



# Genomics, Morphoproteomics, and Treatment Patterns of Patients with Alveolar Soft Part Sarcoma and Response to Multiple Experimental Therapies

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## ABSTRACT

Overexpression of transcription factor 3 in alveolar soft part sarcoma (ASPS) results in upregulation of cell proliferation pathways. No standard treatment algorithm exists for ASPS; multikinase inhibitors [tyrosine kinase inhibitor (TKI)] and immune checkpoint inhibitors (ICI) have shown clinical benefit. To date, no studies have reported on management strategies or sequencing of therapy. We evaluated ASPS treatment patterns and responses in an experimental therapeutics clinic. Genomic and morphoproteomic analysis was performed to further elucidate novel targets. We retrospectively reviewed patients with ASPS treated on clinical trials. Demographic and clinical next-generation sequencing (NGS) profiles were collected. AACR GENIE database was queried to further evaluate aberrations in ASPS. Morphoproteomic analysis was carried out to better define the biology of ASPS with integration of genomic and proteomic findings. Eleven patients with ASPS were identified; 7

received NGS testing and mutations in CDKN2A ( $n = 1$ ) and hepatocyte growth factor ( $n = 1$ ) were present. Ten patients were treated with TKIs with stable disease as best response and 4 patients with ICI (three partial responses). Within GENIE, 20 patients were identified harboring 3 called pathogenic mutations. Tumor mutation burden was low in all samples. Morphoproteomic analysis confirmed the expression of phosphorylated c-Met. In addition, fatty acid synthase and phosphorylated-STAT3 were detected in tumor cell cytoplasm and nuclei. Patients with ASPS have a quiescent genome and derive clinical benefit from VEGF-targeting TKIs. Morphoproteomic analysis has provided both additional correlative pathways and angiogenic mechanisms that are targetable for patients with ASPS. Our study suggests that sequential therapy with TKIs and immune checkpoint inhibitors is a reasonable management strategy.

## Introduction

Alveolar soft part sarcoma (ASPS) is an exceptionally rare tumor of uncertain mesenchymal origin accounting for only 1% of all soft-tissue sarcomas (STS). Contrary to the moniker “alveolar,” the tissue of origin is unknown. Instead, the histopathologic appearance of central necrosis and partitions of connective tissue give the appearance of alveoli (1, 2). ASPS is driven by the fusion of der(17)t(X;17) (p11;q25), which creates the characteristic ASPSCR1-TFE3 fusion, resulting in

significant nuclear overexpression of transcription factor 3 (3). The ASPSCR1-TFE3 fusion causes upregulation of transcripts involved in angiogenesis and proliferation among other pathways (Fig. 1; ref. 4). For patients with metastatic disease, the median survival is over 3 years with median progression-free survival between 4 and 12 months. The most frequent sites of metastasis are lung, bone, and brain (5–7). This indolent disease course is paramount in understanding and interpreting any trial results. Chemotherapy does not have activity in this disease (8), and for decades the standard-of-care was surgical resection including metastatectomy (2). The above mentioned upregulation of angiogenesis transcripts provided evidence for using tyrosine kinase inhibitors (TKI) directed at VEGF. Clinical trials using TKIs, such as, sunitinib (9), cediranib (10), pazopanib (11, 12), and crizotinib (11), have demonstrated activity and have shown clinical responses. Importantly, cediranib performed favorably against placebo showing a 21% response rate versus 0% in placebo with a corresponding progression-free survival of 10.8 months compared with 3.7 months with placebo (13). Combinations of TKIs have been proposed and evaluated in STS (14), but have never been specifically investigated in ASPS. Beyond TKIs, immune checkpoint inhibitors have also shown significant activity in ASPS including near complete responses. This data largely hinges on case reports and subanalysis of larger trials including the phase II trial of axitinib with pembrolizumab for STSs (15–18). This trial was open to all patients with STS, but over a third of all patients enrolled had ASPS. With two exceptions, only patients with ASPS had documented partial responses. In patients with ASPS, the response rate was 54.5% documenting a clear sensitivity of ASPS to checkpoint blockade. To date, no studies have reported on management patterns or sequencing of therapy. No standard-of-care treatment algorithm is available for the treatment of ASPS and these patients are regularly

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**Note:** Supplementary data for this article are available at Molecular Cancer Therapeutics Online (<http://mct.aacrjournals.org/>).

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referred to our phase I clinic. It is with this key factor in mind that we decided to evaluate treatment patterns and responses to therapy in a precision medicine and experimental therapeutics clinic.

We conducted a retrospective review of patients with ASPS treated on clinical trials at the MD Anderson Clinical Department of Investigational Cancer Therapeutics (Houston, TX; phase I Clinical Trials Program) between 2009 and 2017. Demographic information was collected, including diagnosis, age, sex, date of first dose on trial, date of progression, best response by RECIST criteria, and if applicable, date of death and clinical next-generation sequencing (NGS) profiles per-

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on tyrosine 705 (Santa Cruz Biotechnology) were the probes utilized for IHC staining.

### Ethics approval and consent to participate

This retrospective review was approved by The University of Texas MD Anderson Cancer Center Institutional Review Board. All patients on clinical trials signed informed consent according to institutional standards.

### Consent for publication

All authors have consented for the manuscript to be published. Consent to utilize patient information in Fig. 2 was obtained.

### Availability of data and material

Data sharing is not available for the patient information to ensure confidentiality.

AACR GENIE Data available at: <http://genie.cbiportal.org/>

## Results

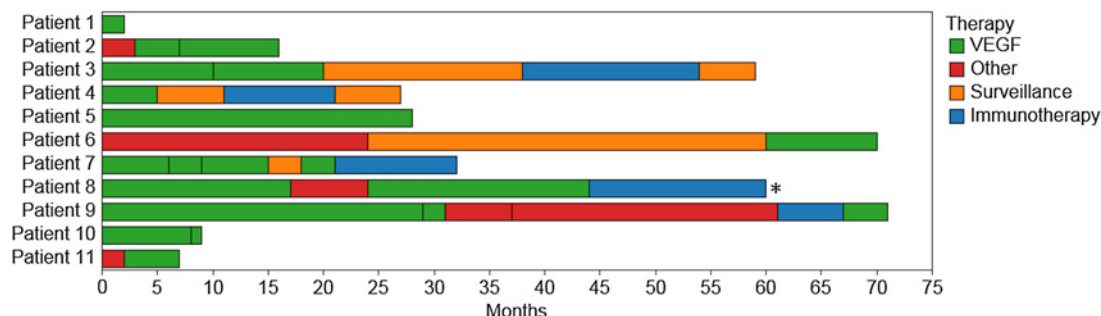
We identified 11 patients with ASPS in our dataset. The median age was 21 years (range, 14–37 years) with a female predominance (F:M, 8:3). Five patients (45%) died, with overall survival durations of 10, 12, 15, 58, and 70 months. Three patients (27%) were lost to follow-up, with times of 3, 6, and 19 years. The 3 patients who were still being followed had been diagnosed 10, 11, and 12 years previously. Among all patients, the most common primary tumor site was the lower extremities ( $n = 7$ ; 64%). The metastatic sites were almost exclusively the brain ( $n = 5$ ; 45%) and lungs ( $n = 11$ ; 100%), although one patient had liver metastasis. Eight patients presented with primary metastatic disease, and 3 developed metastases after initial therapy for curative intent. Among the 7 patients who underwent NGS testing, only two mutations were detected: *CDKN2A* mutation ( $n = 1$ ) and hepatocyte growth factor (*HGF*) amplification ( $n = 1$ ). Ten (91%) patients were enrolled on at least one trial that included TKI-based therapy with either pazopanib ( $N = 3$ ), vandetanib ( $n = 4$ ), or bevacizumab ( $n = 3$ ). The best response achieved was stable disease in 9 patients on a VEGF inhibitor for a median of 12-month duration (Fig. 2). The patient with HGF amplification achieved stable disease for 28 months with multi-kinase TKI (pazopanib) and Histone deacetylase inhibitor (vorinostat) combination. Another patient achieved stable disease for 24 months on a combination of metformin and temsirolimus. Four patients (40%) were treated with checkpoint immunotherapy targeting the PD-1/PD-L1 axis. Three patients experienced a partial response, and 1 had stable disease as the best response, resulting in an overall response

rate to immunotherapy of 75% (Fig. 3). One patient treated with PD-1/PD-L1-directed therapy had a sustained response for 8 months after stopping therapy. All therapies were given sequentially, and no patients received combination TKI and immunotherapy. Within the GENIE database, another 20 patients were identified (Table 1). There were 40 unique cancer-associated genes mutated among the 20 patients. No pathway stood out as recurrently mutated or activated, but 16 aberrant genes are potentially actionable. The majority of patients had one mutation or less (11 patients, 55%). Patients had increasing number of mutations with age. More than half (4/7, 57% patients) under the age of 25 had no potentially actionable mutation. Importantly, almost all mutations are variants of unknown significance with the exception of *RB*, *PTEN*, and *PPP2R1A*. The mutational burden was low with all patients having less than 20% of the genome altered. Copy-number alterations were rare and involved amplification of oncogenes and deletion of tumor suppressors. Morphoproteomic analysis confirmed the strong cytoplasmic expression (1–3+ on a scale of 0–3+) of both p-c-Met (Tyr1234/1235) and FAS and of p-STAT3 (Tyr705) in the nuclei (up to 3+) in the tumor cells of ASPS (Fig. 4).

## Discussion

Despite the small number of patients in our retrospective study, this represents a unique series of patients with ASPS who were enrolled on multiple clinical trials. We observed significant activity across clinical trials and a variety of agents. Previous studies have described partial responses with VEGF multikinase targeting TKIs (Cediranib, sunitinib, pazopanib), but we did not observe any such responses. This is likely because of the small number of patients in this study and the variation in actual agents used. Instead, patients did derive substantial clinical benefit from VEGF-targeting therapy in the form of prolonged stable disease. One important concept emerged from the recent publication by Wilky and colleagues (18): the long duration time to response, over 25 weeks before seeing a partial response. This knowledge was not available to the treating physicians involved in the care of these patients and may perhaps explain why some patients were transitioned from their therapy relatively early. Had patients stayed on therapy, better responses may have been seen.

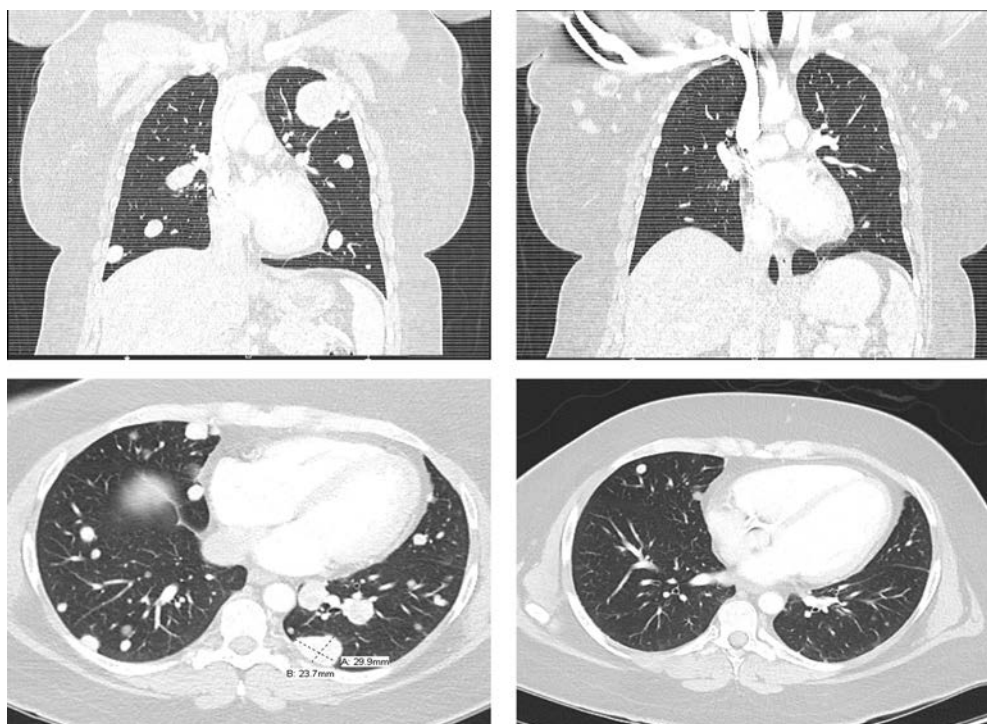
While the concept of stability as an endpoint in an indolent disease is debatable, we know from prior studies that overall survival in untreated patients with metastatic disease is between 3 and 4 years (5, 6). In our study, 8 patients had overall survival duration far longer than 3 years. This is especially impressive as the phase I clinic is naturally a



**Figure 2.**

Types and durations of therapies received by patients with ASPS. Most patients received upfront VEGF-targeted multikinase therapy. Immunotherapy was introduced late in the course of disease, likely related to entry of these agents into the clinic. Some patients had long intervals of stability off therapy representing the natural history of this disease.



**Figure 3.**

Top, patient with ASPS who achieved partial response after treatment with PD-1 axis-targeting therapy. Bottom, another patient with ASPS who achieved partial response after treatment with PD-1 axis-targeting therapy.

referral of last resort for most patients and represents a heavily pre-treated cohort. In addition, one placebo-controlled study of cediranib in ASPS showed that a progression-free survival advantage (10.8 months for cediranib vs. 3.7 months for placebo) resulted in an overall survival advantage at 12 months (96% for cediranib vs. 64% for placebo) (13). This suggests that even in an indolent disease such as ASPS, prolonged stable disease is beneficial to patient survival. In our experience, treatment with VEGF-targeted therapy is worthwhile even if disease stability is the overall goal. Our study was not powered to detect differences between therapeutic regimens because of small numbers of patients and we cannot recommend one particular agent over another. However, based on inpatient observation, we do feel that combinations of drugs performed better than single agents, with acceptable toxicity. Future studies combining a VEGF-directed TKI with another targeted agent in ASPS are of interest. In that regard, morphoproteomic analysis provides the following correlative insights into the biology of ASPS: (i) ASPL-TFE 3 fusion protein in ASPS binds to the MET promoter and induces the expression of c-Met tyrosine kinase and the finding of phosphorylated c-Met indicates that it has been activated by HGF, its ligand (24); (ii) FAS expression represents a correlate to c-Met pathway signaling because inhibition of FAS posttranscriptionally downregulates c-Met expression (25); and (iii) the constitutive activation of the signal transducer and activation of transcription (STAT3) pathway in the tumor cells in the form of phosphorylation of STAT3 on tyrosine 705 with translocation to the nucleus represents a potential autocrine mechanism in providing HGF, the ligand for c-Met given the role of c-src-STAT3 pathway activation as a transcriptional activator of HGF (26, 27) and further correlates with the previous demonstration of activation of platelet-derived growth factor receptor signaling in ASPS (9, 28), which in turn is consistent with the activation of

p-STAT3 (Tyr 705) via the src-STAT3 pathway (29). These observations provide therapeutic targets for relatively low toxicity agents in patients with ASPS, such as crizotinib to interfere with the HGF/c-Met signaling pathway and angiogenesis (11), pazopanib to participate in the inhibition of angiogenesis by VEGF (30, 31), and metformin to downregulate FAS and to interfere in c-Met-driven tumorigenesis (32, 33) and prevent the phosphorylation/activation of the STAT3 pathway (34, 35). Interestingly, the role of metformin is complex with different concentrations in the preclinical studies that support its mechanism of action [5–10 mmol/L metformin (32); 0.5–2 mmol/L (33), and 1.25–5 mmol/L metformin (34)]. These are quite nonphysiologic exposures to metformin (1.8 g dose in human gives ~30  $\mu$ mol/L  $C_{max}$ ). Indeed, the predominant preclinical murine models of anticancer activity of metformin are possibly a guesstimate of higher doses of metformin administration in human studies versus sub-millimolar treatment of cells in culture, which is a caveat in interpretation of preclinical murine metformin studies (36).

One of the longest periods of stable disease (28 months) on therapy was observed in a patient with HGF amplification. High HGF expression has previously been reported in ASPS. Importantly, this correlated with c-Met phosphorylation and activation (37). The patient was treated with pazopanib, which is considered a VEGF inhibiting TKI, but has broad activity against other kinases including c-Met (38). This highlights the possibility that TKI activity in ASPS goes beyond simple VEGF inhibition. Other authors have similarly proposed that TKIs with multikinase activity are more efficacious in ASPS (2), and our experience supports this hypothesis. A similar duration of stable disease (22 months) was seen in a patient on a phase II trial of pazopanib in patients with ASPS, but limited data are available regarding the patient's genomics (12).



**Table 1.** Potentially actionable genomic alterations identified in 20 patients with ASPS sequenced through the AACR GENIE project.

Gene	No. of mutation	Amino acid change	Frequency	Predicted pathogenic	Clinically actionable <sup>a</sup>	Pathway
<b>KMT2D</b>	<b>3</b>	M1098I; R3539Q; Q4557P	<b>15.80%</b>	<b>No</b>	<b>Yes</b>	<b>Histone modification</b>
<b>ERCC5</b>	<b>2</b>	Q680R; G1099A	<b>11.80%</b>	<b>No</b>	<b>Yes</b>	<b>DNA repair</b>
<b>PIK3CA</b>	<b>2</b>	V101L; V196I	<b>10.00%</b>	<b>No</b>	<b>Yes</b>	<b>PI3K</b>
<b>TOP1</b>	<b>1</b>	S97F	<b>7.10%</b>	<b>No</b>	<b>Yes</b>	<b>Topoisomerase</b>
<b>IRS2</b>	<b>1</b>	E1009D	<b>7.10%</b>	<b>No</b>	<b>Yes</b>	<b>Insulin receptor</b>
<b>POLE</b>	<b>1</b>	A1629V	<b>6.30%</b>	<b>Yes</b>	<b>Yes</b>	<b>DNA polymerase</b>
<b>NOTCH3</b>	<b>1</b>	E1725D	<b>5.90%</b>	<b>No</b>	<b>Yes</b>	<b>Transcription factor</b>
<b>ARAF</b>	<b>1</b>	P216L	<b>5.30%</b>	<b>No</b>	<b>Yes</b>	<b>MAPK</b>
<b>ATRX</b>	<b>1</b>	G1712V	<b>5.30%</b>	<b>No</b>	<b>Yes</b>	<b>Tumor suppressor</b>
<b>AXL</b>	<b>1</b>	T797I	<b>5.30%</b>	<b>No</b>	<b>Yes</b>	<b>Jak/Stat</b>
<b>CCND2</b>	<b>1</b>	R165C	<b>5.30%</b>	<b>No</b>	<b>Yes</b>	<b>Cell-cycle</b>
<b>NTRK3</b>	<b>1</b>	Q586K	<b>5.30%</b>	<b>No</b>	<b>Yes</b>	<b>Tyrosine kinase</b>
<b>ATM</b>	<b>1</b>	Y1124F	<b>5.00%</b>	<b>No</b>	<b>Yes</b>	<b>Tumor suppressor</b>
<b>KDR</b>	<b>1</b>	L31P	<b>5.00%</b>	<b>No</b>	<b>Yes</b>	<b>VEGF</b>
<b>NOTCH2</b>	<b>1</b>	C364S	<b>5.00%</b>	<b>No</b>	<b>Yes</b>	<b>Transcription factor</b>
<b>PTEN</b>	<b>1</b>	Y68H	<b>5.00%</b>	<b>Yes</b>	<b>Yes</b>	<b>Tumor suppressor</b>
MTAP	1	I78M	33.30%	No	No	
WAS	1	A418S	33.30%	No	No	
ABCB11	1	A1283V	33.30%	No	No	
DCLRE1C	1	E221G	33.30%	No	No	
DIS3L2	1	Splice_Region	33.30%	No	No	
BCORL1	2	I1022T; G1325C	28.60%	No	No	
CUX1	1	M55I	20.00%	No	No	
SETBP1	1	R625Q	20.00%	No	No	
FANCI	1	R451T	20.00%	No	No	
ETV4	1	G174S	14.30%	No	No	
ZNF217	1	E209K	14.30%	No	No	
TP53	2	R196P; N239D	10.00%	No	No	
INPP4B	1	P17R	7.10%	No	No	
FAT1	1	M220L	6.30%	No	No	
RECQL4	1	E9K	6.30%	No	No	
PMS1	1	D397Y	5.90%	No	No	
TMPRSS2	1	Splice_Region	5.90%	No	No	
PPP2R1A	1	S303Pfs*19	5.60%	Yes	No	
DICER1	1	I829S	5.60%	No	No	
ARID1B	2	R1911K; A329_G330insR	5.60%	No	No	
CBL	1	R835W	5.30%	No	No	
SOCS1	1	V45_P46insRS	5.30%	No	No	
ARID2	1	K304R	5.30%	No	No	
RBI	1	V654Cfs*4	5.00%	Yes	No	

Note: Bolded items are cancer associated potentially actionable genes in the clinic.

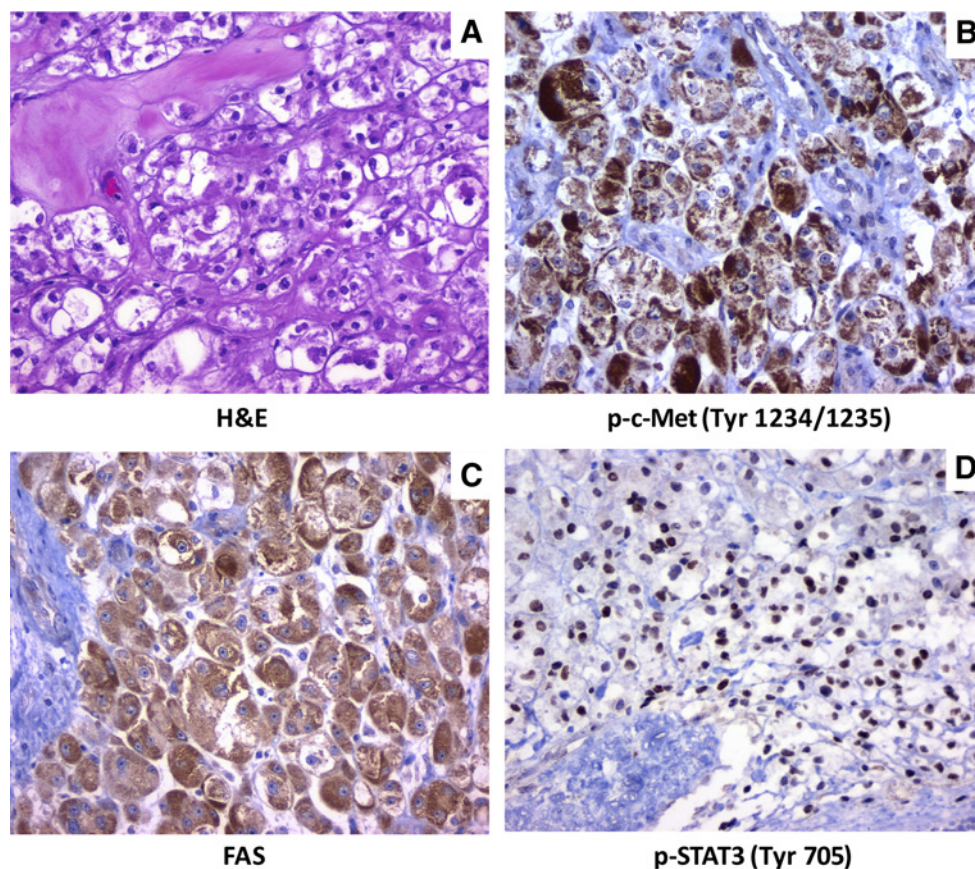
<sup>a</sup>Clinical actionability determined at the time of clinical trial enrollment.

In contrast to VEGF-targeted therapy, immunotherapy shows a clear signal of response in patients with ASPS. In a disease where stability with periods of progression is the norm, true partial responses are unusual and provocative. Such findings have previously been reported either as individual cases (15) or as a sub-analysis of a larger study (16). At the 2018 Connective Tissue Oncology Society meeting, Geraldine O'Sullivan Coyne presented the first stage of the phase II study of atezolizumab in ASPS. Eighteen patients were evaluable with 7 observed partial responses (39%), without unexpected toxicity (39). The use of metformin as suggested by morphoproteomic analysis is relevant in this regard because metformin reduces PD-L1 by endoplasmic reticulum-associated degradation (40).

Clinical-grade DNA hybrid capture–targeted exome sequencing did not identify readily actionable cooccurring mutations in our patients. We investigated this further by analyzing patients with ASPS

sequenced in the AACR GENIE database. We did find 16 potentially actionable aberrant genes. However, the association between increasing age and number of mutations suggests that at least some of these aberrations are passengers accumulated over time, rather than true driver mutations. This is further bolstered by the fact that almost all mutations are variants of unknown significance. This phenomenon of increasing mutations with age has previously been documented (41). In addition, sequencing data support a low tumor mutational burden in this disease. Unfortunately, genomic profiling does not appear to answer why multikinase VEGF directed or immunotherapy works in patients with ASPS. One of the major limitations of this manuscript is lack of comprehensive immunoprofiling. Future studies including genomic, proteomic, and immunoprofiling are underway. This may potentially unravel resistance and response mechanisms to immune checkpoint inhibitors. From our study, it is clear that genomic mutations and copy-number variations do not hold the key to



**Figure 4.**

Morphoproteomic analysis in the patient with ASPS depicts the hematoxylin and eosin (H&E; **A**) and up to 3+ brown-chromogenic expression of p-c-Met (Tyr 1234/1235) and fatty acid synthase (FAS) in the cytoplasmic compartment of the tumor cells (**B** and **C**, respectively) and p-STAT3 (Tyr 705) with variable expression up to 3+ translocated to the tumoral nuclei (**D**) on a scale of 0–3+ (original magnifications  $\times 200$ ).

understanding this disease. Further studies should focus on the oncogenic mechanism of the ASPS-TFE3 gene fusion perhaps utilizing next-generation–based RNA-sequencing and morphoproteomics to unlock the mystery of this nebulous and mercurial disease.

## Conclusion

Patients with ASPS have a quiescent genome and derive clinical benefit from VEGF-targeting multikinase agents. In light of the frequently indolent and variable course of ASPS, stable disease is a difficult endpoint to interpret. In contrast, immune checkpoint blockade yields clear partial responses. No guidelines exist for management of patients with ASPS, except to avoid traditional chemotherapy. Our study suggests that sequential therapy with TKIs disrupting not only VEGF, but other signaling pathways demonstrated by morphoproteomics is a reasonable management strategy. In line with other reports, prolonged treatment may be required to see significant clinical activity. For patients in whom a true response is needed, immunotherapy targeting the PD-1/PD-L1 axis is recommended. Above all, enrollment in clinical trials is paramount.

## Disclosure of Potential Conflicts of Interest

A.P. Conley is a consultant at Bayer, Deciphera, and Genentech. D.S. Hong is a consultant at Alpha Insights, Axiom, Janssen, Merrimack, Medscape, Numab, Pfizer, Seattle Genetics, Takeda, Trieza, Adaptimmune, Baxter, Bayer, Genentech, GLG, Group H, Guidepoint Global, Infinity, reports receiving a commercial research grant from Abbvie, Adaptimmune, Genmab, Ignyta, Infinity, Kite, Kyowa, Lilly, LOXO, Merck, Medimmune, Mirati, Amgen, MiRNA, Molecular Templates, Mologen, NCI-CTEP, Novartis, Pfizer, Seattle Genetics, Takeda, AstraZeneca, Bayer, BMS, Daiichi-Sanko, Eisai, Fate Therapeutics, Genentech,

and reports receiving other commercial research support from LOXO, MiRNA, ASCO, AACR, SITC, Genmab, has ownership interest (including patents) in Molecular Match, OncoResponse, and Presagia. A. Naing reports receiving a commercial research grant from NCI, EMD Serono, Regeneron, Merck, BMS, Pfizer, CytomX Therapeutics, Neon Therapeutics, Calithera Biosciences, TopAlliance Biosciences, Eli Lilly, Kymab, MedImmune, PsiOxus, Immune Deficiency Foundation, Healios Onc. Nutrition, Atterocor, Amplimmune, ARMO BioSciences, Karyopharm Therapeutics, Incyte, Novartis, has received speakers bureau honoraria from CytomX Therapeutics and Novartis, and has provided expert testimony for ARMO BioSciences (travel and accommodation). C.E. Herzog is a member, data monitoring committee at Merck, Sharp, and Dome and reports receiving a commercial research grant from Roche Genentech and Array Biopharm. S. Patel is a consultant at Novartis, Epizyme, Daiichi Sankyo, Dova, Bayer and reports receiving a commercial research grant from Blueprint Medicines. V. Subbiah is a consultant/advisory board member at LOXO Oncology/Eli Lilly, Helsinn, R-Pharma US, Incyte, QED, Novartis, Medimmune and reports receiving a commercial research grant from LOXO Oncology/Eli Lilly, Blueprint Medicines, Fujifilm, Pharmamar, D3, Pfizer, Multi-vir, Amgen, ABBVIE, Agensys, Boston Biomedical, Idera, Turning Point therapeutics, Exelixis, Inhibrx, Altum, Medimmune, Dragonfly Therapeutics, Takeda, Roche/Genentech, Novartis, Bayer, GSK, Nanocarrier, Berghealth, Incyte, and Northwest Biotherapeutics. No potential conflicts of interest were disclosed by the other authors.

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**Other (developed morphoproteomics as a discipline and have contributed to the writing, review and revision of the manuscript):** R.E. Brown

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# Molecular Cancer Therapeutics

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