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The utility of TP53 and PIK3CA mutations as prognostic biomarkers in salivary adenoid cystic carcinoma

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ABSTRACT

Objectives: Despite wide excision and post-operative irradiation, loco-regional and/or metastatic recurrence is a significant clinical problem in salivary adenoid cystic carcinoma (SACC). Reliable biomarkers are required to tailor post-treatment surveillance to patients at highest risk of recurrence. We sought to determine the utility of *TP53* and *PIK3CA* mutations as prognostic biomarkers in SACC.
Materials and methods: DNA was extracted from archival tumour blocks of 145 SACC patients from 66 UK referral centres and sequenced for *TP53* and *PIK3CA* mutations. Clinical, pathological and outcome data were analysed to determine the impact of the genomic alterations on disease recurrence and overall survival (OS).
Results: TP53 and *PIK3CA* mutations were identified in 8% (10/121 successful analyses) and 2% (3/121) of cases, respectively. There were too few *PIK3CA* mutations in this cohort for informative further analysis. *TP53*-mutated SACC had significantly shorter median OS (5.3 vs. 16.3 years, $p = 0.019$) and lower 10-year survival (48% vs. 81%) compared with *TP53* wild-type ACC. Solid-pattern histopathology was more frequent in *TP53*-mutated SACC (50% vs. 15%, $p = 0.27$).
Conclusion: TP53-mutated recurrent and metastatic SACC was associated with shorter OS, which was significant when combined with published genomic data sets. Stratifying by *TP53* status, in addition to established clinical, pathological and genomic biomarkers, may usefully inform follow-up strategy.

Introduction

Salivary adenoid cystic carcinoma (SACC) is a rare salivary gland cancer with an annual incidence rate of 0.5/100,000 per year [1]. It recurs in most patients despite intensive treatment of localised disease, usually with wide excision and post-operative radiotherapy [1]. Standard clinical and histopathological factors associated with shorter disease-free and overall survival include TNM stage, tumour site, solid pattern histology, margin status, perineural invasion, age and sex [2]. These factors can be used to gauge the risk of disease recurrence and predict overall survival (OS) following diagnosis. However, uncertainty persists regarding the most appropriate clinical and radiological follow-up after curative-intent local therapy and there is, as yet, a dearth of additional prognostic biomarkers to support clinical decision-making.

Recent advances in the understanding of SACC genomics provide one avenue for the development of prognostic biomarkers. Oncogenic fusion and overexpression of the myeloblastosis (Myb) transcription factor or the Myb homologue (Mybl1) are seen in almost all SACC patients [3]. Multiple studies have evaluated the value of *MYB* as a prognostic marker in SACC. Although results are often limited by small sample sizes, both the presence of *MYB* translocation [4] and the level of Myb protein expression [5] have been associated with higher relapse rates and worse

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clinical outcomes. However, more recent systematic reviews have demonstrated that *MYB* mutation and myb protein expression have minimal or no prognostic potential [6,7]. In contrast to *MYB/MYBL1* alterations, other genetic alterations in ACC are low-frequency events [8] providing a significant barrier to their clinical evaluation as prognostic biomarkers.

NOTCH pathway activation is the most frequent alteration in addition to *MYB* or *MYBL1* [8]. *NOTCH* gain-of-function mutations are identified in 11–29% of patients with SACC [9–13]. The presence of NOTCH gain-of-function mutations has been shown to correlate with shorter disease-free and overall survival and worse outcomes with cytotoxic chemotherapy in comparison with *NOTCH* wild-type SACC [9].

Recent analysis of publically available genomic datasets or single institution case series confirm that *TP53* mutations are seen in up to 5–10% [8,12] and *PIK3CA* mutations in up to 5–22% of SACC patients [8,14]. We therefore sought to evaluate the utility of *TP53* and *PIK3CA* mutations as prognostic biomarkers in SACC. To overcome the primary challenge of studying sufficient patients for a statistically meaningful analysis, we established a UK-wide salivary gland cancer referral network in collaboration with the NHS England Genomics Hub, through which patients were referred to assess their suitability for clinical trial therapies and to participate in translational and biomarker research.

Patients and methods

Patient consent and clinical data collection

From April 2017 to September 2019 145 consecutive patients with histologically confirmed adenoid cystic carcinoma underwent clinical review at a tertiary cancer center (The Christie NHS Foundation Trust, Manchester, UK). Patients provided written informed consent to donate their formalin-fixed paraffin-embedded (FFPE) archival tumour samples to the Manchester Cancer Research Centre (MCRC) Biobank to undergo clinical, pathological and genomic data collection, analysis and publication as part of the study ELLA-01. This study was granted research ethics approval under the MCRC Biobank Research Tissue Bank Ethics (NHS NW Research Ethics Committee 18/NW/0092) and was performed in accordance with the Declaration of Helsinki. Comprehensive clinical data were collected including details on stage at diagnosis, previous treatments received and response to treatment where available, and dates of recurrence and of last follow-up or survival.

Next-generation sequencing and histopathology classification

DNA was extracted from archival FFPE samples and underwent next-generation sequencing at the Manchester Center for Genomic Medicine National Health Service Genomics Laboratory Hub to sequence for *TP53* and *PIK3CA* mutations (Qiagen GeneRead DNAseq Targeted Panel V2). The assay covered the entire coding region of the *TP53* gene and the whole coding region of *PIK3CA* with the exception of a 31 nucleotide sequence at the 5′ end of exon 5 (hg19 chr3:178,921,332 to 178,921,362). A custom bioinformatic pipeline validated to detect single nucleotide variants and indels (<40 base pairs) to 4% variant allele frequency was used to detect variants. Where identified, variants were classified using a combination of American College of Medical Genetics guidelines and Association for Molecular Pathology tiering [15,16] with reference to publically available resources including Catalogue Of Somatic Mutations In Cancer v19 [17–19], and other subscription-based resources including Human Gene Mutation Database Professional (Qiagen). In 2/145 cases in whom NGS analysis had failed with this approach, *TP53* and *PIK3CA* mutation status was available from prior commercially sourced next generation sequencing (Roche, Foundation Medicine). Centralised review of the pathology sample was performed by an accredited head and neck cancer histopathologist (GB, Manchester University NHS Foundation Trust) in 70 patients for whom haematoxylin and eosin sections were available from the diagnostic tumour blocks. For assessment of solid component, the presence of any solid component was noted, and in addition, whether over a cut off of 30% tumour area had solid morphology was noted as both have been associated with a more aggressive phenotype [20–22]. To detect *MYB* gene rearrangement, FFPE sections were processed using the ZytoLight Spec *MYB* dual colour break apart probe (Zytovision, Z-2143-200) as per manufacturer's instructions and imaged using a Zeiss Axio Imager M1 fluorescence microscope to determine the predominant signal pattern in multiple areas across the sample.

Statistical analysis

To determine the association between mutational status and clinicopathological characteristics, two-sided Fisher's exact test was used. Recurrence-free survival (RFS) and overall survival (OS) were calculated using Kaplan-Meier analysis. RFS was defined as time from diagnosis to relapse or death from disease, whichever event occurred first. RFS was censored at data cut-off at date of last follow up. OS was defined as time from diagnosis to death of any cause; those patients alive at data cut-off were censored. Median RFS and OS were reported and the difference between survival curves calculated using the log rank test. P values were two-sided, $P < 0.05$ was accepted as statistically significant. For mutation status, age, sex, the presence of solid histopathology and peri-neural invasion, Cox proportional hazards models were fitted for both risk of recurrence and death, supplementary Table 1. Hazard ratios were calculated with 95% confidence intervals. Statistical analysis was performed using SPSS Statistics (version 25) and Kaplan-Meier curves visualised using GraphPad Prism (version 8).

Results

145 patients with histological confirmation of SACC were referred from 66 Local or Regional Cancer Centres within the United Kingdom National Health Service for centralised clinical review at a single Tertiary Cancer Centre (The Christie NHS Foundation Trust, Manchester, UK). Archival FFPE tumour blocks were collected and *TP53* and *PIK3CA* status were determined in 121/145 (83%) patients, the remaining 17% of samples having insufficient or poorly preserved DNA for successful NGS analysis. Baseline patient demographics for these patients are summarised in Table 1. Most patients were female (60%) and the median age was 48 years (range 16–79). Consistent with this cohort being evaluated for clinical trial therapies, loco-regional or distant recurrence was present in 96% (116/122) of patients, with lung metastases being the most frequent site of recurrence as previously reported in SACC [20,23]. On histopathology review, 32% had areas of solid morphology consistent with previous reports [2]. Perineural invasion was seen in the diagnostic biopsy samples in 32%, in keeping with previous reporting rates of 30% to 100% [24,25]. As no patients had entire surgical resection samples available for review, this may have attributed to the relatively low observed rate in this study. The apparent match between tumours showing perineural invasion and those showing a solid component was a noted irregularity and therefore reviewed to confirm that these values were correct. Vascular invasion is a further emerging histological marker of poor prognosis in surgically treated patients [26]. This is infrequent in adenoid cystic carcinoma and ideally requires examination of a standardised tumour resection specimen. Only biopsy samples or incomplete resection specimens were released for central pathology review for this study. Of 79 samples available for analysis, 9 (11%) demonstrated evidence of vascular invasion. The majority of patients 105/121 (86%), did not receive systemic therapy. Of the remaining 16 patients (14%), 11 patients had one line of therapy, 4 patients had 2 or more lines and 1 patient had unknown therapy. Consistent with a lack of standard systemic therapy options, fourteen different regimens were used amongst these patients, and the details are summarised in Supplementary Table 2.

Table 1
Baseline characteristics of adenoid cystic carcinoma patients (n = 121).

Characteristic	N	%
Sex		
Male	48	40
Female	73	60
Disease Site		
Major salivary gland	64	53
Minor salivary gland	57	47
Disease recurrence		
Yes	116	96
No	5	4
Site of recurrence		
Local	36	30
Liver	21	17
Lung	84	69
Bone	15	12
Other	15	12
Solid Component		
Present	22	18
Absent	50	41
NOS~	49	41
Perineural invasion		
Present	22	18
Absent	50	41
NOS	49	41

~Histology was centrally reviewed in 70/121 cases.

To determine the frequency of *TP53* and *PIK3CA* mutations in this cohort, DNA extracted from FFPE samples underwent next-generation sequencing and bioinformatic analysis. *TP53* mutations were identified in 8% (10/121) of SACC patients and *PIK3CA* mutations in 2% (3/121). The nucleotide and associated amino acid change for these mutations are summarised in Table 2 alongside the clinical characteristics of these patients. Given the low frequency of *PIK3CA* mutations in this cohort, subsequent clinical and pathological analysis was performed on the *TP53* mutated cohort alone. Fig. 1A shows the locations of the mutations which were identified within the *TP53* gene in 10/121 ACC patients. The majority of *TP53* mutations (83%) occurred within the DNA-binding domain. To investigate whether the distribution of mutations within the *TP53* gene seen in our cohort was consistent with previous reports, we identified 928 ACC samples within a publically available genomic repository (cBio Cancer Genomics Portal: cBioPortal [27,28]). The majority of these cases (n = 673) were genomic analysis without any accompanying clinical data deposited by Foundation Medicine (Roche). The remainder were from published data sets including Memorial Sloan Kettering (MSK-IMPACT) study (n = 94), MSK (n = 88) and Sanger/MD Anderson Cancer Centre (n = 59). Consistent with our cohort, *TP53* mutation was seen in 9% of these patients (84/928). The locations of these mutations were also found to be concentrated within the DNA binding domain (Fig. 1B) although 9/84 (11%) were within the tetramerization domain.

The distribution of clinical characteristics in SACC patients (n = 121) by *TP53* status is shown in Table 3. Sex, age and site of primary tumour were evenly distributed between the patients irrespective of *TP53* status. There was no statistical difference between individual sites of recurrence, although the presence of liver metastases was seen in 19% of *TP53* wild-type patients and in none with TP53 mutation. The presence of solid component is an established factor associated with adverse risk in SACC. To further assess for any association between *TP53* mutation status and these risk factors, centralised histology review was performed

Table 2
TP53 and PIK3CA mutations and clinical characterstics.

Base change	Variant allele frequency	Amino acid change	Predicted Functional effect	Histology	Sex	Age	Primary site	TNM	Site of metastasis	Perineural invasion
TP53 mutations										
c.584 T > C	7% reads	Ile195Thr	Loss of function	Solid > 30%	F	62	Parotid	TXN0M0	Lung	Absent
c.1146delA	29% reads	Lys382fs*40	Loss of function	Tubular / cribriform	F	78	Sublingual	TXNXM1	Lung	Absent
c.428_429delTG	13% reads	Val143fs*5	Loss of function~	Tubular / cribriform	M	47	Submandibular	T4bN2cM1	Lung	Absent
c.668delC	73% reads	Pro223fs*24	Loss of function	Solid > 30%	F	48	Trachea	T4N2M0	Lung and lymph node	Absent
c.814G > T	4% reads	Val272Leu	Loss of function	Tubular / cribriform	F	64	Ethmoid sinus	T4bN0M0	No recurrent metastatic disease	Absent
c.527G > A	4% reads	Cys176Tyr	Loss of function	Tubular / cribriform	F	72	Post nasal space	T1N0M0	No recurrent metastatic disease	Present
c.832C > T	17% reads	Pro278Ser	Loss of function	Solid < 30% and tubular / cribriform	F	74	Parotid	TxNxM0	Lung, liver, skin, bone	Present
c.373A > G	14% reads	Thr125Ala	Partially functional	Tubular / cribriform	F	56	Parotid	T3N0M0	Lung, local	Present
c.329G > C	50% reads	Arg110Pro	Loss of function	Solid > 30%	M	40	Submandibular	T3N0M0	Lung	Present
c.467G > C	8% reads	Arg156Pro	Loss of function ~~	Solid < 30% and tubular / cribriform	F	37	Parotid	T1N0M0	Lung	Present
PIK3CA mutations										
c.1633G > A	11% reads	Glu545Lys	Activating	Tubular / cribriform	F	69	Parotid	T1N1M0	Local, lymph node	Absent
c.1258 T > C	18% reads	Cys420Arg	Activating ~	Tubular / cribriform	M	47	Submandibular	T4bN2cM1	Lung	Absent
c.1633G > A	8% reads	E545K	Activating	Tubular / cribriform	F	34	Larynx	T4aN0M1	Lung	Absent

~ This patient has co-existent TP53 Val143fs*5 and two PIK3CA mutations: Cys420Arg 18% and Glu545Lys 8%.
~~ This patient has two co-existent TP53 mutations – c.467G > C Arg156Pro 8% and c.824G > A Cys275Tyr 4%.

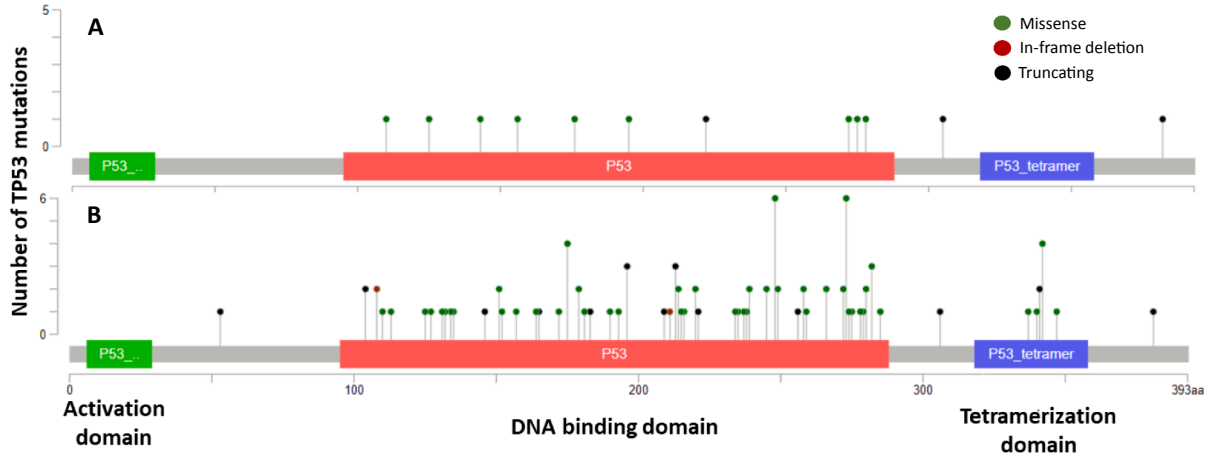

Fig. 1. Distribution of TP53 mutations in adenoid cystic carcinoma. Individual variants and their distribution within the TP53 gene are shown by circles. Mis-sense are shown in green, in-frame deletions shown in brown and truncating mutations in black. (A) show the distribution of TP53 mutations identified in this study of ACC patients and (B) shows the distribution of TP53 mutations of ACC patients within the cBioPortal analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

on 60/121 (50%) of *TP53* wild-type patients and this was compared with histological characteristics for all *TP53* mutated patients. Although the small numbers in the *TP53* mutated group limits the power of the statistical analysis, solid component and peri-neural invasion were both seen more frequently in patients with *TP53* mutations, being detected in 50% of patients with *TP53* mutations and in 15% of patients with wild-type *TP53* (p = 0.27).

To investigate whether *TP53* mutations may be co-existent with *MYB* gene rearrangement, and therefore may represent a putative sub-group of *MYB* altered SACC or in contrast whether *TP53* mutations and *MYB* alterations may be mutually exclusive. *MYB* gene rearrangement was assessed by fluorescent in situ hybridisation (FISH) in 40 SACC patients (4 *TP53* mutated and 36 *TP53* wildtype). *MYB* rearrangement was seen in 50% of the *TP53* mutated and 73% of the *TP53* wild-type SACC patients (p = 0.15). Fluorescent in situ hybridisation images are demonstrated in Fig. 2.

During a median follow-up from diagnosis of 6.6 years (range 3–465 months), 31/121 (26%) patients had died during the follow up of this study, death occurred in 40% of patients with *TP53* mutation and in 23% with *TP53* wild-type SACC (p = 0.14). Of the 121 patients, 97 (80%) were initially treated with surgical resection with curative intent. Of the 10 patients with a *TP53* mutation, resection with curative intent was possible in 8 patients consistent with the proportion of all SACC patients in this study. As resection margin status is known to impact on survival, we sought to analyse this, however, histopathological assessment of operative resection margins was only available from 16 patients. Adjuvant photon radiotherapy was given in 79/121 patients (65%), 2 patients received adjuvant proton bean therapy and 2 treated with upfront palliative carbon-ion. No patients were lost to follow up. Fig. 3 shows the Kaplan-Meier analysis for RFS (Fig. 3A) and OS (Fig. 3B) from diagnosis for patients with *TP53*-mutated and wild-type SACC. For patients without metastatic disease at diagnosis (n = 101), the median RFS was shorter in patients with *TP53* mutation (44 vs. 50 months, p = 0.25, Fig. 3A). For all SACC patients, the median OS was shorter in the presence of *TP53* mutation (64 vs. 196 months, p = 0.06; Fig. 3B). When cox proportional hazards models were fitted for the variables including mutation status, age, sex, and the presence of solid component or perineural invasion, only age was associated with a significantly increased risk of recurrence (HR 2.69; 95% confidence interval 1.45–5.01) in univariate analysis (Supplementary Table S1).

To determine whether our finding that *TP53* mutation was associated with adverse clinical outcomes in SACC is consistent with other institutional data, we extracted the overall survival data from 144 SACC patients available within cBioPortal with and without *TP53* mutation (11 *TP53* mutated and 133 *TP53* wild-type) and performed Kaplan-Meier survival analysis. Consistent with our findings, there was a shorter median overall survival for patients with *TP53* mutation although this difference did not reach statistical significance. The median overall survival for *TP53*-mutated ACC was 29 vs. 169 months in *TP53* wild-type ACC (P = 0.106; HR 2.13, 95% confidence interval 0.83–5.44 Fig. 4A). To increase the statistical power of the current study, we combined the overall survival data in the UK cohort (n = 121) with that extracted from cBioPortal (n = 144) to develop a cohort of 265 ACC patients with data on both *TP53* mutation and overall survival time (21 with *TP53* mutation, 244 *TP53* wild-type). This confirmed that OS was significantly shorter in patients harboring *TP53* mutations with a median OS of 64 vs.196 months (HR, 2.26 95% confidence interval 1.12–4.56; P = 0.019; Fig. 4B).

Discussion

The primary aims of this study were to determine the utility of *TP53* and *PIK3CA* mutations as prognostic biomarkers in SACC. *TP53* mutations were identified in 8% of ACC patients, consistent with the expected 5–10% frequency [8,27,28].The presence of *TP53* mutations was associated with a shorter recurrence-free and significantly shorter overall survival with a trend towards higher frequency of solid component.

Somatic mutations in the *TP53* gene are one of the most frequent alterations in human cancer. Large-scale genomic sequencing studies have confirmed that approximately half of all cancers harbour a *TP53* mutation, although the frequency and distribution of mutations can vary significantly between tumour types [29–31]. Most *TP53* mutations are missense and result in loss of function [32]. Of 11 *TP53* mutations identified in this study, 10 have previously been categorised using functional transactivation assays as loss-of-function, with no evidence of gain-of-function. One missense alteration (*TP53* Thr125Ala) is partially functional and likely to represent a rare hypomorphic allele. The majority of missense mutations occur in the DNA-binding domain, implying that this feature of the p53 protein is crucial for tumour suppression [32], and all the missense alterations identified in this study were also within the DNA binding domain. Alternate p53 mutant alleles have been reported which may reflect selection of function or gain-of-function that promote tumorigenesis and drive chemotherapy-resistance, invasion and metastasis [33]. *TP53* mutations encountered in cancer may acquire some combination of these opposing loss- or gain-of-function characteristics [34]. None of the *TP53* variants detected in this study fell into a

Table 3
Clinical and pathological characteristics of TP53 mutated and wild-type salivary adenoid cystic carcinoma.

	TP53 MT (n = 10)		TP53 WT (n = 111)		P value
	N	%	N	%	
Sex					
Male	2	20%	46	41%	0.31
Female	8	80%	65	59%	
Disease Site					
Major salivary gland	7	64%	57	51%	0.33
Minor salivary gland	3	36%	54	49%	
Disease recurrence					
Yes	10	100%	106	95%	0.99
No	0	0%	5	5%	
Site of recurrence					
Local	3	30%	33	30%	0.99
Lung	9	82%	75	68%	0.50
Bone	0	0%	15	14%	0.6
Liver	0	0%	21	19%	0.21
Other	1	9%	15	14%	0.99
Solid component					
Present	5	50%	17	15%	0.27
Absent	5	50%	43	39%	
NOS~	0	0%	51	46%	
Perineural invasion					
Present	5	50%	17	15%	0.27
Absent	5	50%	43	39%	
NOS	0	0%	51	46%	
TNM					
T Stage					
TX	0	0%	1	1%	
T1	1	9%	7	6%	
T2	0	0%	7	6%	
T3	1	9%	7	6%	
T4	2	18%	10	9%	
N Stage					
NX	0	0%	1	1%	
N0	2	18%	23	21%	
N1	0	0%	4	4%	
N2	2	18%	4	4%	
M Stage					
MX	0	0%	0	0%	
M0	3	27%	30	27%	
M1	1	9%	2	2%	
UNKNOWN	6	60%	79	71%	

~Histology was centrally reviewed in 70/121 cases.

known gain-of-function category. There are, however, comparatively few documented gain-of-function *TP53* variants and this status would only be allocated following intensive functional laboratory investigation.

In addition to *TP53* mutations, this study aimed to evaluate the utility of *PIK3CA* mutations as a prognostic biomarker in SACC. Despite analysing almost the entire *PIK3CA* gene, we identified a lower than expected frequency of *PIK3CA* mutations, 2% compared to 5% in the cBioPortal dataset and 22% in a recently published institutional series [14]. This relatively low frequency in our cohort precluded any further clinical and pathological investigation. Although it is not possible formally to test this hypothesis within the current study, one possibility is that this relative difference in frequency may be related to the timing of the biopsy that was analysed in relation to the disease course. If, as has been clearly demonstrated, mutations are acquired during evolution from a primary tumour to a metastasis [8,35], analysis of tissue samples collected at metastasis may detect a higher frequency of genetic alterations compared with analysis of primary tumours.

There have been recent advances in the development of drug therapies in SACC with lenvatinib showing 15% response rates in a biomarker-unselected population [36]. However, there remains an urgent need for the development of new and effective drug therapies. Current approaches being studied in biomarker-selected patients include the gamma secretase inhibitor, AL101, in SACC with *NOTCH* gain-of-function mutations (NCT03691207), and all-*trans* retinoic acid (ATRA) is being studied in biomarker-unselected patients (NCT03999684). Although *TP53* mutation has been proposed as a putative biomarker to predict response to the Wee1 inhibitor, AZD1775, based on patients in early phase trials benefiting from this drug being enriched for the presence of *TP53* mutations [37], the utility of *TP53* mutations as a predictive biomarker is yet to be borne out.

In this cohort of SACC patients, we have shown co-existence of *TP53* mutations and *MYB* gene rearrangements suggesting that *TP53* mutation may make up a distinct sub-set of *MYB* rearranged SACC with a different disease course. Given the critical role of *TP53* in maintaining genomic integrity, ongoing studies are interrogating the hypothesis that *TP53* altered ACC defines a novel subset of SACC with a distinct mutational burden. Such a differential mutational landscape may be exploited to identify differential therapeutic approaches with both genetically targeted therapies and immune therapies.

Beyond providing an understanding of individual genomic drivers of cancer in isolation, clinically annotated biorepositories such as that developed for this study will be invaluable for understanding tumour-immune-microenvironment interactions in SACC. The development of immune checkpoint inhibitors has revolutionised the way cancer is treated; however, these immunotherapies are only effective in a subset of tumour types and significant responses to immune checkpoint blockade are rarely reported in SACC [38,39]. The challenge currently facing oncology is to design improved immunotherapies and treatment strategies that encompass more tumour types. Advancements in next-generation sequencing (bulk RNA-seq, single-cell RNA-seq and mass cytometry by time-of-flight (CyTOF)) have enabled researchers to gain unprecedented insight into the immune landscape of multiple human tumours, revealing potential biomarkers and targets for new immunotherapies [40–42]. The microenvironment of SACC has been shown to be

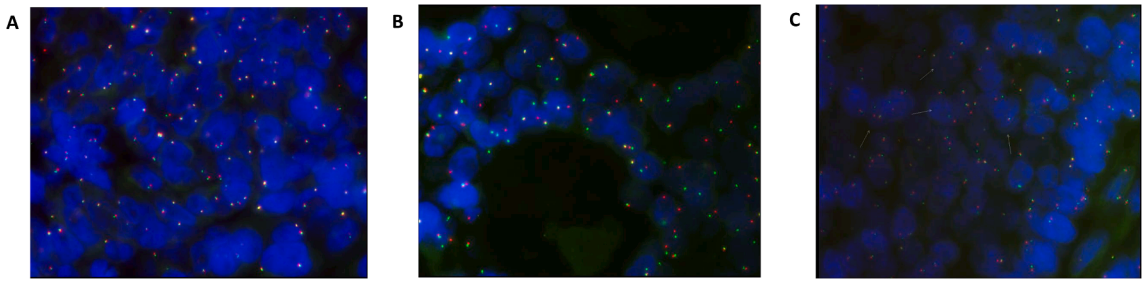

Fig. 2. (A) Cells show two intact fusion signals indicating no evidence of MYB gene rearrangement. (B) Cells show one intact fusion signal and clearly separated red and green signals, indicative of MYB gene rearrangement. (C) Cells show one intact fusion signal (i.e. red and green signals co-localised) and red and green signal separated by a small distance. This may be suggestive of an intrachromosomal rearrangement of MYB rather than a standard translocation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

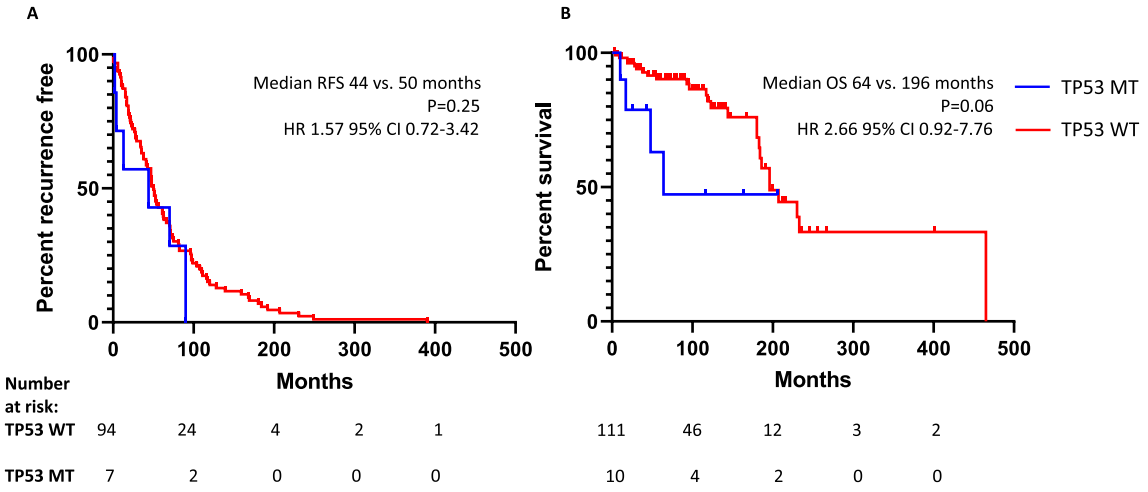

Fig. 3. (A) Kaplan-Meier estimates of recurrence free survival of adenoid cystic carcinoma patients without metastatic disease at diagnosis with TP53 mutation (n = 7) versus wild-type TP53 (n = 94). (B) Kaplan-Meier estimates of overall survival in patients with TP53 mutation (n = 10) versus wild-type (n = 111).

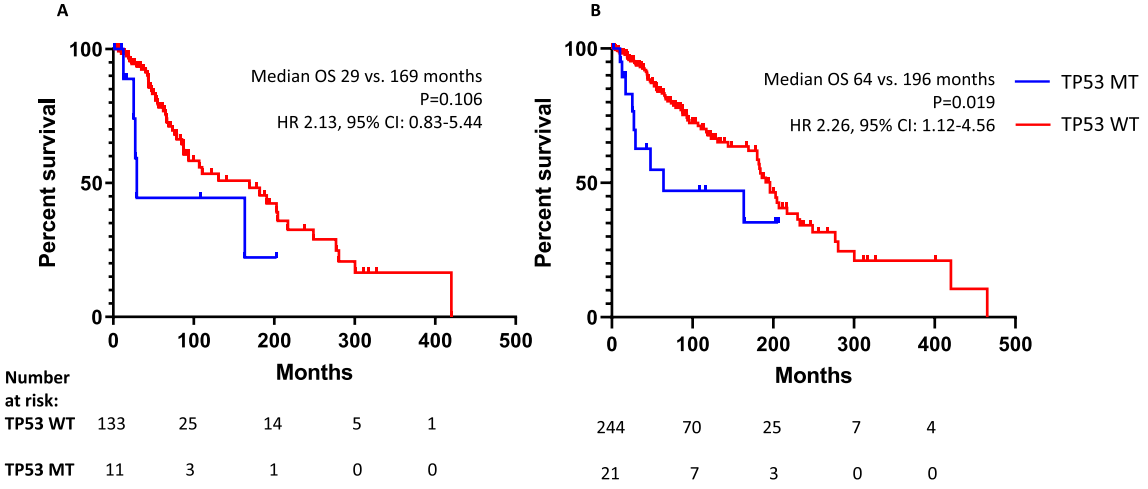

Fig. 4. (A) Kaplan-Meier estimates of overall survival for ACC patients by TP53 mutation status from cBioPortal (n = 144) in patients with TP53 mutation (n = 11) versus wild-type TP53 (n = 133). (B) Kaplan-Meier estimates of OS from combined data cBioPortal and UK data-sets in patients with TP53 mutation (n = 21) versus wild type (n = 265).

largely depleted of immune cells [43–45]. Using bulk RNA-seq SACC has been characterised by T-cell exclusion and an increased presence of myeloid-derived suppressor cells and M2-polarised macrophages, leading to an immunosuppressive microenvironment that correlates with tumour recurrence [46]. The rarity of fresh samples is a major challenge that faces SACC research. Large patient cohorts are required in order for correlations between clinical data and immune profiling to have sufficient statistical power, and findings in SACC studies that focus solely on fresh samples will likely be limited in their significance. The advent of Imaging Mass Cytometry, combining CyTOF with imaging, means biorepositories of FFPE tumours will be an invaluable resource to the salivary oncology field, enabling researchers to understand both the immune phenotype and spatial relationships occurring within the tumour microenvironment.

Regarding study limitations, the authors acknowledge that the cohort of patients included in the study were limited to those evaluated for trial therapies which could introduce the possibility of selection bias. Other variables such as advanced stage, radiotherapy resistance, lymph node involvement, slow kinetic of relapse and growth and high grade transformation have been shown to have a negative impact on prognosis [47]. Due to the large geographical nature of the data set, incomplete data for variables such as the TNM stage, resection margin, PNI and tumour grade lead to small numbers in sub-groups and subsequent multivariate analysis was not possible. This highlights the limitations of incomplete clinical data when analysing referrals from multiple cancer centres. Further limitations include the use of biopsy material analysed for solid component, *peri*-neural and *peri*-vascular invasion which may have underestimated the incidence.

Conclusion

In conclusion, we have shown when combining clinical and genomic data from the UK cohort from 66 cancer centres, with the cBioPortal clinical and genomic data, that TP53 mutation was associated with shorter overall survival in recurrent and metastatic salivary ACC. This study highlights the challenges associated with real-world clinical, genomic and pathological data acquisition, where limited data on multiple prognostic factors restricts the ability to perform multi-variate survival analysis. These findings raise the possibility that stratifying by genomic predictors of clinical outcomes may usefully inform follow-up strategy in addition to established clinical, pathological and genomic biomarkers. Further studies are required to translate these findings to clinical practice.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' Contributions

Clinical data collection was performed by HA, SR, BH and RM; genetic and pathology data collection was performed by AW and GB; cBioPortal analysis and visualisation was performed by SR; MYB cytogenetic analysis was performed by CH; all authors contributed to analysis and interpretation of the data; the manuscript was prepared by HA and RM and all authors contributed to the final version of the manuscript.

Ethics approval and consent to participate

This study has research ethics approval under the MCRC Biobank Research Tissue Bank Ethics (NHS NW Research Ethics Committee 18/NW/0092). All participants provided written informed consent to donate their samples to the MCRC Biobank. The study was performed in accordance with the Declaration of Helsinki.

Data availability

For our comparative analysis we used publicly available data from the Adenoid cystic carcinoma project (2019) hosted on cBioPortal https://www.cbioportal.org/study/summary?id=acc_2019. Clinical and genomic data generated though the course of the current study can be made available to approved researchers with appropriate ethical and legal agreements.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2020.105095.

References

[1] Ellington CL, Goodman M, Kono SA, Grist W, Wadswort T, Chen AY, et al. Adenoid cystic carcinoma of the head and neck Incidence and survival trends based on 1973–2007 Surveillance, Epidemiology, and End Results data. Cancer 2012;118(18):4444–51.

[2] Ganly I, Amit M, Kou L, Palmer FL, Migliacci J, Katabi N, et al. Nomograms for predicting survival and recurrence in patients with adenoid cystic carcinoma. An international collaborative study. Eur J Cancer 2015;51(18):2768–76.

[3] Drier Y, Cotton MJ, Williamson KE, Gillespie SM, Ryan RJH, Kluk MJ, et al. An oncogenic MYB feedback loop drives alternate cell fates in adenoid cystic carcinoma. Nat Genet 2016;48(3):265–72.

[4] West RB, Kong C, Clarke N, Gilks T, Lipstick J, Cao H, et al. MYB expression and translocation in adenoid cystic carcinomas and other salivary gland tumors with clinicopathologic correlation. Am J Surg Pathol 2011;35(1):92–9.

[5] Mitani Y, Li J, Rao PH, Zhao P-H, Bell D, Lippman SM, et al. Whole-genome sequencing of salivary gland adenoid cystic carcinoma. Clin Cancer Res 2010;16(19):4722–31.

[6] Almeida-Pinto YD, Santos Costa SF, Andrade BAB, Altemani A, Vargas PA, Abreu LG, et al. t(6;9)(MYB-NFIB) in head and neck adenoid cystic carcinoma: A systematic review with meta-analysis. Oral Dis. 2019 Jul;25(5):1277–82.

[7] Liu X, Chen D, Lao XL, Liang Y. The value of MYB as a prognostic marker for adenoid cystic carcinoma: Meta-analysis. Head Neck 2019 May;41(5):1517–24.

[8] Ho AS, Ochoa A, Jayakumaran G, Zehir A, Mayor C-V, Tepe J, et al. Genetic hallmarks of recurrent/metastatic adenoid cystic carcinoma. J Clin Investig 2019;129(10):4276–89.

[9] Ferrarotto R, Mitani Y, Diao L, Guijarro I, Wang J, Zweidler-McKay P, et al. Activating NOTCH1 mutations define a distinct subgroup of patients with adenoid cystic carcinoma who have poor prognosis, propensity to bone and liver metastasis, and potential responsiveness to Notch1 inhibitors. J Clin Oncol 2016;35(3):352–60.

[10] Sajed DP, Faquin WC, Carey C, Severson EA, Afrogheh A, Johnson C, et al. Diffuse staining for activated NOTCH1 correlates with NOTCH1 mutation status and is associated with worse outcome in adenoid cystic carcinoma. Am J Surg Pathol 2017;41(11):1473–82.

[11] Stephens PJ, Davies HR, Mitani Y, Van Loo P, Shlien A, Tarpey PS, et al. Whole exome sequencing of adenoid cystic carcinoma. J Clin Invest 2013;123:2965–8.

[12] Ho AS, Kannan K, Roy DM, Morris LG, Ganly I, Katabi N, et al. The mutational landscape of adenoid cystic carcinoma. Nat Genet 2013;45:791–8.

[13] Ross JS, Wang K, Rand JV, Sheehan CE, Jennings TA, Al-Rohil RN, et al. Comprehensive genomic profiling of relapsed and metastatic adenoid cystic carcinomas by next-generation sequencing reveals potential new routes to targeted therapies. Am J Surg Pathol 2014;38:235–8.

[14] Hanna GJ, Bae JE, Lorch JH, Scoenfeld JD, Tishler RB MDN, et al. Long-term outcomes and clinicogenomic correlates in recurrent, metastatic adenoid cystic carcinoma. Oral Oncol 2020;106:104690.

[15] Richards S, Aziz N, Bale S, Bick D, Das S, Gasteir-Foster G, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet Med 2015;17(5):405–24.

[16] Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer. J Mol Diagn 2017;19(1):4–23.

[17] Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013;31(11):1023–31.

[18] Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: the catalogue of somatic mutations in cancer. Nucleic Acids Res 2019, 2019,;47(D1):D941–7.

[19] Cancer.sanger.ac.uk [Internet] COSMIC v91, released 07-APR-20. Available from: https://cancer.sanger.ac.uk/cosmic.

[20] Bhayani MK, Yener M, El-Naggar A, Garden A, Hanna EY, Weber RS, et al. Prognosis and risk factors for early-stage adenoid cystic carcinoma of the major salivary glands. Cancer 2012;118(11):2872–8.

[21] Perzin KH, Gullane P, Clairmont AC. Adenoid cystic carcinomas arising in salivary glands; a correlation of histologic features and clinical course. Cancer 1978;42:265–82.

[22] Szanto PA, Luna MA, Tortoledo ME, White RA. Histologic grading of adenoid cystic carcinoma of the salivary glands. Cancer 1984;54(6):1062–9.

[23] Sung MW, Kim KH, Kim JW, Min YG, Seong WJ, Roh JL, et al. Clinicopathologic predictors and impact of distant metastasis from adenoid cystic carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 2003;129(11):1193–7.

[24] Dantas AN, Morais EF, Paiva Macedo RA, Tinoco JM, Morais M. Clinicopathological characteristics and perineural invasion in adenoid cystic carcinoma: a systematic review. Brazilian J Otorhinolaryngol 2015;81(3):329–35.

[25] Amit M, Eran A, Billan S, Fridman E, Na'ara S, Charas T, et al. Perineural spread in noncutaneous head and neck cancer: new insights into an old problem. J Neurol Surgery Part B, Skull Base 2016;77(2):86–95.

[26] Martins-Andrade B, Santos-Costa S, Santana M, Altemani A, Vargas P, Fregnani E, et al. Prognostic importance of the lymphovascular invasion in head and neck adenoid cystic carcinoma: A systematic review and meta-analysis. Oral Oncol 2019;93:52–8.

[27] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discovery May 2012;2:40.

[28] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. 2013;6:pl1.

[29] Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008;455(7216):1069–75.

[30] Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature 2009;458 (7239):719–24.
[31] Gao J, Aksoy BA, Dogrusoz U, Dresdener G, Gross B, Sumer O, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6(269):p11.
[32] Shirole NH, Pal D, Kastenhuber ER, Senturk S, Boroda J, Pisterzi P, et al. TP53 exon-6 truncating mutations produce separation of function isoforms with pro-tumorigenic functions. Elife. 2016;5:e17929. https://doi.org/10.7554/eLife.17929.
[33] Aschauer L, Muller PAJ. Novel targets and interaction partners of mutant p53 Gain-Of-Function. Biochem Soc Trans 2016;44(2):460–6.
[34] Kastenhuber ER, Lowe SW. Putting p53 in Context. Cell 2017;170(6):1062–78.
[35] Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. NEJM 2012;366(10):883–92.
[36] Tchekmedyian V, Sherman EJ, Dunn L, Tran C, Baxi S, Katabi N, et al. Phase II study of lenvatinib in patients with progressive, recurrent or metastatic adenoid cystic carcinoma. J Clin Oncol 2019;37(18):1529–37.
[37] Bauer TM, Jones SF, Greenlees C, Cook C, Jewsburry PJ, Mugundu G, et al. A Phase 1b, open-label, multi- cener study to assess the safety, tolerability, pharmacokinetics, and anti-tumour activity of AZD1775 monotherapy in patients with advanced solid tumours: expansion cohorts. J Clin Oncol 2016;34(15_suppl). TPS2608-TPS2608.
[38] Schoenfeld JD, Mahmood U, Chen Y-H, Mak RH, Lorch JH, Hanna GJ, et al. A randomized phase II study of pembrolizumab with or without radiation in patients with recurrent or metastatic adenoid cystic carcinoma. J Clin Oncol 2019; 37(15_suppl). 6082–6082.
[39] Tchekmedyian V, Sherman EJ, Dunn L, Fetten JV, Michel LS, Kriplani A, et al. A phase II trial cohort of nivolumab plus ipilimumab in patients (Pts) with recurrent/metastatic adenoid cystic carcinoma (R/M ACC). J Clin Oncol 2019;37 (15_suppl). 6084–6084.
[40] Chevrier S, Levine JH, Zanotelli VRT, Silina K, Schulz D, Bacac M, et al. An Immune Atlas of Clear Cell Renal Cell Carcinoma. Cell 2017;169(4):736–49.
[41] Lavin Y, Kobayashi S, Leader A, Amir ED, Elefant N, Bigenwald C, et al. Innate Immune Landscape in Early Lung Adenocarcinoma by Paired Single-Cell Analyses. Cell 2017;169(4):750–65.
[42] Friebel E, Kapolou K, Unger S, Nunez NG, Utz Srushing EJ, et al. Single-Cell Mapping of Human Brain Cancer Reveals Tumor-Specific Instruction of Tissue-Invading Leukocytes. Cell 2020;181(7):1626–42.
[43] Mosconi C, Almeida de Arruda JA, Rodrigues de Farais AC, Oliveira GAQ, Moura de Paula H, Fonesca FP, et al. Immune Microenvironment and Evasion Mechanisms in Adenoid Cystic Carcinomas of Salivary Glands. Oral Oncol 2019;88:95–101.
[44] Chang H, Kim JS, Choi YJ, Cho J-G, Woo J-S, Kim A, et al. Overexpression of PD-L2 is associated with shorter relapse free survival in patients with malignant salivary gland tumors. Onco Targets Ther 2017;10:2983–92.
[45] Sridharan V, Gjini E, Liao X, Chau NG, Haddad RI, Severgnini M, et al. Immune Profiling of Adenoid Cystic Carcinoma: PD-L2 Expression and Associations With Tumor-Infiltrating Lymphocytes. Cancer Immunol Res 2016;4(8):679–87.
[46] Linxweiler M, Kuo F, Katabi N, Lee M, Nadeem Z, Dalin MG, et al. The Immune Microenvironment and Neoantigen Landscape of Aggressive Salivary Gland Carcinomas Differ by Subtype. Clin Cancer Res 2020. https://doi.org/10.1158/1078-0432.ccr-19-3758.
[47] Jang S, Patel PN, Kimple RJ, McCulloch TM. Clinical outcomes and Prognostic Factors of Adenoid Cystic Carcinoma of the Head and Neck. Anticancer Res 2017; 37(6):3045–52.