conditioning Solution 1

(CC1). Endogenous biotin was blocked with the relevant kit. Tissue sections were incubated with the anti-human p53 antibody for 32 minutes at 37°C in BenchMark® XT autostainer and revealed with the iVIEW DAB detection kit, yielding a brown reaction product. After the staining run was complete, the slides were removed from the autostainer, the sections lightly counterstained with Mayer's haematoxylin, dehydrated, and mounted with permanent mounting medium. As negative control, the primary antibody was substituted with a Ventana dispenser, filled with a non-immune mouse serum at the same concentration.

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Microdissection

One paraffin block was selected to represent the tumour from each patient. Six to ten serial sections (4 µm thickness) were obtained from the paraffin-embedded tissue blocks. The first and last sections were stained with haematoxylin and eosin to confirm that the intervening blank slides contained the lesional material. Tissue fragments were microdissected using stereomicroscopic visualisation from blank, deparaffinised slides. Areas that contained large amounts of blood, necrosis, or contaminating normal tissue were avoided in microdissection. Post-microdissected slides were evaluated to confirm accurate sample microdissection.

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TP53 mutation analysis

TP53 mutation analysis was performed in 12 AVL and 8 AS. DNA was extracted from microdissected paraffin-embedded tissues, using a manual protocol¹⁷. PCR primers for exons 4-9, encoding the DNA binding domain of TP53 gene, were designed to obtain fragments of no more than 300 bp. Amplification reactions were performed with 60 ng of DNA, 10 ng of each primer, 200 µM dNTPs, 1x PCR reaction

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buffer, and 2.5 U Taq polymerase in a final volume of 25µl. PCR products were directly sequenced on both strands using an ABI PRISM 3130XL Genetic Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA).

In silico analysis

Unknown mutations were tested by bioinformatics. We used two dedicated softwares: SIFT (Sorting Intolerant From Tolerant: <http://blocks.fhcrc.org/sift/SIFT.html>) and POLYPHEN (prediction of functional effect of human nsSNPs: <http://genetics.bwh.harvard.edu/pph/>).

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RESULTS

Clinical findings

Clinical data are summarised in Table 1. Patients with AVL were all female, ranging from 47 to 69 years of age. Eleven patients underwent conservative surgery for breast carcinoma. In an additional case, a radical bilateral mastectomy was performed for metachronous bilateral breast carcinoma. All patients received post-operative external radiotherapy with doses varying from 44 to 60 Gy. Lesions were clinically described as solitary or multiple erythematous, skin-coloured or transparent papules, vesicles or discrete nodules/plaques, appearing within the prior irradiation field with a latency interval from 19 to 192 months after radiotherapy (mean 80.7, median 73). No oedema or ecchymoses were described. Other skin lesions at the site of radiotherapy have not been detected. In ten cases, lesions were completely removed by local excision, whereas in two cases an incision biopsy was performed.

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Follow-up data were available for all AVL patients. None of the patients with AVL developed an AS or recurrent lesions or metastases, and they all remained well 3 to 146 months after diagnosis (mean 42.6, median 34).

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Patients with AS were all female, ranging from 53 to 90 years of age. They all had an antecedent history of radiotherapy following conservative/radical surgery for ipsilateral breast carcinoma. Most of the lesions were described as multiple purple-erythematous plaques or nodules on mammary irradiated skin. One patient had a purple-erythematous ring-shaped patch, whereas in another case AS presented as a diffuse breast oedema (*peau d'orange*). The latent period between radiation therapy for breast carcinoma and the diagnosis of AS ranged from 72 to 168 months (mean 115.2, median 96) after radiotherapy for the five patients whose anamnestic data were available. Patients were treated with radical mastectomy in four cases; in three patients initial treatment consisted in incomplete excision. Follow-up data were available in 7 patients. Four are alive 12 to 41 months after diagnosis (mean 25.2, median 24). Three patients died of metastatic breast carcinoma from 12 to 16 months after the diagnosis of cutaneous AS.

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Histopathological findings

At low power, AVL appeared as relatively well-circumscribed, wedge-shaped proliferations of markedly dilated irregular vascular spaces located in the dermis (Figures 1, 2). Vascular proliferations often projected the overlying normal epidermis upwards, thus explaining the elevated clinical appearance of the lesion (Figure 3). In other cases, lesions were flat, with a plaque-like morphology. Vascular spaces were lined by a single layer of endothelial cells with plump-oval or flattened nuclei (Figure 4A-B). Endothelial cells occasionally protruded into the lumen in a hobnail pattern

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(Figure 4C). Numerous small projections of stroma covered by a single layer of endothelium were a constant finding (Figures 4D). The papillae were either attached to the internal surface of the vessels or apparently lying free in the lumen. Other lesions descended into the deep dermis. Vascular spaces became smaller and their lumina more irregular, angulated or slit-like. Dissection of dermal collagen was only focally and rarely encountered. Mitoses were found in one case. Lumina were empty or filled with a small amount of proteinaceous material. Erythrocytes were seen within vascular spaces in a small number of lesions, whereas extravasated red blood cells or “blood lakes” were not observed. The stroma contained a lymphohistiocytic infiltrate with a variable number of plasma cells that varied from mild to dense.

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AS were multifocal or diffuse vascular proliferations showing infiltration into the subcutis. Some cases also showed infiltration into the underlying breast parenchyma. Tumours were all high-grade and were composed of solid aggregates of epithelioid or spindle-cells with slit-like spaces containing intraluminal and extravasated red blood cells. In all cases, tumour cells displayed prominent nuclei, brisk mitotic activity, and an infiltrative growth pattern with prominent dissection between dermal collagen bundles. In three AS, areas consistent with AVL were observed at the periphery of the malignant tumour (Figures 5-6).

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Immunohistochemical findings

p53 immunostaining was evaluated in 10/12 AVL, with variable expression and heterogeneous distribution within single cases (Table 2, Figure 7). In 4/10 (40%) cases p53 expression was detected in less than 20% of positive cells, whereas in another 4/10 (40%) cases, p53 was expressed by a variable percentage between

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20% and 50% of cells. In one case (10%), no p53 stained cell was detectable, whereas in another case (10%), the percentage of stained cells was more than 50%.

p53 protein was expressed in 7/8 (87.5%) AS. In four cases (50%)

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immunostaining was less than 20% of the neoplastic cells. In two cases (25%), the percentage of positive cells was comprised between 20% and 50%, whereas in one case (12.5%) positive cells constituted more than 50% of the lesion.

AS with peripheral AVL, resembling areas showed no difference in p53 protein expression.

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TP53 mutation analysis

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Overall, we identified some TP53 variation in 10/12 (83.3%) AVL and in 7/8 (87.5%) AS. Detailed results of TP53 mutational analysis are summarised in Table 2.

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The most common variation in both categories was the P72R polymorphism in exon 4, found in 6/12 (50%) AVL and in 5/8 (62.5%) AS. The F54L variation in exon 4 was detected in 5/12 (42%) AVL but in none of the AS. Interestingly, one AS sample carried a pathogenetically relevant disruptive mutation c.592delG, a frameshift deletion in exon 6, causing a premature stop codon (Figure 8). The other unknown mutations were analysed in silico with two different bioinformatic tools, and seemed to be non-damaging (non-disruptive) mutations, except for K319N in exon 9, which was found in 2/12 (16.6%) AVL and 2/8 (25%) AS (Table 3).

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DISCUSSION

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TP53 mutation analysis has never previously been applied to the evaluation of AVL molecular profiles. In this study, we found TP53 variations in the majority (83%)

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of AVL and in a similar percentage (87.5%) of post-radiation cutaneous AS. The most common variation in both categories was the P72R polymorphism in both AVL and AS, and the F54L variation in exon 4 detected in AVL. Interestingly, in one angiosarcoma sample, but in none of the AVL samples, we found a pathogenetically relevant disruptive mutation c.592delG in exon 6, a frameshift deletion causing a premature stop codon, resulting in a truncated p53 protein. Although the recognition of AVL as a morphological precursor to AS is still a controversial issue, the common mutational pathway suggested by our data provides preliminary results in support of this hypothesis, and also information relevant to the pathogenesis of atypical and malignant vascular proliferations of irradiated breast skin. It is conceivable that the *TP53* mutations occur early in the tumorigenesis of these atypical vascular proliferations, and may be responsible in part for the initiation or potentiation of endothelial cell growth. Subsequently, the angiosarcoma lesions could be exposed to additional stimuli, giving rise to the frankly malignant phenotype.

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The TP53 proline 72 arginine (P72R) polymorphism was a common mutation harboured in AVL and AS categories. *TP53* mutations causing a malfunction of the p53 pathway are frequently encountered in many different types of human cancers¹⁹. The core domain of p53 is highly conserved and confers DNA-binding specificity to the protein. Variations at this site make up 98% of the transforming mutations in p53¹⁸. Conversely, polymorphic sequence variations are expected to have modest or no cancer-related biological effect¹⁹. The TP53 codon 72 has been extensively studied because it affects the gene coding sequence, generating polymorphic variants with different biochemical and biological activities. The P72R variant has been associated with cancer susceptibility and worse prognosis^{19, 20, 21, 22, 23, 24, 25, 26, 27, 28}. In addition, codon 72 polymorphism has been shown to modulate *TP53*-

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dependent apoptosis, and therefore, modify sensitivity to chemotherapeutic agents²⁹. Nevertheless, meta-analyses based on several studies investigating the susceptibility to lung and cervical cancer found no association between TP53 codon 72 polymorphism and cancer risk^{30, 31}. Methodological issues could have contributed to heterogeneity among previously reported results^{30, 31}.

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In agreement with previous studies, we found that p53 immunohistochemistry did not always correlate with the mutational status of the *TP53* gene³². p53 immunohistochemistry in AVL and AS revealed similar staining characteristics in both pattern and quantity. This further supports the biological and molecular similarities between these apparently distinct entities. Nuclear p53 immunostaining was variable in cases harbouring *TP53* mutations, suggesting that *TP53* mutations may be only one of the factors resulting in an altered p53 signal system.

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Very little has been done at the molecular level to increase our understanding of the genetic alterations involved in malignant transformation of endothelial cells.

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Transgenic mice that were deficient in *p53* demonstrated a relatively high incidence (23%) of cutaneous AS, suggesting that alterations in the *p53* gene may be pathogenetically important¹⁰. In human tissues, Naka et al¹³ analysed *p53* mutations in paraffin-embedded specimens from 33 patients with AS by PCR single-stranded polymorphism, followed by direct sequencing, and found 17 cases with *p53* mutations, concluding that *p53* mutations are a major pathway for the development of human AS. At the protein level, overexpression of p53 and MDM-2 protein has been found to correlate with the increased VEGF expression that is found in nearly 80% of AS³³. Another study also supported the hypothesis that VEGF and the VEGF receptor system play a role in proliferation and growth of AS by showing that tumour

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cells of the haemangiosarcoma cell line ISO-HAS contain a *p53* mutation and express the mRNA of VEGF and its 2 receptors, flt-1 and KDR³⁴.

▲ AVL described in the current study displayed a fully benign clinical course, in agreement with the vast majority of cases so far described.^{1-5, 7-8, 35}

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Histopathologically, our cases met the diagnostic criteria proposed by Fineberg and Rosen¹. In 1999, Diaz-Cascajo et al. first proposed the term “benign lymphangiomatous papules (BLAP)” on the basis of the clinical and histological findings together with the absence of recurrences or distant metastases in their series and in previously reported cases². Requena et al suggested the consideration of BLAP separated from more atypical vascular proliferations resembling benign lymphangioendothelioma (acquired progressive lymphangioma), patch-stage Kaposi’s sarcoma, and well-differentiated AS⁸. In 2008, Patton et al⁵ reinforced the observation and subclassified AVL in to lymphatic type (LT AVL) and vascular type (VT AVL). LT AVL were described as circumscribed proliferations of ectatic thin-walled vessels confined to the dermis; the vessels were often back to back and showed hobnail endothelial cells that protruded into the lumina⁵. In contrast, VT AVL showed round to linear capillary vessels randomly arranged throughout the dermis, and 40% of these lesions displayed significant cytological atypia⁵. Interestingly, VT AVLs were considered to have a higher risk of association with AS ~~than~~ with LT AVL⁵. In our series, 2 cases could be classified as LT AVL, and 3 VT AVL cases displayed a vascular pattern, but in the majority of cases features of both patterns were almost equally represented, in support of the AVL morphological heterogeneity.

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▲ The morphological profile of AVL showing progression to AS is yet to be clearly defined. In previous studies, AVL showing progression to AS were described showing prominent nucleoli, occasional infiltration of the subcutis, mitotic figures, and

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moderate to significant cytological atypia^{3, 4, 5}. As previously emphasised⁵, we agree that histopathology in the context of post-radiation vascular proliferation of the breast skin should be interpreted with extreme caution, especially when specimens represent only partial sampling of the lesion. Clinical features may be diagnostically helpful since all the patients who experienced an adverse outcome after an initial diagnosis of AVL were older, had a shorter interval from radiations as compared to those who showed no recurrence or progression, and were associated with erythema, induration, inflammatory changes, discoloration and telangiectasias⁴. In any event, these latter morphological observations, coupled with our preliminary findings, suggest an initial common mutational pathway involving p53 alterations, which favours the hypothesis that indeed AVL and AS may represent a morphologic continuum, implying that AVL could, at least in some cases, be the actual precursor of AS or incipient AS. However, the risk of progression to malignancy is likely to be quite small given the limited number of AVL showing progression to AS described so far.

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In conclusion, as this is the first report on the involvement of p53 in AVL, replication of these findings in other studies is encouraged. Advances in the search for molecular changes of radiation-induced atypical vascular proliferations may increase our understanding of the relationship between AVL and AS, and lead to improved diagnosis and treatment choices for affected patients.

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TABLE 1

Clinical features of the 12 atypical vascular lesions and 8 angiosarcomas developed in the field of irradiation of breast skin

Age/ Sex	Primary disease	Surgery	Latency interval* (months)	Clinical presentation	Treatment	Pathology
67/F	Bilateral BC	Radical, bilateral	192	Multiple papules and vesicles with clear fluid on the left breast	CE. Other lesions are still present.	Atypical vascular lesion
64/F	Jpsilateral BC	Conservative	96	Single erythematous nodule	CE	Atypical vascular lesion
47/F	Jpsilateral BC	Conservative	19	Single erythematous plaque	CE	Atypical vascular lesion
69/F	Jpsilateral BC	Conservative	74	Erythematous papule and nodule	CE	Atypical vascular lesion
49/F	Jpsilateral BC	Conservative	75	Erythematous plaque/nodule	CE	Atypical vascular lesion
58/F	Jpsilateral BC	Conservative	141	Nodular lesion	IB	Atypical vascular lesion
63/F	Jpsilateral BC	Conservative	60	Erythematous papule	IB	Atypical vascular lesion
66/F	Jpsilateral BC	Conservative	48	Erythematous papule	CE	Atypical vascular lesion
61/F	Jpsilateral BC	Conservative	57	Nodular lesion	CE	Atypical vascular lesion
65/F	Jpsilateral BC	Conservative	72	Erythematous papule	CE	Atypical vascular lesion
47/F	Jpsilateral BC	Conservative	96	Erythematous itchy nodular lesion	CE	Atypical vascular lesion
56/F	Jpsilateral BC	Conservative	39	Whitish nodular lesion	CE	Atypical vascular lesion
55/F	Jpsilateral BC	Conservative	96	Multiple erythematous plaques	RS	Angiosarcoma
76/F	Jpsilateral BC	Conservative	144	Multiple erythematous plaques	RS	Atypical vascular lesion
81/F	Jpsilateral BC	Conservative	168	Erythematous ring-shaped patch	RS	Atypical vascular lesion
80/F	Jpsilateral BC	Conservative	NA	Erythematous plaque/nodular lesion	Incomplete excision	De novo angiosarcoma
79/F	Jpsilateral BC	Conservative	96	Erythematous plaque/nodular	RS	De novo angiosarcoma

TABLE 2

Results of p53 protein expression and *TP53* mutation analysis in post-radiation

atypical vascular lesions (n = 12) and angiosarcomas (n = 8)

#	Diagnosis	Age/ Sex	p53 IHC	Ex4	Ex5	Ex6	Ex7	Ex8	Formatted ... [47]
1	AVL	67/F	NE	F54L; P72R	WT	WT	WT	WT	Formatted ... [48]
2	AVL	64/F	++	WT	WT	WT	WT	WT	Formatted: No underline, Font color: Auto ... [49]
3	AVL	47/F	+++	P72R	WT	WT	WT	WT	Formatted ... [50]
4	AVL	69/F	++	I150T; F54L; P72R	WT	WT	WT	WT	Formatted ... [51]
5	AVL	49/F	+	F54L; P72R	WT	WT	WT	WT	Formatted ... [52]
6	AVL	58/F	+	WT	V147I	WT	WT	WT	Formatted ... [53]
7	AVL	63/F	NE	WT	WT	WT	WT	WT	Formatted ... [54]
8	AVL	66/F	++	F54L	WT	WT	WT	WT	Formatted ... [55]
9	AVL	61/F	+	P72R	WT	WT	WT	WT	Formatted ... [56]
10	AVL	65/F	++	WT	WT	WT	WT	WT	Formatted ... [57]
11	AVL	47/F	+	P72R	WT	WT	WT	WT	Formatted ... [58]
12	AVL	56/F	-	F54L	WT	WT	WT	WT	Formatted ... [59]
13	AS	55/F	+	P72R	WT	WT	WT	WT	Formatted ... [60]
14	AS	76/F	++	E68G; P72R	T155T	WT	WT	WT	Formatted ... [61]
15	AS	81/F	-	P72R	WT	WT	WT	WT	Formatted ... [62]
16	AS	80/F	+	WT	WT	c.592delG	WT	WT	Formatted ... [63]
17	AS	79/F	+	P72R	WT	WT	WT	WT	Formatted ... [64]
18	AS	53/F	++	WT	WT	WT	WT	WT	Formatted ... [65]
19	AS	67/F	+	P72R	WT	WT	WT	WT	Formatted ... [66]
20	AS	90/F	+++	WT	WT	WT	WT	WT	Formatted ... [67]

AVL: atypical vascular lesion; AS: angiosarcoma; p53 immunohistochemical (IHC) results: -, no stained

cells were detectable; +, the percentage of stained cells was less than 20%; ++, stained cells were

comprised between 20% and 50%; +++, the percentage of stained cells was more than 50%; NE, not evaluable; WT: wild-type.

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TABLE 3

In silico analysis of *TP53* mutations by two different bioinformatic tools

Mutation	SIFT	PolyPhen
I50T c.149T>C	tolerated	benign
F54L c.162C>A	tolerated	benign
E68G c.203A>G	tolerated	benign
P72R c.215C>G	tolerated	benign
V147I c.439G>A	not tolerated	benign
K319N c.957G>T	not tolerated	possibly damaging

SIFT: Sorting Intolerant From Tolerant (<http://blocks.fhcrc.org/sift/SIFT.html>); POLYPHEN: prediction of functional effect of human nsSNPs (<http://genetics.bwh.harvard.edu/pph>)

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FIGURE LEGENDS

Figure 1

Atypical vascular lesion showing dilated vascular spaces in the upper dermis surrounded by a moderate chronic inflammatory infiltrate (original magnification x20).

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Figure 2

Atypical vascular lesion characterised by more irregular, anastomosing, markedly-dilated vascular spaces in the superficial dermis (original magnification x10).

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Figure 3

Atypical vascular lesion, vascular channels proliferation in the upper dermis results in an upward projection of the overlying epidermis clinically featuring an exophytic papule (original magnification x10).

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Figure 4

Atypical vascular lesion, A) vascular spaces are lined by a single layer of endothelial cells with plump-oval or flattened nuclei; B) empty vascular lumens, surrounded by a brisk mononuclear inflammatory infiltrate; C) endothelial cells occasionally protrude into the lumen in a hobnail pattern; D) small stromal projections covered by a single layer of endothelium are detected within the vascular lumen (original magnification x40).

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Figure 5

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A) High-grade angiosarcoma of irradiated breast skin (original magnification x5) and atypical vascular lesion (asterisk) in the same excision specimen; B) the angiosarcoma shows frankly malignant epithelioid and spindle cells with significant mitotic activity and vasoformative structures (original magnification x40); C) neoplastic cells show infiltration of deep dermis and so called “blood lakes” are observed (original magnification x20); D) at the periphery of angiosarcoma (asterisk in A), dilated vascular spaces lined by inconspicuous endothelial cells and reminiscent of atypical vascular lesions are observed (original magnification x40)

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Figure 6

A) High-grade multifocal angiosarcoma of irradiated breast skin and atypical vascular lesion (asterisk) in the same excision specimen (original magnification x5); B) neoplastic cells exhibit significant cytological atypia and an infiltrative pattern of growth dissecting dermal collagen bundles (original magnification x20); C) small slit-like vascular channels lined by atypical endothelial cells are seen in contiguity with more dilated, lymphatic-like vessels lined by cytologically bland endothelial cells and suggestive of atypical vascular lesions (original magnification x20); D) at higher power, enlarged nuclei and prominent nucleoli characterise severely atypical endothelial cells in angiosarcoma (original magnification x40).

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Figure 7

Immunohistochemical p53 staining pattern in endothelial cell nuclei of atypical vascular lesions (A-B, upper row) and angiosarcomas (C-D, upper row) is heterogeneous even in the same lesion (original magnification x40).

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Figure 8

Identification of the c.592delG mutation in exon 6 of the *TP53* gene by direct DNA sequencing in one angiosarcoma sample.

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119x90mm (300 x 300 DPI)

Review

119x90mm (300 x 300 DPI)

review

119x90mm (300 x 300 DPI)

Review

119x90mm (300 x 300 DPI)

Review

252x188mm (96 x 96 DPI)

Review

119x90mm (300 x 300 DPI)

Figure 8