Case Report

Personalized Immunotherapy Achieves Complete Response in Metastatic Adenoid Cystic Carcinoma Despite Lack of Conventional Biomarkers

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Posted Date: 6 August 2024

doi: 10.20944/preprints202408.0349.v1

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Abstract: There is currently no effective treatment strategy for the recurrent/metastatic adenoid cystic carcinoma (R/M ACC). Furthermore, recent single-agent and combination immunotherapy trials have failed in unselected ACC cohorts unlike non-ACC salivary gland cancers. Genomic profiling revealed no actionable targets but *NOTCH1* and *KDM6A* frameshift and *CTCF* splice site mutations (no *MYB/L* fusion) with a low TMB, MSS and negative PD-L1. We recommended anti-PD-1 plus anti-CTLA-4 combination based on TMB 2-fold greater than median TMB in ACC, tumor harboring multiple immunogenic frameshift or splice site mutations, and PD-L1 negativity. Accordingly, we achieved a complete response in a radiotherapy and chemotherapy-refractory patient with locally recurrent lacrimal gland ACC and lung metastasis following personalized immunotherapy in combination with integrative therapeutics. Therefore, it is crucial to assess not only conventional immune biomarkers but also patient-specific parameters.

Keywords: personalized immunotherapy; precision oncology; immune checkpoint inhibitor (ICI); integrative therapies; adenoid cystic carcinoma (ACC); frameshift mutation; splice site mutation

Introduction

Adenoid cystic carcinoma (ACC) is a rare type of cancer that typically originates from secretory glands, constituting 1.5-2% of all head and neck cancers [1]. The major salivary glands account for the majority of the cases, followed by minor salivary glands[1,2]. Lacrimal glands (LG) are structurally similar to the salivary glands but LG ACCs are much rarer in frequency and much poorer in prognosis [3,4]. Still, ACC at both anatomical regions share many common properties, including high rates of perineural invasion and convergent genomic profiles [4]. ACC is a biphasic tumor composed of myoepithelial and epithelial cellular components, and is divided into 3 histological patterns: cribriform and tubular (mostly low grade 1/2), and solid (generally grade 3), where the loss of myoepithelial cells is often associated with aggressive solid histology [5]. ACC usually exhibits high rates of locoregional recurrence or distant metastasis, warranting long-term surveillance. Initial treatment plan usually includes surgery and/or adjuvant radiotherapy (RT - based on surgical margin), with no effective chemotherapy (CT) regimen existing. Recurrent/metastatic (R/M) ACC patients are usually incurable, and the systemic therapies are palliative in nature. Therefore, molecularly-guided therapies are urgently needed.

Response to immune checkpoint inhibitors (ICIs) has been initially described and approved in unresectable or metastatic tumors (tissue/site agnostic) with TMB higher than 10 muts/Mb[6], and microsatellite instability-high (MSI-H) solid tumors[7]. Although previous studies reported a low TMB in the ACC[5,8], diverse single or dual immunotherapy approaches have been investigated in

 doi:10.20944/preprints202408.0349.v1

these patients. The objective response rates (ORR - with or without radiotherapy), however, have been scarce in unselected ACC cohorts[9]. As of recently, TMB is open to debate due to cancer type-dependent variations. Accordingly, multiple new parameters potentially affecting and/or predicting the benefit from immuno-oncology drugs have emerged, including but not limited to cancer type-dependent neoantigenic repertoire[10], clonality/subclonality of the alterations[11], mutational signatures[12,13], type of alterations[14], and functions of the mutated genes and their involvement in immune processes[15]. The efficacy of ICIs may also be improved when combined with chemotherapy, targeted therapies or agents that modify tumor microenvironment (TME). Combined inhibition of non-redundant PD-1 and CTLA-4 immune checkpoints, especially in tumors with baseline negative PD-L1 status, increases infiltration and expansion of the activated (not exhausted) effector T cells in the tumor periphery and triggers unique cellular responses compared with monotherapy[16,17]. Likewise, high-dose intravenous vitamin C (IVC) is able to facilitate immune cell infiltration in the TME, thereby augmenting the activity of the ICIs in a T cell-dependent manner with high tolerability and minimal toxicity[18–21]. Curcumin could suppress immune-related oncogenic pathways such as nuclear factor kappa B (NF-κB), and function as an adjuvant to boost immune response and immunotherapy efficacy[22]. Consequently, the response rates could be improved by a holistic approach encompassing the consideration of not only TMB, MSI or PD-L1 but also these emerging markers as well as by rational drug combinations.

In short, it is critical to perform in-depth characterization of each tumor to offer new treatment modalities thus improve patient outcomes and/or quality of life. Here we achieved a durable and complete tumor regression in an R/M ACC patient through dual immunotherapy and IVC/bioavailable oral curcumin (BOC) combination despite negative immunotherapy biomarkers. This highlights the importance of considering not only conventional markers but also patient-specific factors. Altogether, personalized immunotherapy could maximize the likelihood of treatment success.

Case Presentation

Diagnosis and Pathology: In 2012, a 39-year-old Caucasian female with no family history of cancer or inherited diseases was admitted to the hospital with swelling in the left eye and was diagnosed with left lacrimal gland ACC (solid variant/type). The patient underwent a lateral orbitotomy with a positive surgical margin. The pathological staging of the tumor was T3N0M0 (stage III – 4x3x1.5 cm). Macroscopically, the solid area was found to continue at the margins of the specimen. Microscopically, the cysts showed an invasive malignant tumor in a stroma with fibrosis and occasional myxoid changes. The tumor was composed of solid and focal cribriform nests of basaloid cells, and showed infiltration into partially preserved normal acinar structures and perineural areas (Figure 1). Immunohistochemical (IHC) staining revealed that p63 and calponin were negative in tumor cells, while ESA (EPCAM) and c-Kit (CD117) are diffusely but patchy positive. Tumor cells were stained diffusely positive for phospho-NF-κB p65 (S536), and weak-to-moderate positive for c-Kit in a separate analysis (Figure 1).

 doi:10.20944/preprints202408.0349.v1

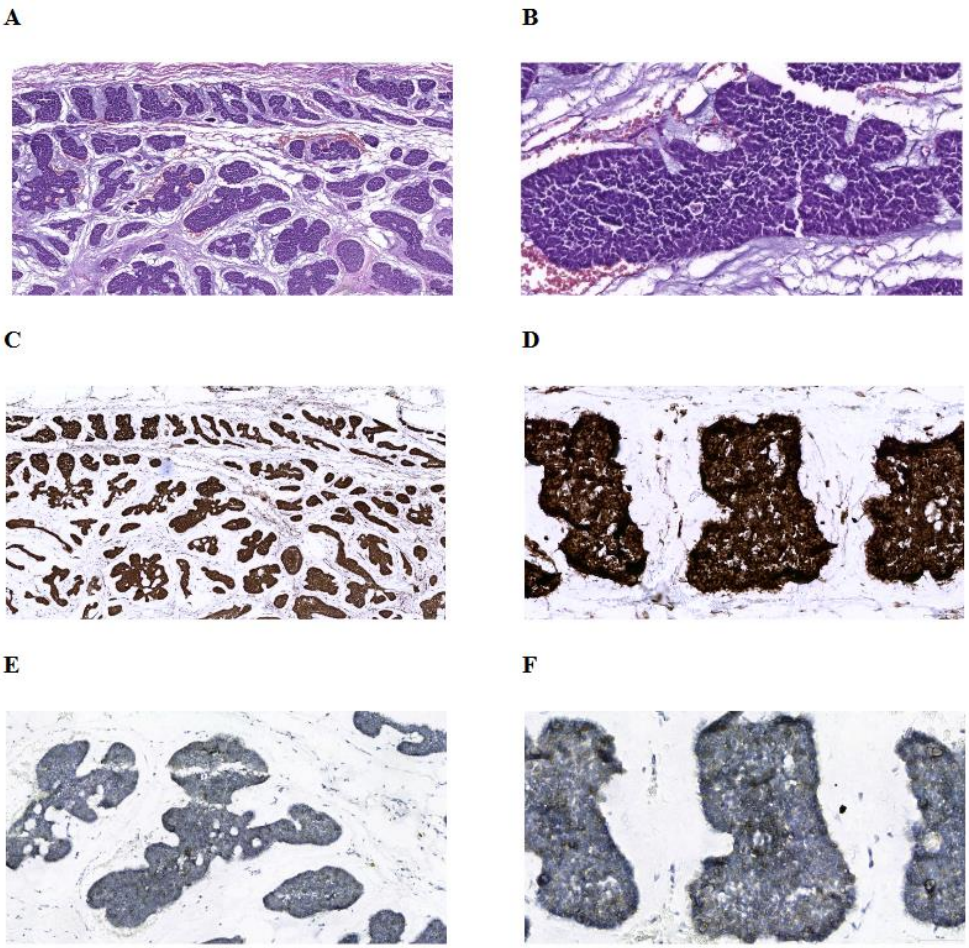

Figure 1. The photomicrograph shows an adenoid cystic carcinoma specimen. There are solid and focal cribriform nests of basaloid cells (Hematoxylin-eosin staining, **A** - ×100, **B** - ×400). Immunohistochemistry showing a positive NF-κB p65 p-S536 staining (ab86299 at 1/200 - 1 g/ml, detected by DAB, **C** - ×40 and **D** - ×400). IHC showing weak-to-moderate focal positive c-Kit (CD117) staining (**E** - x200 and **F** - x400).

Treatment: In 2012, after lateral orbitotomy with positive surgical margin, the patient was treated with adjuvant RT (60 Gray = Gy) without CT. In 2015, a palpable growth at lower outer quadrant of the eye was observed and considered local recurrence by radiologic evaluation. She later underwent another operation that removed orbital contents, bones and adjacent contents with negative surgical margins. No adjuvant RT/CT was planned considering previous RT and complete resection. In October 2018, partial maxillectomy was performed due to maxillary recurrence detected during routine check-up. Due to the positive surgical margin, adjuvant concurrent chemoradiotherapy (CRT - 60 Gy plus cisplatin) followed by cisplatin/doxorubicin was planned. Although there was a suspected lung metastasis around this time, a mass on zygomatic bone and ocular cavity as well as multiple metastases in the lung were confirmed in February 2019. She was later admitted to our clinic for a second opinion, and we recommended a CGP test to design a personalized treatment plan. Due to post-surgery complications, the patient received adjuvant CRT starting from March 2019 through May 2019. She experienced grade 2 nausea and asthenia. The patient was later offered carboplatin plus paclitaxel considering the lung metastases, which she refused after receiving two cycles (July – August 2019). Moreover, response evaluation in August 2019 did not show a significant response at primary or metastatic sites.

CGP-guided Treatment: According to the FoundationOne® CDx (F1 CDx) CGP results (March 2019), the specimen harbors *NOTCH1* D2442fs*35 (VAF: 45.6%), *CTCF* splice site 223+1G>A (48.8%), and *KDM6A* P1107fs*13 (48.2%) alterations with a TMB of 4 muts/Mb, MSS and negative PD-L1 (TPS: 0% - Dako 22C3 pharmDx™). Variants of unknown significance (VUS) include *KDM5A* G1116E

 doi:10.20944/preprints202408.0349.v1

(47.2%), *MSH2* A2T (53.9%), *MSH6* A36V (52.1%), *MYC* V280del (29.1%), and *PIK3C2G* Y676H (46.9%). The specimen was negative for any *MYB*/*MYBL* fusions by the CGP. Consequently, dual immunotherapy (ipilimumab 50 mg in total, nivolumab 400 mg – approved by health authorities) in combination with high-dose intravenous vitamin C (IVC - 1.5 g/kg biw on consecutive days, frequency was later reduced) and BOC (NovoCurmin by Dyna Sci – 2x2 capsules) were started in October 2019. The BOC was utilized to impair NF-κB signaling pathway, which we have successfully used in our previous ACC case based on the same rationale[23]. The IVC, on the other hand, was included to augment immunotherapy response and induce tumor-selective DNA damage. The control PET/CT scan in February 2020 showed noticeable regression of lung metastases, but the mass on zygomatic bone was still present. We interpreted this as pseudoprogression but the patient did not consent to a new biopsy, and stereotactic body radiation therapy (SBRT) was planned by an external clinic. Subsequently, she was treated with SBRT (CyberKnife® - 30 Gy). We decided to continue the immunotherapy for 3 more months by also considering the potential abscopal effect and synergy between the immunotherapy and RT. The patient complained of grade 2 asthenia and TSH levels were found to be elevated during this period. She was diagnosed with thyroiditis and later developed hypothyroidism, probably due to the immunotherapy[24]. Accordingly, thyroid hormone replacement therapy was started. Apart from this, the treatment was well-tolerated with only mild adverse events (AEs). After 3 months, a complete regression was observed in both lung parenchyma and zygoma. No tumor was observed at 1-year follow-up (Figure 2 and 3). The last immunotherapy was given in December 2020, and there was no disease progression or serious AEs. The patient was tested positive for COVID-19 in March 2021. Overall, we had achieved a radiologic complete response and long-term progression-free survival (PFS) before the patient died due to COVID-19 pneumonitis at the end of April 2021.

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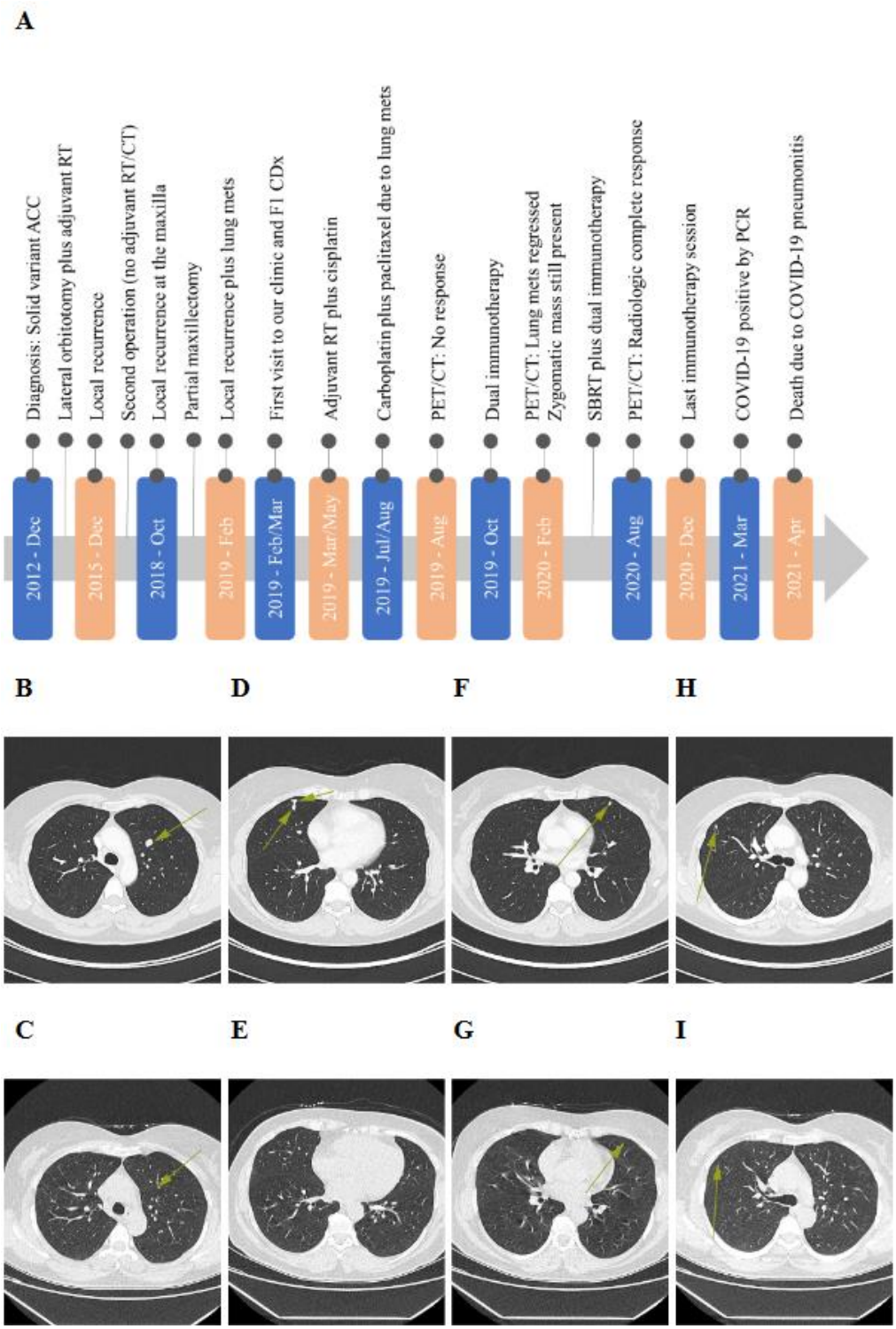

Figure 2. Timeline of disease status and treatment (**A**). CT images showing the presence and absence of lung nodules before and after the treatment (August 2019: **B, D, F, H** – August 2020: **C, E, G, I**).

 doi:10.20944/preprints202408.0349.v1

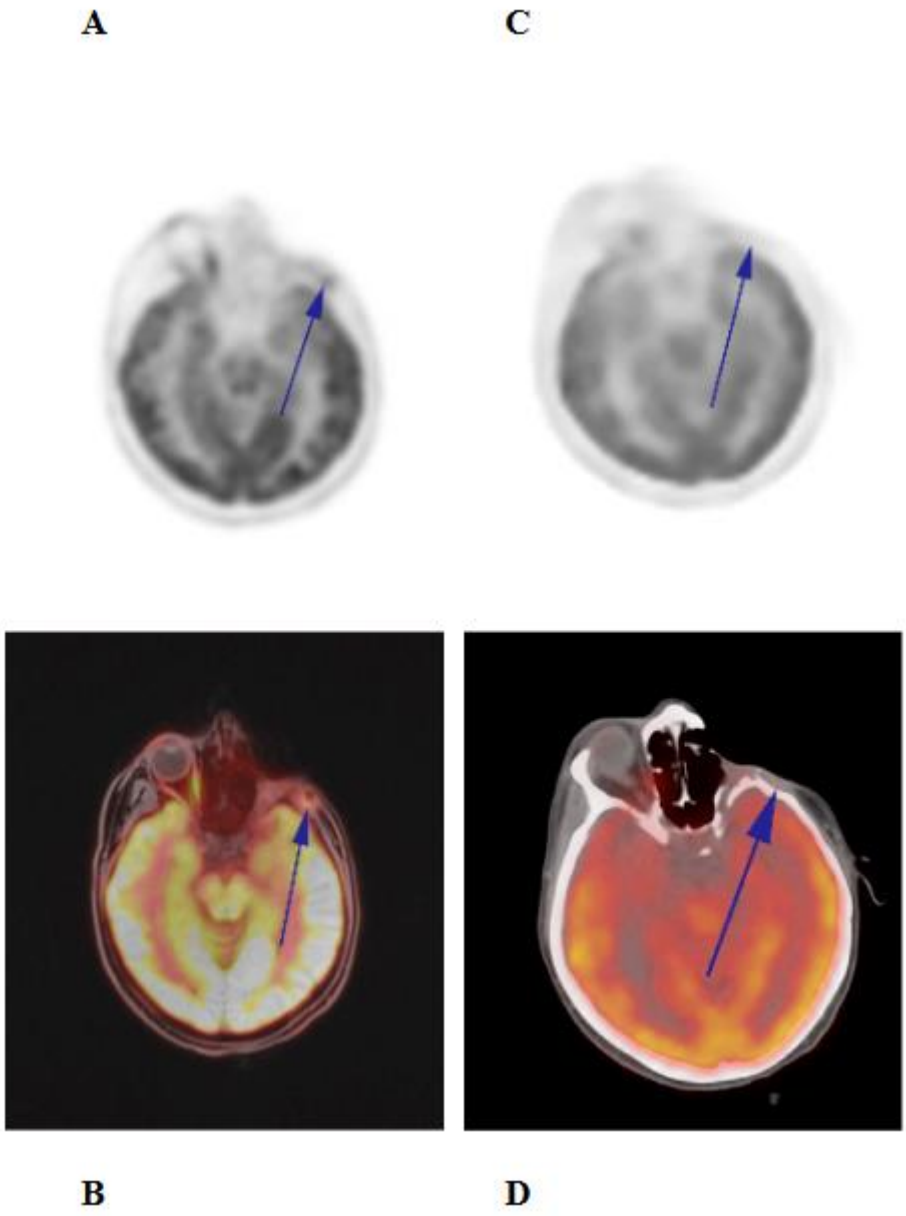

Figure 3. The PET/CT images showing marked tumor regression (**A, C**) and showing no pathologic FDG uptake (**B, D**) following the treatment.

Discussion

ACC is a rare and enigmatic cancer characterized by biological diversity and lack of biomarkers to guide targeted treatment approaches. The current standard-of-care (SOC) is surgery followed by adjuvant RT, sometimes together with CT. Of patients receiving radiotherapy, more than half will eventually develop local or distant recurrence. Single agent or combinatorial CT, on the other hand, provided minimal clinical benefit. Therefore, alternative approaches such as targeted agents and immunotherapy should be explored.

Receptor tyrosine kinase (RTK) inhibitors yielded no-to-low objective responses although a considerable proportion (~40%) of ACC tumors do harbor alterations in tyrosine kinase genes[8,25]. Therefore, targeted approaches are currently far from being solid treatment options. The frequency of somatic mutations was associated with solid histology[26]. There were frequent alterations in *NOTCH* genes with the majority seen in *NOTCH1*[8,26]. Unlike *MYB-NFIB* fusions and *TERT* promoter alterations, *NOTCH1* mutations are linked to decreased survival[8], and more common among tumors with solid histology and liver/bone metastases[8,27]. A separate study reported an

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ACC subtype characterized by *NOTCH* activating mutations (unlike LOF mutations in HNSCC) and enrichment of solid histology[5]. Furthermore, those with *NOTCH1* activating mutations had even shorter overall survival (OS) compared with *NOTCH1* inactivating mutations. Some *NOTCH* alterations could confer sensitivity to gamma secretase inhibitors (GSIs). However, our patient was not expected to benefit from GSI treatment as nonsense or frameshift mutations removing C-terminal PEST degron domain stabilize the NICD and require ligand-dependent activation or extracellular negative regulatory region (NRR) mutation for complete activation. The mutations in chromatin remodeling genes are also commonly observed in the ACC tumors[8,26]. Our patient has alterations in 3 such genes: *KDM6A*, *KDM5A* and *CTCF*. *NOTCH1* mutations are mutually exclusive with *TERT* alterations, but exhibit co-occurrence pattern with *KDM6A*, suggesting a cooperation between them. Accordingly, *KDM6A* alterations lead to poor survival among ACC patients. CTCF is a transcriptional repressor of c-Myc and could result in MYC upregulation or increased activity when it has a LOF mutation, driving a more aggressive phenotype and disease course[5]. Alterations in PI3K signaling genes (a VUS in *PIK3C2G*) were also typically observed in tumors with a solid histology, an aggressive subset of the ACC tumors[26]. In brief, tumor mutational profile and burden of the patient was compatible with solid variant ACC, thus associated with worse prognosis.

Tumors are not isolated entities but form dynamic interactions with the surrounding stroma, endothelial cells and multiple types of immune cells, enabling alternative therapeutic approaches such as ICIs[28]. To date, a couple of markers have been utilized to predict response to and benefit from the ICIs[29,30], such as high tissue TMB (tTMB ≥10 muts/Mb)[6], MSI-H or dMMR status [31], or high PD-L1[32]. However, there are many patients without response to immunotherapy despite the presence of these markers and those with robust responses without these markers. In line, threshold for high TMB has recently been questioned as it is challenging to determine a single, fixed cut-off in a tumor-agnostic manner. TMB failed to estimate benefit from the ICIs in many cancer types[33], and is an inadequate predictor of response to immunotherapy[34]. Previous studies have revealed a low median TMB of 0.3-2 muts/Mb[5,8], and less than 5 and 2% of all ACC cases harbor more than 10 and 20 muts/Mb, respectively[35], excluding >95% of the ACC patients from receiving immunotherapy. Recent studies and meta-analyses also challenged the value of PD-L1 expression as a predictive or prognostic biomarker and revealed that it has limited utility in various cancer types[32,36]. Furthermore, PD-L1 expression is quite uncommon or non-existent in the ACC specimens[37,38]. There is also no consensus on whether high or low PD-L1 (by which scoring parameter) or which cut-off for positive or negative staining predicts better clinical outcome, even in different studies of the same cancer type[39].

Phase 1b KEYNOTE-028 study of pembrolizumab in advanced salivary gland carcinoma (SGC) reported no ORR or durable responses in the ACC[40]. Nivolumab had a low ORR of 8.7% and a non-progressive rate of 33.3% at 6 months in NISCAHN phase 2 trial in patients with SGC[41]. A phase 2 trial of nivolumab plus ipilimumab in advanced SGC found that the combination had limited efficacy in the ACC[41,42]. However, in-depth analysis of the responding patients (2 confirmed and 1 unconfirmed PR) revealed that our patient similarly harbors more immunogenic *KDM6A* and *NOTCH1* frameshift/truncation mutations, a potentially higher TMB (4 muts/Mb by F1 CDx – at least 2-fold higher than the median TMB) than the responding patients (25-45 muts by WES) as 306 muts by WES correspond to 8 muts/Mb by F1 CDx[43], and negative PD-L1 status. We recommended dual immunotherapy based on the idea that remodeling the TME by facilitating T cell initiation and trafficking via anti-CTLA-4 could convert the tumor to an immunologically hot state that can be later targeted by anti-PD-1. In sum, the current biomarkers used to guide immunotherapy are not sufficient for distinguishing the patients that may or may not benefit from the treatment. Therefore, an elaborate examination of all possible contributing factors should be routinely performed during clinical decision-making.

It is critical to follow a patient-centric approach to identify case-specific immune biomarkers and practice precision immunotherapy. Clonality or subclonality of the alterations present in a tumor has recently been recognized as a potential biomarker. Considering the co-occurrence pattern between the mutations in our patient and their variant allele frequency (~50%), they are likely to be clonal.

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While clonal neoantigens trigger T cell immunoreactivity and sensitivity to ICI, those of subclonal nature do not[33,44]. Similarly, a meta-analysis concluded that it is not quantity but quality of the mutations that determines the efficacy of immunotherapy in a cancer-type dependent manner[45]. Recent studies have also identified several gene alterations and pathways that could predict response or resistance to immune checkpoint blockade (ICB) better than TMB alone[46,47]. For instance, deleterious *NOTCH* alterations causing downregulation of NOTCH signaling in NSCLC or *NOTCH* signaling mutations in colorectal cancer (CRC) were found to be a predictive biomarker of favorable response to ICI[48,49], whereas it was elevated NOTCH signaling in small cell lung cancer[50]. In melanoma, *NOTCH1* expression was shown to cause immunosuppressive tumor microenvironment while its inhibition enhanced immunotherapy efficacy [51]. Briefly, conflicting results render *NOTCH1* especially unique as its role in immunotherapy should be meticulously defined on a per patient basis or at least in a cancer type-dependent manner. Similar to *NOTCH1*, *KDM6A* may serve as a favorable or unfavorable marker in metastatic urothelial carcinoma patients with TMB-H or in the general population, respectively[52,53]. Based on our patient's outcome, we concluded that these mutations might augment immunotherapy efficacy in the ACC. Even in mismatch repair (MMR)-proficient tumors, IVC may enhance ICI efficacy by functioning as an adjuvant[18,54], which is currently tested in CRC patients in a pilot study[55]. In multiple preclinical models, vitamin C together with anti–PD-1 and anti–CTLA-4 induced apparent tumor growth inhibition or regression[18]. The IVC also induces oxidative stress and epigenetic reprogramming to cause cancer-selective DNA damage and cell death[56,57]. Similarly, curcumin may act as an immunomodulatory agent[22], impair proteasome activity[58] and induce NF-κB inhibitory events to prevent tumor growth and progression in preclinical cancer models[59]. We have previously used it in combination with imatinib and achieved a complete metabolic response in a c-Kit and phospho-NF-κB positive metastatic ACC[23], as both proteins are highly expressed and associated with disease progression in the ACC[60,61]. Bortezomib, another proteasome and NF-κB inhibiting agent, with doxorubicin provided high disease control rates in R/M ACC patients in a phase 2 trial[62]. Another important factor has lately emerged as a predictive marker is the type of alteration. Frameshift (Fs) and indel mutations carry a greater immunogenic potential as they elevate neoantigen abundance and mutant-binding specificity[63–65], partly stemming from high number of base and thus amino acid changes that drive changes in protein structure to expand epitope repertoire[66], unlike SNVs. In a real-world pan-cancer study, tumors with low TMB but Fs alterations had better PFS compared to those with low TMB without Fs alterations[67]. This could pave the way for development of personalized and potent cancer vaccines[68,69]. In head and neck cancer, the Fs mutations, including those in *NOTCH1*, were found to be enriched in the responders to anti-PD-1/PD-L1 therapies[70]. Pan-cancer splice site mutations may also generate more immunogenic peptides than missense mutations, with a positive correlation to high PD-L1 and PD-1 expression as well as to high T cell immune activity[71]. These genomic parameters could be complemented by immunophenotypic data to better discriminate between immune cold and hot tumors, thus response to the ICIs[72].

Collectively, ACC is an unpredictable and heterogeneous cancer type that exhibits high recurrence risk. Furthermore, the current status in the clinic or population-based clinical trials is zero-to-low response rates, underlying the need for personalized treatment. This necessitates tailor-made medicine with a detailed examination of each alteration and/or potential biomarker in a patient sample unlike one-size-fits-all approaches. Thus, we could gain new fronts in our fight against cancer and transform the current treatment landscape in the ACC.

Acknowledgments: We are grateful to the Foundation Medicine, Inc. Molecular Tumor Board (Mehlika Hazar-Rethinam, PhD and Saad A. Khan, MD) for providing insight on molecular understanding of the tumor upon our request.

Data Availability Statement: All the data generated for the study are available in this article or from the corresponding author upon request.

Informed Consent: Written informed consent was obtained from the patient for participation, treatment and publication of her anonymized data in this scientific publication.

Conflicts of Interest Statement and Funding: The authors declare that they have no conflict of interest. The authors did not receive any funding for the publication.

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