 doi:10.20944/preprints202107.0068.v1

Article

Genomic Landscape of Angiosarcoma: A Targeted and Immunotherapy Biomarker Analysis

Andrea Espejo-Freire [1], Andrew Elliott [2], Andrew Rosenberg[1], Philippos Apolinario Costa[1], Priscila Barreto[1], Emily Jonczak[1], Gina D'Amato[1], Ty Subhawong[1], Junaid Arshad[3], Julio A. Diaz-Perez[1], W. Michael Korn[2], Matthew J. Oberley2, Daniel Magee[2], Don Dizon[5], Margaret von Mehren[6], Moh'd M. Khushman[7], Atif Mahmoud Hussein[8], Kirsten Leu[9], Jonathan C. Trent[1].

[1] University of Miami Sylvester Comprehensive Cancer Center, Jackson Memorial Hospital; andrea.espejofreire@jhsmiami.org; arosenberg@med.miami.edu; philippos.costa@jhsmiami.org; priscila.barretocoe@jhsmiami.org; eej18@med.miami.edu; gina.damato@med.miami.edu; tsubhawong@med.miami.edu; julio.diazperez@jhsmiami.org; jtrent@med.miami.edu
[2] Caris Life Sciences; aelliot@carisls.com; wmkorn@carisls.com, moberley@carisls.com; dmagee@carisls.com
[3] The Oncology Institute of Hope and Innovation; dr.junaidarsh@gmail.com
[5] Lifespan Cancer Institute, Rode Island Hospital; don.dizon@lifespan.org
[6] Fox Chase Cancer Center; margaret.vonmehren@fccc.edu
[7] The University of Alabama at Birmingham; mkhushman@uabmc.edu
[8] Memorial Health Care System; ahussein@mhs.net
[9] Nebraska Cancer Specialists; kleu@nebraskacancer.com
* Correspondence: Jonathan C. Trent, jtrent@med.miami.edu

Simple Summary: Angiosarcomas (AS) are rare highly aggressive sarcomas with limited therapeutic options. Genomic sequencing techniques have identified recurrent genetic abnormalities. Nevertheless, the association of these findings with etiology, site of origin, prognosis, and therapeutic implications is not well understood. We analyzed data of Next Generation Sequencing (NGS) and Whole Transcriptome Sequencing (WTS) in a cohort of 143 AS cases. We identified a distinct genomic biology according to AS primary site. Cases of head and neck AS have primarily Immunotherapy (IO) response markers, and mutations in TP53 and POT1. On the other hand, breast AS is enriched for cell cycle alterations, predominately MYC amplification. Additionally, a microenvironment abundant with immune cells is present in a minority of cases but distributed evenly among primary sites. Our findings can facilitate the design and optimization of therapeutic strategies for AS according to its biology at different primary sites.

Abstract: We performed a comprehensive analysis of angiosarcoma (AS) genomic biomarkers and their associations with the site of origin. We aimed to describe the genomic landscape of AS in a cohort of 143 cases of AS profiled by Caris Life Sciences. Data of Next Generation Sequencing (NGS) with a 592 gene panel was available for the entire cohort. Fifty-three cases had data of Whole Exome Sequencing (WES) which we used to study the microenvironment phenotype. Immunotherapy (IO) response biomarkers: Tumor Mutation Burden (TMB), Microsatellite Instability (MSI) and PD-L1 status were included. IO-response markers were present in 36.4% of the cohort and in 65% of head and neck AS (H/N-AS) ($p<0.0001$). H/N-AS cases had predominantly mutations in TP53 (50.0%, $p=0.0004$), POT1 (40.5%, $p<0.0001$) and ARID1A (33.3%, $p=0.5875$). In breast AS, leading alterations were MYC amplification (63.3%, $p<0.0001$), HRAS (16.1%, $p=0.0377$), and PI3KCA (16.1%, $p=0.2352$). A microenvironment with a high immune signature, associated with better response to IO, was present in 13% of the cases. This signature was evenly distributed among different primary sites. We found that the molecular biology for AS varies significantly according to the primary site. Our findings can facilitate the design and optimization of therapeutic strategies for AS to overcome resistance to IO and targeted therapies.

 doi:10.20944/preprints202107.0068.v1

Keywords: Angiosarcoma; biomarkers; tumor microenvironment; immunotherapy, next generation sequencing, whole transcriptome sequencing.

1. Introduction

Angiosarcomas (AS) are highly aggressive sarcomas accounting for only 2% of all soft-tissue-sarcomas (STS)[1]. Unfortunately, even when presenting as localized disease, the five-year OS is only 60%. Once patients have locally advanced or metastatic disease, responses to cytotoxic chemotherapy are frequently seen but are short lived, leading to a median OS of only 9-15 months[2,3]. Moreover, despite evidence of upregulation of vascular specific receptor tyrosine kinases, VEGF blockade provides just a 2-3-month survival benefit [4–7].

The majority of cases of AS occur sporadically (primary) or are related to radiation therapy or chronic lymphedema (secondary) [8,9]. Sequencing techniques have identified of some recurrent somatic genetic abnormalities; however, their associations with risk factors, anatomic site, prognosis, and therapeutic implications need further study. The first identified genetic alteration was KDR (AKA VEGFR2) which harbors point mutations in 10% of cases of primary or secondary breast AS [10]. Some mutations appear to be distinct to cases of primary and secondary AS. This is the case of MYC amplification, reported in 50 to 100% of cases of radiation associated AS but not in primary AS [11,12]. Other recurrent reported alterations are TP53, PI3KCA, POT1, RAS, BRAF, PTPRB, PLCG1 and APC [8,13,14]. Most recently, results of Whole Exome Sequencing (WES) of 47 samples from 36 patients self-registered to the Angiosarcoma Project were published. In this cohort, the authors reported that mutations in TP53 and KDR are mutually exclusive, with 89% of KDR mutations found in primary breast AS compared to 82% of TP53 present in non-primary breast AS (p=0.02). Additionally, nine out of ten PI3KCA alterations were seen in primary breast AS (P=0.0003) [13]. These findings indicate a deeper exploration into the biology of AS according to etiology and primary site is needed.

There is growing evidence that immunotherapy is efficacious in the treatment of AS. A phase II study on the use of immunotherapy for advanced STS (Alliance A091401) showed that one AS patient had an objective response [15]. Subsequently, a retrospective analysis of seven AS cases treated with immunotherapy revealed a response rate of 71% (5/7) at 12 weeks, including one case of complete response [16]. The Angiosarcoma Project showed that median TMB is significantly higher in patients with Head and Neck AS (HN-AS) ($p=1.10x10^{-5}$). In this cohort, 3 out of 10 patients with HN-AS received immunotherapy (IO), and two of them achieved exceptional responses (both had a very high TMB; 78 and 138 mutations/MB). In contrast, none of the three patients with AS other than HN treated with immunotherapy responded to the therapy[13]. As these responses are not homogeneous for a given histology, additional efforts to determine other potential immunotherapy response markers are in progress.

Here, we analyzed genomic data of Next Generation Sequencing (NGS) and Whole Transcriptome Sequencing (WTS) from 143 cases. To our knowledge, this is the largest cohort of AS cases with genomic data. We described a distinct AS biology according to primary site and show potential biomarkers to guide therapeutic studies.

2. Materials and Methods

We retrospectively analyzed the data of 143 AS tumors profiled by Caris Life Sciences from 2015-2019. The histologies of 'Angiosarcoma', 'Angiomyosarcoma', or 'Lymphangiosarcoma' were included. Clinical characteristics including age, sex, site of origin, site of biopsy, status of metastatic vs. primary were tabulated. No data of prior exposure to radiation therapy was available. NGS enriched for 592 cancer-related whole-gene targets was performed on each tumor. We included pathogenic mutations and copy number amplification in the analysis. WTS was performed on 53 tumors and used for microenvironment cell population (MCP)-counter analysis as described by Becht et al [17]. Hierarchical

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 July 2021 doi:10.20944/preprints202107.0068.v1

clustering of MCP-counter Z-scores was used to identify subgroups based on tumor microenvironment profiles.

Biomarkers classically associated with response to IO (TMB-High [≥10/Mb], MSI-High, and PD-L1 [IHC ≥ 2+ and 5%]) were included. A sarcoma pathologist at Sylvester Comprehensive Cancer Center reviewed hematoxylin & eosin (H&E) slides to confirm the diagnosis. Additionally, we annotated data of cell morphology, biopsy anatomical site, grade, necrosis, lumen formation, and intra and peritumoral inflammatory infiltrate. The inflammatory infiltrate was graded as follows: 0 – no inflammatory cells observed, 1 – corresponding to <5% of the cellularity, 2 – corresponding to 5-30% of the cellularity and 3 for >30% of the cellularity.

Cytologic, molecular and genomic results were evaluated according to the primary tumor site. Statistical analyses were performed using Chi-square or Fisher's exact tests where appropriate. Wilcoxon Method was used for comparison between groups and p-values were adjusted for multiple hypothesis testing by the Benjamini & Hochberg procedure.

3. Results

The median age of the cohort was 67 (range 22-89), 61% were female, and 29% were classified as metastatic/recurrent. The number of cases by location were head and neck (n=44, 31%), breast (n=31, 22%), extremity (n=16, 11%), viscera (n=28, 20%), skin at other locations (n=11, 8%), and unknow (n=13, 9%). Table 1 shows the H&E histologic characteristics of the cases. Figure 1 shows the spectrum of density of inflammation within cases of AS.

Table 1. Primary site distribution and histologic characteristics of cases

Angiosarcoma subgroup	All	Head&Neck	Breast	Visceral	Extremity	Cutaneous	Unknown	P-value
Sample size, N (%)	143 (100%)	44 (30.8%)	31 (21.7%)	28 (19.6%)	16 (11.2%)	11 (7.7%)	13 (9.1%)	
Morphology								
Epithelioid	46 (32.9%)	19 (43.2%)	8 (26.7%)	9 (32.1%)	3 (18.8%)	3 (30.0%)	4 (36.4%)	
Spindle	9 (6.4%)	0 (0.0%)	3 (10.0%)	4 (14.3%)	0 (0.0%)	1 (10.0%)	1 (9.1%)	0.16
Mixed	85 (60.7%)	25 (56.8%)	19 (63.3%)	15 (53.6%)	13 (81.3%)	6 (60.0%)	6 (54.5%)	
Grade								
1	2 (1.4%)	0 (0.0%)	1 (3.3%)	1 (3.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
2	78 (55.7%)	21 (47.7%)	21 (70.0%)	15 (53.6%)	8 (50.0%)	5 (50.0%)	8 (72.7%)	0.52
3	60 (42.9%)	23 (52.3%)	8 (26.7%)	12 (42.9%)	8 (50.0%)	5 (50.0%)	3 (27.3%)	
Vessel formation								
Yes	117 (83.6%)	35 (79.5%)	28 (93.3%)	23 (82.1%)	12 (75.0%)	9 (90.0%)	9 (81.8%)	0.43
No	23 (16.4%)	9 (20.5%)	2 (6.7%)	5 (17.9%)	4 (25.0%)	1 (10.0%)	2 (18.2%)	
Inflammatory infiltrate								
0	8 (5.7%)	1 (2.3%)	2 (6.7%)	3 (10.7%)	2 (12.5%)	0 (0.0%)	0 (0.0%)	
1	105 (75.0%)	31 (70.5%)	28 (93.3%)	19 (67.9%)	11 (68.8%)	8 (80.0%)	7 (63.6%)	0.11
2	25 (17.9%)	10 (22.7%)	0 (0.0%)	6 (21.4%)	3 (18.8%)	2 (20.0%)	4 (36.4%)	
3	2 (1.4%)	2 (4.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Location of infiltrate								
Periphery	8 (6.1%)	2 (4.7%)	1 (3.6%)	3 (12.0%)	1 (7.1%)	0 (0.0%)	1 (9.1%)	
Intratumoral	31 (23.5%)	11 (25.6%)	4 (14.3%)	6 (24.0%)	4 (28.6%)	3 (30.0%)	3 (27.3%)	0.73
Both	92 (69.7%)	30 (69.8%)	23 (82.1%)	16 (64.0%)	9 (64.3%)	7 (70.0%)	7 (63.6%)	
Neutrophils present								
Yes	30 (22.7%)	11 (25.6%)	3 (10.7%)	8 (32.0%)	5 (35.7%)	1 (10.0%)	2 (18.2%)	0.29
No	102 (77.3%)	32 (74.4%)	25 (89.3%)	17 (68.0%)	9 (64.3%)	9 (90.0%)	9 (81.8%)	

Note: 4 samples (1 breast, 1 cutaneous, and 2 unknown) did not have H&E slides available for review.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 July 2021 doi:10.20944/preprints202107.0068.v1

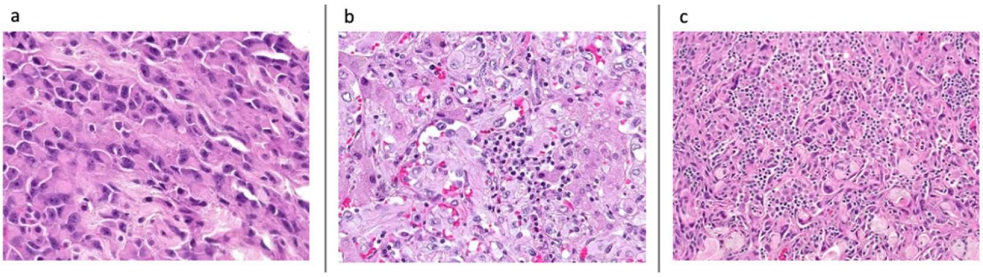


Figure 1. Illustration of the spectrum of density of inflammation within angiosarcomas. a) grade 1 - <5% of cells are inflammatory cells. b) grade 2 - <30% of cells are inflammatory cells. c) grade 3 - >30% of cells are inflammatory cells..

3.1 Markers of Immunotherapy Response

Predictive IO-response biomarkers were the most common marker in the entire cohort, present in 36.4% of cases (TMB-High in 26%, PD-L1+ 21.8%, MSI-High 0.7%). Predictive IO-response biomarkers were the highest in the H-N AS subgroup, with TMB-High observed in 63.4% H-N AS cases (n=26/41; p<0.0001), a significant increase compared to other sites. Fourteen cases of H-N AS (33%) were positive for PD-L1 by IHC, 11 of which were concurrently TMB-High. Only 1 case of H-N AS had dMMR/MSI-high status. Of note, breast AS had the lowest frequency of IO-response biomarkers. Figure 2. Shows IO-response biomarkers.

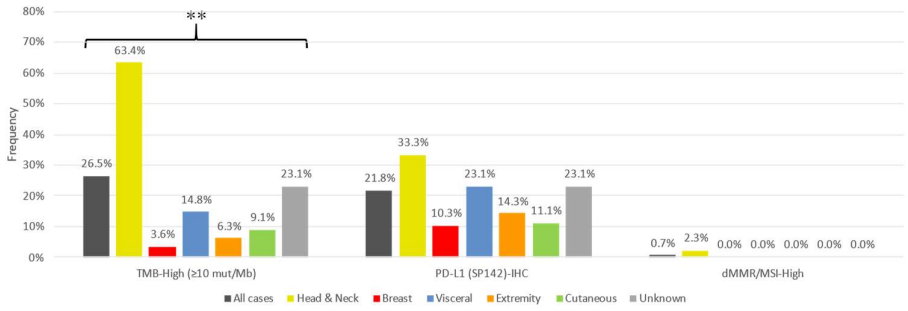


Figure 2. Immunotherapy response biomarkers vary according to primary site. ** Head and neck angiosarcoma cases have a significantly higher predominance of TMB-High with p<0.0001. PDL-1 positivity is present among the different sites.

3.2 Genetic alterations

The most common genetic alterations were TP53 (29%), MYC amp (23%), ARID1A (17%), POT1 (16%), and ATRX (13%). Genetic alterations were distinct according to primary site. In H-N AS, TP53 mutations are present in 48.8% (n=21/43; p=0.0002), POT1 in 41.9% (n=18/43; p<0.0001), and ARID1A in 31.3% (n=5/16; p=0.7331). On the other hand, cell cycle pathway aberrations were common in breast AS, with MYC amplification present in 63.3% (n=19/30; p<0.0001). Mutations in HRAS were present in 16.1% (n=5/31; p=0.0155) and PI3KCA in 16.1% (n=5/31; p=0.1489). At other cutaneous sites and extremity cases, some distinct alterations appear more common; however, conclusions are difficult to obtain from the small number of cases at these sites. Figure 3 shows the genetic alterations in AS.

3.3 Microenvironment Phenotype

Using the MCP-counter method to study the sarcoma microenvironment, we defined four distinct immune classes based on microenvironment cell population abundance. Hierarchical clustering identified subgroups with distinct microenvironment profiles that were consistent with those described by Petitprez et al[18]. Fifty-three cases

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 July 2021 doi:10.20944/preprints202107.0068.v1

with available WTS data were distributed as follows: Immune-High – B lineage high (13.2%), Vascularized – Endothelial cells high (24.5%), Immune-Desert (41.5%) and Heterogeneous – Moderate abundance (20.8%). Immune class signatures were evenly distributed among different primary sites. Interestingly, the Immune-High group had the lowest median TMB = 6 muts/MB (range 3-17). (Figure 4. Microenvironment phenotype in AS).

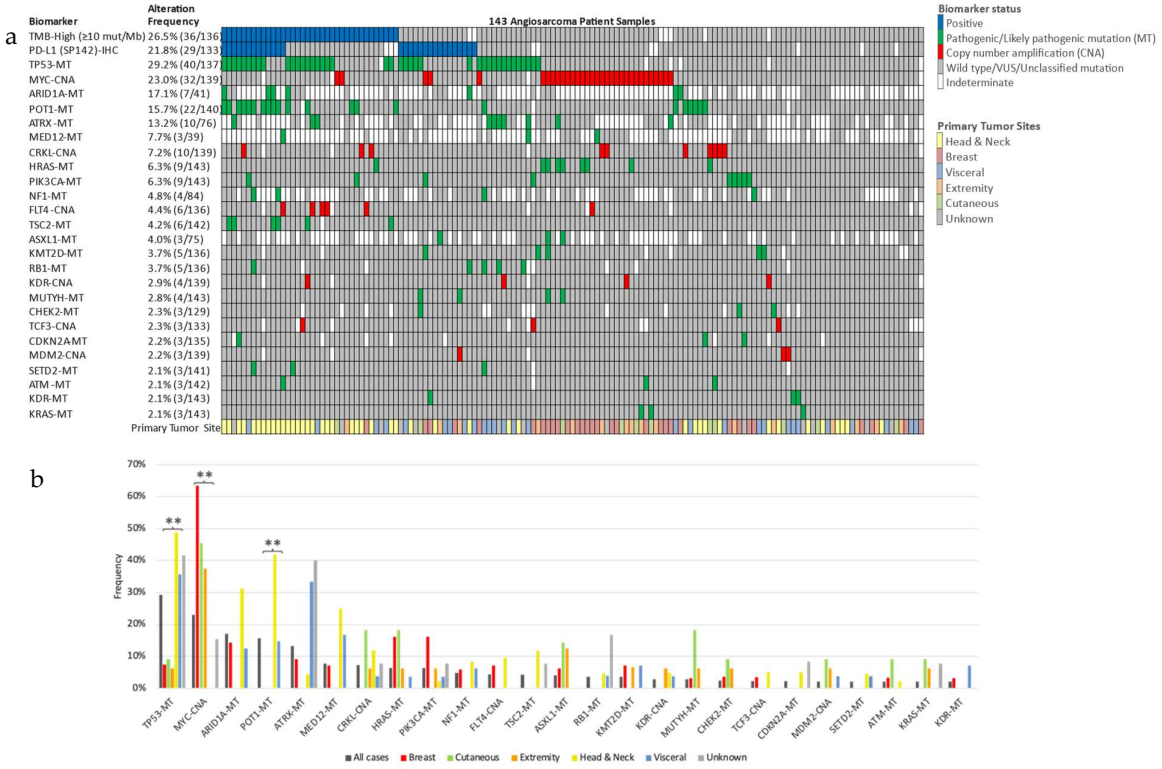
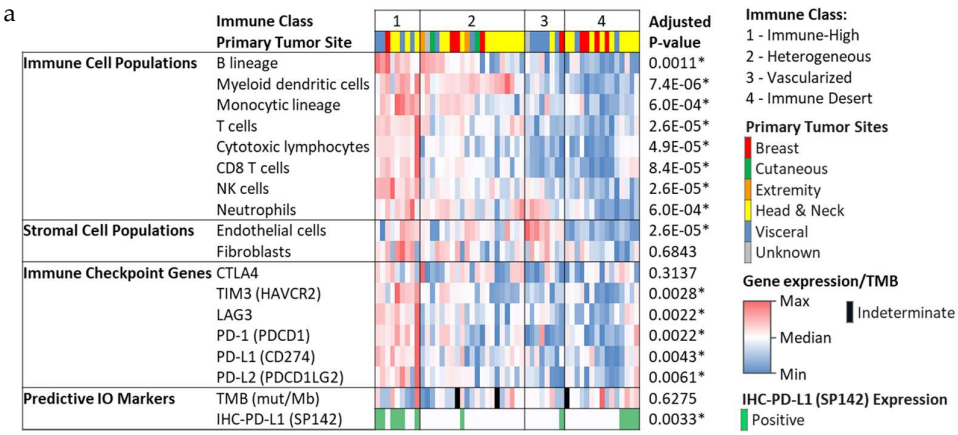


Figure 3. Genomic landscape of angiosarcoma shows distinct pattern according to primary site. a) Oncoprint for the entire cohort of 143 cases. b) Alterations by primary site.**p<0.0001

Next, we compared the microenvironment of the AS cohort with a cohort of melanoma (n=1255). Interestingly, we saw that the microenvironment of angiosarcoma has an overall similar immune profile to that of melanoma, but with enrichment of specific cell populations, including endothelial and myeloid dendritic cells (Figure. 4b). While the median abundance of CD8+ T cell and B cell populations was lower in AS compared to melanoma, the difference was not statistically significant.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 July 2021 doi:10.20944/preprints202107.0068.v1

b

Min Max

Cell population	Median Abundance AS	Median Abundance MM	Fold-change AS/MM	P-value
Endothelial cells			9.63665625	0.0000
Myeloid dendritic cells			3.42470247	0.0000
Fibroblasts			1.57788321	0.0001
T cells			1.55824842	0.0002
Neutrophils			1.1367466	0.0420
NK cells			1.04409958	0.3530
Monocytic lineage			1.02002445	0.0658
CD8 T cells			0.7656233	0.2081
Cytotoxic lymphocytes			0.61703868	0.0011
B lineage			0.55998515	0.6904

Figure 4. a) Microenvironment phenotype in angiosarcoma. b) Comparison of tumor microenvironment between angiosarcoma and melanoma.

Finally, when analyzing the microenvironment according to the tabulated histologic characteristics observed by H&E, we observed that the cases with an inflammatory infiltrate of grade 2 or 3 had a higher number of T cell, CD8+ cells, Cytotoxic T cells, NK, and B cells by MCP counter method. Importantly, B-cell abundance in cases with grade 2 or 3 infiltrate was significantly higher than in cases of grade 0 or 1 (p=0.034). Expression of Immune related markers TIM3, LAG3, PD-1 (PDCD1), PD-L1 (CD274), PD-L2 (PDCD1LG2) was also more abundant in the cases with grade 2 or 3 inflammatory infiltrate. For PDL-1 this was statistically significant (p=0.038). Figure 5. Microenvironment findings according to histological features on H&E.

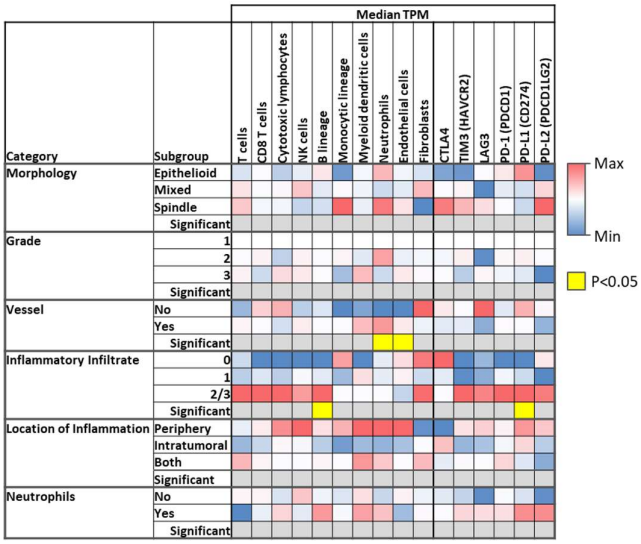


Fig. 5 The presence of a grade 2 or 3 inflammatory infiltrate observed on H&E microscopy correlates significantly with higher B cell abundance and PDL-1 expression

4. Discussion

To our knowledge, this is the largest cohort of AS genomic biology described to date. Our findings confirm previous studies that show that AS has a distinct biology depending on their primary site and etiology. Here, we confirm that classical IO-response markers are common in AS, being present in about one third of the cases. Over 60% of cases of H-N AS have markers of IO-response. This corroborates with the retrospective series by Florou et al., where most responders were cases of H-N AS (4/5 cases). Similarly, the 2 cases of outstanding responses noted in the Angiosarcoma Project were cases of H-N AS.

 doi:10.20944/preprints202107.0068.v1

Interestingly, in the cohort described by Florou et al., the case that achieved a long-lasting complete response had low TMB with only 0.9 muts/MB. This should call the attention to the fact that IO-response markers are not the sole determinant for IO response.

Here we also describe that a microenvironment with a high immune signature and abundance of B-cells is present in about 13% of the cases and is evenly distributed among different primary sites. A signature of B-cell lineage abundance regardless of high or low CD8+ T cell infiltration was found to be a predictor of response to PD1 blockage and PFS in soft-tissue-sarcomas[18]. Additionally, in other solid tumors, distinct microenvironment characteristics have shown predictor capabilities to IO and other targeted therapies[19,20]. Importantly, we reviewed the H&E slides of 138 of the patients (4 cases had no available H&E slide) and described the tumor inflammatory infiltrate. We saw that B-cell abundance by WTS and PDL-1 expression was associated with the presence of an inflammatory infiltrate of grade 2 or 3 as assessed by light microscopy. This strategy could be optimized by incorporating immunohistochemistry to compare the predictive phenotypes determined by the MCP-counter method.

Certain genetic alterations of AS are more common at specific primary sites. Consequently, further studies to overcome IO resistance and increase effectiveness of targeted therapies accounting for specific alterations are warranted. Therapeutic strategies that target TP53, POT1 and ARID1A could be of value in H-N AS. In cases with a TP53 mutation, we found that 67% had concomitant markers of IO response. We should investigate strategies using agents that target TP53 truncating mutations, especially for cases of H-N AS that are resistant to IO. ARID1A is one of the most common alterations encountered in out cohort. Being part of the chromatin remodeling complex SWI/SNF, its deficiency results in EZH2 overactivity. In epithelioid sarcoma, we have observed success with the use of EZH2 inhibitor tazemetostat. Prospective evaluation of tazemetostat in ARID1A mutated AS should be considered[21]. Over 60% of cases of B-AS show MYC amplification. In our cohort, we do not have data about the association of the cases with prior radiation therapy. However, from prior reports, there is strong evidence that MYC amplification is almost exclusively seen in cases of radiation associated AS[11,12]. Other common mutations found in B-AS are PI3K and HRAS, and appear to be mostly found in cases of primary B-AS. These differences should be accounted for when devising differential strategies to treat radiation associated B-AS and primary B-AS.

Finally, we cknowledge that an important constraint of our study is the limited clinical data which was obtained from the requisition forms submitted by the ordering physician. In the study of rare tumors like AS, this type of genomic analysis should be performed in retrospective cohorts with available clinical data of responses to the different therapeutic strategies to further understand their therapeutic effects.

5. Conclusions

In this comprehensive analysis of the genomic landscape of AS, we observed a distinct AS biopsy according to primary site. The particularities likely represent a different etiologic phenomenon and biologic behavior. For this reason, further studies of retrospective cohorts to confirm and expand on the therapeutic implications are needed. Additionally, these findings should be accounted for when designing prospective trials for AS. Finally, incorporating similar genomic testing in the correlative studies of prospective trials can help us build effective predictive tools to combat this deadly aggressive disease.

Author Contributions: Conceptualization, Andrea Espejo-Freire and Jonathan Trent; Data curation, Andrew Elliot, Andrew Rosenberg and W. Michael Korn; Formal analysis, Andrew Elliot, W. Michael Korn, Matthew J. Oberley and Daniel Magee; Investigation, Andrea Espejo-Freire, Andrew Elliot and Andrew Rosenberg; Project administration, Andrea Espejo-Freire and Jonathan Trent; Supervision, Jonathan Trent; Writing – original draft, Andrea Espejo-Freire; Writing – review & editing, Andrew Elliot, Andrew Rosenberg, Philippos Apolinario Costa, Priscila Barreto Coelho, Emily

Jonczak, Gina D'Amato, Ty Subhawong, Junaid Arshad, Julio A. Diaz-Perez, W. Michael Korn, Matthew J. Oberley, Daniel Magee, Don Dizon, Margaret von Mehren, Moh'd M. Khushman, Atif Mahmoud Hussein, Kirsten Leu and Jonathan Trent.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted in accordance with guidelines of the Declaration of Helsinki, Belmont report, and U.S. Common rule. In keeping with 45 CFR 46.101(b) (4), this study was performed utilizing retrospective, deidentified clinical data. Therefore, this study is considered IRB exempt and no patient consent was necessary from the subjects.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: Andrew Elliott – Caris employee; W. Michael Korn – Caris employee, consultant of Merck; Matthew Oberley – Caris employee with equity; Daniel Magee – Caris employee with stock. The remaining authors declare no conflict of interest with the data presented herein.

References

[1] F. Ducimetière *et al.*, "Incidence of sarcoma histotypes and molecular subtypes in a prospective epidemiological study with central pathology review and molecular testing," *PLoS One*, vol. 6, no. 8, 2011.

[2] M. G. Fury, C. R. Antonescu, K. Van Zee, M. E. Brennan, and R. G. Maki, "A 14-year retrospective review of angiosarcoma: clinical characteristics, prognostic factors, and treatment outcomes with surgery and chemotherapy," *Cancer J.*, vol. 11, no. 3, pp. 241–247, 2005.

[3] J. K. Mito *et al.*, "A Comparison of Outcomes and Prognostic Features for Radiation-Associated Angiosarcoma of the Breast and Other Radiation-Associated Sarcomas," *Int. J. Radiat. Oncol. Biol. Phys.*, vol. 104, no. 2, pp. 425–435, 2019.

[4] M. Agulnik *et al.*, "An open-label, multicenter, phase II study of bevacizumab for the treatment of angiosarcoma and epithelioid hemangioendotheliomas," *Ann. Oncol.*, vol. 24, no. 1, pp. 257–263, 2013.

[5] A. Kollár *et al.*, "Pazopanib in advanced vascular sarcomas: an EORTC Soft Tissue and Bone Sarcoma Group (STBSG) retrospective analysis," *Acta Oncol. (Madr).*, vol. 56, no. 1, pp. 88–92, 2017.

[6] R. G. Maki *et al.*, "Phase II study of sorafenib in patients with metastatic or recurrent sarcomas," *J. Clin. Oncol.*, vol. 27, no. 19, pp. 3133–3140, 2009.

[7] I. L. Ray-Coquard *et al.*, "Paclitaxel given once per week with or without bevacizumab in patients with advanced angiosarcoma: A randomized phase II trial," *J. Clin. Oncol.*, vol. 33, no. 25, pp. 2797–2802, 2015.

[8] V. Florou, "Current and Future Directions for Angiosarcoma Therapy," *Curr. Treat. Options Oncol.*, 2018.

[9] R. J. Young, N. J. Brown, M. W. Reed, D. Hughes, and P. J. Woll, "Review Angiosarcoma," vol. 11, no. October, 2010.

[10] C. R. Antonescu *et al.*, "KDR activating mutations in human angiosarcomas are sensitive to specific kinase inhibitors," *Cancer Res.*, vol. 69, no. 18, pp. 7175–7179, 2009.

[11] J. Manner *et al.*, "MYC high level gene amplification is a distinctive feature of angiosarcomas after irradiation or chronic lymphedema," *Am. J. Pathol.*, vol. 176, no. 1, pp. 34–39, 2010.

[12] T. Guo, L. Zhang, N.-E. Chang, S. S. R. G. Maki, and C. R. Antonescu, "Consistent MYC and FLT4 Gene Amplification in Radiation-Induced Angiosarcoma But Not in Other Radiation-Associated Atypical Vascular Lesions Tianhua," *Cancer*, vol. 396, no. January, pp. 389–396, 2011.

[13] C. A. Painter *et al.*, "The Angiosarcoma Project: enabling genomic and clinical discoveries in a rare cancer through patient-partnered research," *Nat. Med.*, vol. 26, no. 2, pp. 181–187, 2020.

[14] S. Behjati *et al.*, "Recurrent PTPRB and PLCG1 mutations in angiosarcoma," *Nat. Genet.*, vol. 46, no. 4, pp. 376–379, 2014.

[15] S. P. D'Angelo *et al.*, "Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): two open-label, non-comparative, randomised, phase 2 trials," *Lancet Oncol.*, vol. 19, no. 3, pp. 416–426, 2018.

[16] V. Florou *et al.*, "Angiosarcoma patients treated with immune checkpoint inhibitors : a case series of seven patients from a single institution," vol. 7, pp. 1–8, 2019.

 doi:10.20944/preprints202107.0068.v1

[17] E. Becht *et al.*, "Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression," *Genome Biol.*, vol. 17, no. 1, pp. 1–20, 2016.

[18] F. Petitprez *et al.*, "B cells are associated with survival and immunotherapy response in sarcoma," *Nature*, vol. 577, no. 7791, pp. 556–560, 2020.

[19] N. Riaz *et al.*, "Tumor and Microenvironment Evolution during Immunotherapy with Nivolumab," *Cell*, vol. 171, no. 4, pp. 934-949.e15, 2017.

[20] D. F. McDermott *et al.*, "Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma," *Nat. Med.*, vol. 24, no. 6, pp. 749–757, 2018.

[21] J. K. Alldredge and R. N. Eskander, "EZH2 inhibition in ARID1A mutated clear cell and endometrioid ovarian and endometrioid endometrial cancers," *Gynecol. Oncol. Res. Pract.*, vol. 4, no. 1, pp. 1–9, 2017.