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Molecular Case Studies

RESEARCH ARTICLE

Personalized oncogenomic analysis of metastatic adenoid cystic carcinoma: using whole-genome sequencing to inform clinical decision-making

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Abstract Metastatic adenoid cystic carcinomas (ACCs) can cause significant morbidity and mortality. Because of their slow growth and relative rarity, there is limited evidence for systemic therapy regimens. Recently, molecular profiling studies have begun to reveal the genetic landscape of these poorly understood cancers, and new treatment possibilities are beginning to emerge. The objective is to use whole-genome and transcriptome sequencing and analysis to better understand the genetic alterations underlying the pathology of metastatic and rare ACCs and determine potentially actionable therapeutic targets. We report five cases of metastatic ACC, not originating in the salivary glands, in patients enrolled in the Personalized Oncogenomics (POG) Program at the BC Cancer Agency. Genomic work-up included whole-genome and transcriptome sequencing, detailed analysis of tumor alterations, and integration with existing knowledge of drug–target combinations to identify potential therapeutic targets. Analysis reveals low mutational burden in these five ACC cases, and mutation signatures that are commonly observed in multiple cancer types. Notably, the only recurrent structural aberration identified was the well-described *MYB-NFIB* fusion that was present in four of five cases, and one case exhibited a closely related *MYBL1-NFIB* fusion. Recurrent mutations were also identified in BAP1 and BCOR, with additional mutations in individual samples affecting NOTCH1 and the epigenetic regulators ARID2, SMARCA2, and SMARCB1. Copy changes were rare, and they included amplification of MYC and homozygous loss of CDKN2A in individual samples. Genomic analysis revealed therapeutic targets in all five cases and served to inform a therapeutic choice in three of the cases to date.

[Supplemental material is available for this article.]

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INTRODUCTION

Adenoid cystic carcinomas (ACCs) are relatively uncommon malignant epithelial neoplasms that can arise in a variety of gland-bearing organs most commonly in the head and neck region. They account for ~1% of all head and neck malignancies and 10% of salivary gland tumors (Coca-Pelaz et al. 2015). ACCs demonstrate three main histologic patterns: solid, cribriform, and tubular (Cheng et al. 1992). Low-grade (grade 1–2) ACCs exhibit a combination of cribriform and tubular patterns and are associated with slower rates of disease progression, including a 5-yr survival rate of ~90%, with <40% survival at 15 yr. Conversely, high-grade (grade 3) ACC, based on the presence of a significant proportion of tumor showing solid growth pattern, is associated with advanced-stage and more frequent development of late-onset distant metastases (Liu et al. 2012).

Because of the typically slow growth pattern of ACC, historically it has been difficult to assess the efficacy of systemic therapy in metastatic disease, and at present there are no reliable therapeutic options for long-term disease control of ACC (Chae et al. 2015; Coca-Pelaz et al. 2015). Molecular and genome-sequencing studies have begun to elucidate the genetic landscape of ACC in efforts to understand its pathophysiology and potentially alter its clinical course (Ross et al. 2014; Bell et al. 2016; Rettig et al. 2016).

The use of whole-genome sequencing to inform clinical decision-making is an active area of research. Through the Personalized Oncogenomics project (POG) at the British Columbia Cancer Agency (Vancouver, Canada), we have established a pipeline that generates a comprehensive molecular analysis of individual cancer patients with advanced malignancies (Laskin et al. 2015). The aims of these analyses are both to further elucidate and catalog cancer genomes in a metastatic setting and to provide patients with rational treatment options by using genomic data to identify available systemic therapies or active clinical trials. By performing whole-genome and transcriptome sequencing of five patients with ACC, we confirmed a role for the well-characterized *MYB*- and *MYBL1-NFIB* gene fusions in ACC pathogenesis and identified actionable targets that helped to inform therapy decisions in three of the five patients.

RESULTS

Clinical and Histological Data

Between 2012 and September 2017, five patients with ACC were enrolled in POG with an average age of 46.2 (SD 9.68) at time of diagnosis. Of the five cases, three patients presented with tumors originating from the head and neck. The other two cases presented with primary tumors at the trachea (POG 1 and POG 2), which are rare but described in the literature (Gondivkar et al. 2011). Interestingly, none of our cases had tumors originating from the major or minor salivary glands, though POG 3 with primary ACC of the hard palate had evidence of invasion into the salivary gland. All patients had evidence of metastatic disease prior to POG analysis. Primary treatments included localized debulking surgery (in four of five cases, including laser bronchoscopy in POG 1) and radiation therapy (in all five cases, including brachytherapy in POG 1). Patient 4 received systemic therapy involving vinorelbine followed by gemcitabine and carboplatin prior to enrollment in the POG program. The average time from diagnosis to enrollment in POG was 8.6 yr ranging from 1 to 24 yr (SD 9.07), thereby highlighting the indolent but persistent nature of ACCs (Table 1).

Biopsies were obtained from either primary site recurrences or metastases for POG analysis. Notably, histopathologic evaluation of our samples correlated with their clinical course. For example, biopsy of POG 1 revealed a grade 2 ACC characterized by cribriform and

Table 1. Patient characteristics

Patient	Sex	Age at Dx	Year of Dx	Location of primary	Metastases sites	Treatment before POG biopsy	Year of POG consult	Biopsy site
1	M	45	2009	Distal trachea	Lung	Multiple laser bronchoscopies, XRT (45 Gy), brachytherapy	2013	Trachea
2	F	44	1991	Trachea	Lung	Partial tracheotomy, XRT (55 Gy), lung met wedge resection	2015	Lung nodule
3	M	34	2007	Right hard palate	Lung	Resection, XRT	2016	Lung nodule
4	M	61	2015	Left maxillary sinus/orbit	Lung, liver, T11 vertebra	Left orbital exenteration and reconstruction, XRT (60 Gy)	2016	Liver
5	M	47	2011	Floor of mouth	Tongue, mylohyoid, myoglossus, lung	XRT (70 Gy)	2016	Floor of mouth

Dx, diagnosis; POG, Personalized Oncogenomics program; XRT, radiotherapy.

tubular patterns, and no evidence of perineural or lymphovascular invasion, which correlates with slower progression over several years (Fig. 1A). Conversely, biopsy of POG 4 revealed a grade 3 ACC characterized by cribriform and solid patterns as well as extensive perineural and lymphovascular invasion, which is reflected in its rapid progression and distant metastases to the bone (Fig. 1B).

Mutational and Expression Landscape of ACC

Whole-genome and transcriptome sequencing was performed on the five samples, and we identified somatic mutations in 112 genes among the cases (Supplemental Table 1). An average of 21.40 protein coding single-nucleotide variants (SNVs) were identified between samples (ranging from 12 to 39). The number of protein coding small insertions and deletions (indels) ranged from one to seven across the five samples, with an average of 3.40. Notably, POG 5 had a considerably higher number of SNVs than the other samples (Fig.

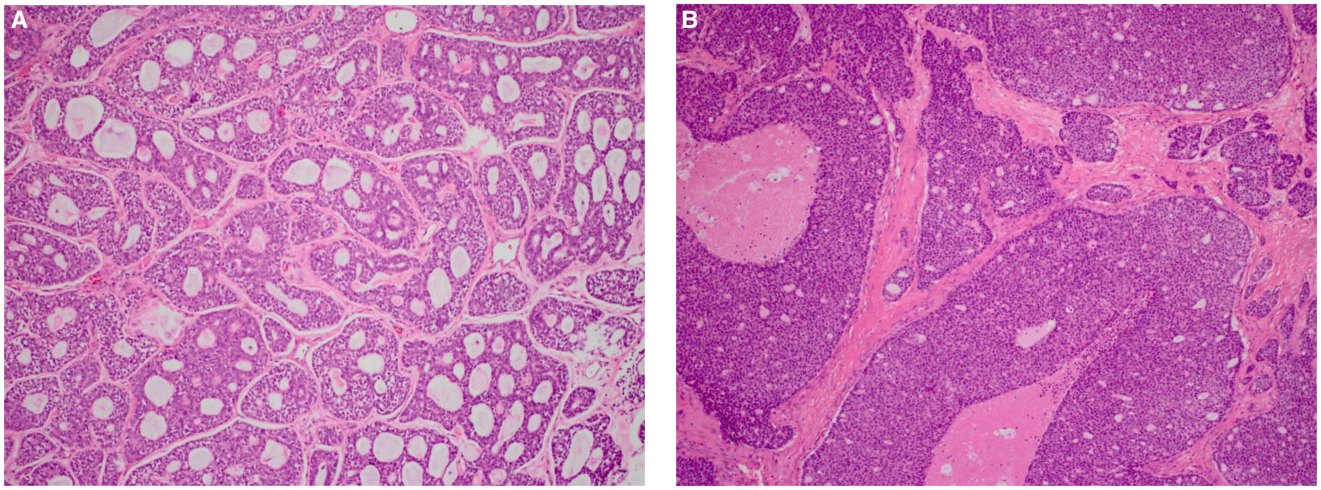

Figure 1. Hematoxylin and eosin staining images of representative POG ACC cases at 100× original magnification: (*A*) POG 1, (*B*) POG 2.

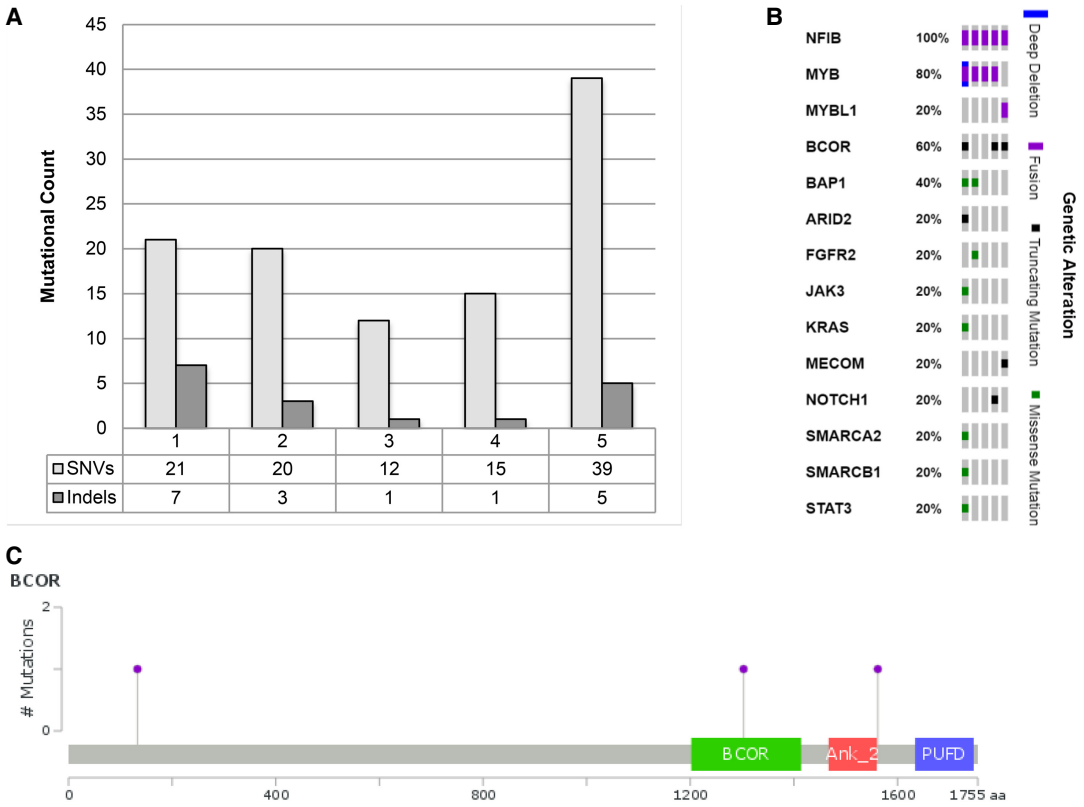

Figure 2. Mutational landscape of ACC. (*A*) Number of single-nucleotide variations (SNVs) and short insertion and deletions (indels) in five cases of ACC. (*B*) Oncoprint depicting small mutations and recurrent fusions in known cancer-related genes, POG1 to POG5 from *right* to *left*. (*C*) Frameshifting mutations observed in BCOR occur throughout the protein, at amino acids 133, 1303, and 1562.

2A). The only genes identified with greater than one mutation among these five samples were *BAP1*, manifesting as SNVs in POG 4 and POG 5, and *BCOR*, manifesting as a truncating SNV in POG 2 and frameshifting indels in POG 1 and POG 5 (Fig. 2B). Notably, BCOR is mutated in <15% of tumors of any type across TCGA, but contains putative loss-of-function mutations in 60% (3 of 5) of samples in our cohort (Fig. 2C). *BAP1* is a tumor suppressor that encodes a deubiquitinating enzyme regulating key cellular pathways (Wang et al. 2016), whereas *BCOR* is a *BCL6* corepressor mutated in various sarcomas and hematological diseases (Tanaka et al. 2017).

Despite the low frequency of mutations, sequencing of the entire genomes produces sufficient variants for analysis of mutational signatures (Alexandrov et al. 2013) in ACC. All five cases showed evidence of C>T base changes consistent with deamination associated with natural aging processes (Table 2; Supplemental Fig. 1A). In addition, all but one case showed evidence of signature 5, which has an unknown etiology but is commonly observed across cancer types. Furthermore, four of five cases showed evidence for signature 16, also with no known association. Examining the relationship between signature probabilities, we observed a significant anticorrelation between signatures 5 and 16 in all four cases with elevated signature 16, suggesting bleed of signal between these two similar mutation signatures (Supplemental Fig. 1B; Spearman $\rho < -0.6$). This provides evidence that the presence of signature 16 may be driven by signature 5 elevation rather than representing an additional source of mutations.

Table 2. Variant table

Sample	Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	dbSNP/ COSMIC ID	Genotype
POG 1	*BCOR*	X	c.398_399insAC	p.Ala134fs	ins	Frameshift		Homozygous
POG 1	*MECOM*	3	c.1636C>T	p.Gln546*	snv	Truncating		Heterozygous
POG 2	*BCOR*	X	c.3961G>T	p.Glu1321*	snv	Truncating		Heterozygous
POG 2	*NOTCH1*	9	c.2825G>A	p.Cys942Tyr	snv	Missense		Heterozygous
POG 2	*NOTCH1*	9	c.4045_4052del GCTCGTAC	p.Ala1349fs	del	Frameshift		Heterozygous
POG 4	*BAP1*	3	c.188C>G	p.Ser63Cys	snv	Missense	COSM96362	Homozygous
POG 4	*FGFR2*	10	c.1144T>C	p.Cys382Arg	snv	Missense	rs121913474; COSM915496	Heterozygous
POG 5	*ARID2*	12	c.2989C>T	p.Gln997*	snv	Truncating		Heterozygous
POG 5	*BAP1*	3	c.374A>C	p.Glu125Ala	snv	Missense		Heterozygous
POG 5	*BCOR*	X	c.4685_4700del GCACTTGGGACTTCTA	p.Gly1562fs	del	Frameshift		Heterozygous
POG 5	*JAK3*	19	c.1765G>A	p.Gly589Ser	snv	Missense		Heterozygous
POG 5	*KRAS*	12	c.34G>C	p.Gly12Arg	snv	Missense	rs121913530	Heterozygous
POG 5	*SMARCA2*	9	c.3638G>C	p.Arg1213Pro	snv	Missense		Heterozygous
POG 5	*SMARCB1*	22	c.110G>C	p.Arg37Pro	snv	Missense		Heterozygous
POG 5	*STAT3*	17	c.1467T>A	p.Asn489Lys	snv	Missense		Heterozygous

HGVS, Human Genome Variation Society; dbSNP, Single Nucleotide Polymorphism database; COSMIC, Catalogue of Somatic Mutations in Cancer; ins, insertion; snv, single-nucleotide variant; del, deletion.

In addition to a low mutation load, we observed only a low level of copy-number alterations in these tumors, with genomes of three samples predominantly copy neutral with small numbers of deletions, and POG 4 and POG 5 showing in addition some limited regions of amplification (Supplemental Fig. 2). No genes were subject to homozygous deletion or amplification in more than one sample. POG 4 contained a homozygous deletion of CDKN2A/B (p16) and corresponding decrease in expression. POG 5 showed amplification on 8q24 containing MYC and associated increased MYC expression; MYC also showed increased expression in POG 2 and POG 4 but without associated amplification.

We additionally compared gene expression in our five samples to 40 different primary tumor types, including 37 primary (sub)types from the Cancer Genome Atlas (TCGA) (The Cancer Genome Atlas Research Network et al. 2013). The frequency of mutations in our cases was notably low, with percentiles of 36%, 29%, 21%, and 22% for samples 1–4, respectively, relative to TCGA. Sample 5 had a higher mutation rate, presenting in the 65th percentile relative to TCGA tumors. Of note, this comparator set of cancers does not contain any samples of ACC. However, four of the five samples showed similarity to primary breast cancer (BRCA) despite originating from different tissues (Supplemental Fig. 3). POG 3 showed a similar pattern but with increased similarity to thoracic tumors, likely corresponding to the lung nodule biopsy site of this tumor. To examine further the association with breast cancer, we used the AIMS classifier (Paquet and Hallett 2015) to determine if any of the ACCs had further similarities to specific breast cancer subtypes. We found that POG 1 and POG 4 were classified as basal, corresponding to mostly triple negative breast tumors.

Together, this analysis demonstrates that there are no striking mutational processes active in ACC, consistent with the low mutational burden in these tumors. Furthermore, the lack of characterization of ACC in TCGA demonstrates a rationale for in-depth genomic analysis of ACC.

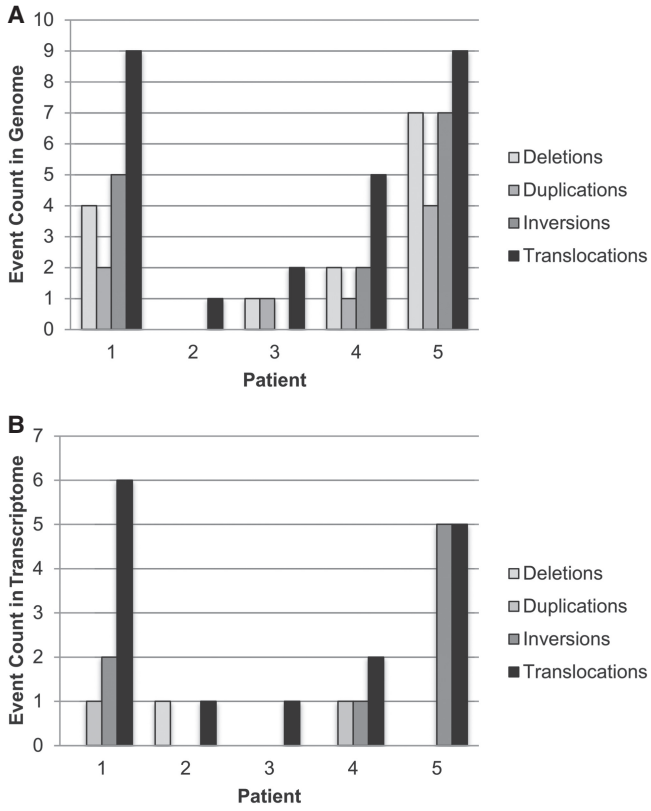

Figure 3. Structural variants in the (*A*) genome and (*B*) transcriptome of five cases of ACC.

Structural Variations

To evaluate structural variations in our five samples, we assessed gene fusion products at the genomic and transcriptomic level (Fig. 3A,B). Previous work has implicated the t(6;9) translocation between the *MYB* oncogene and the transcription factor *NFIB* as a prominent driver of ACC pathophysiology found in the majority of tumors (Mitani et al. 2010; Gonda and Ramsay 2016). Our analysis elucidated that four of five samples exhibited the *MYB-NFIB* translocation at the genome and transcriptome level, with POG 3, POG 4, and POG 5 creating identical fusion products with chromosomal breaks after exon 8 of *MYB* (Fig. 4A). POG 2 also harbored the *MYB-NFIB* translocation, but with the chromosomal break after exon 12 (Fig. 4B). Of note, POG 1 exhibited a more recently characterized t(8;9) translocation between *MYBL1*, a distinct *MYB*-family transcription factor, and *NFIB*, demonstrated in both the genome and transcriptome sequencing (Fig. 4C; Brayer et al. 2016; Mitani et al. 2016). Interestingly, the *MYB-NFIB* fusion product was the only translocation present in the transcriptomes of POG 2 and 3. Furthermore, *MYB, MYBL1,* and *NFIB* were not involved in any structural variations with other genes in our cases. Our data therefore confirms the importance of the *MYB-NFIB* translocation in the pathogenesis of ACC, as a variation of the fusion that was present in the transcriptome of all of our cases.

Identification of Potential Therapeutic Targets and Clinical Outcomes

Targeted therapies for advanced ACC are currently under investigation in early phase clinical trials. Because of the rarity of ACC and the relatively low mutational burden, most of drugs in trials have been selected based on observations from preclinical studies. Table 3 highlights

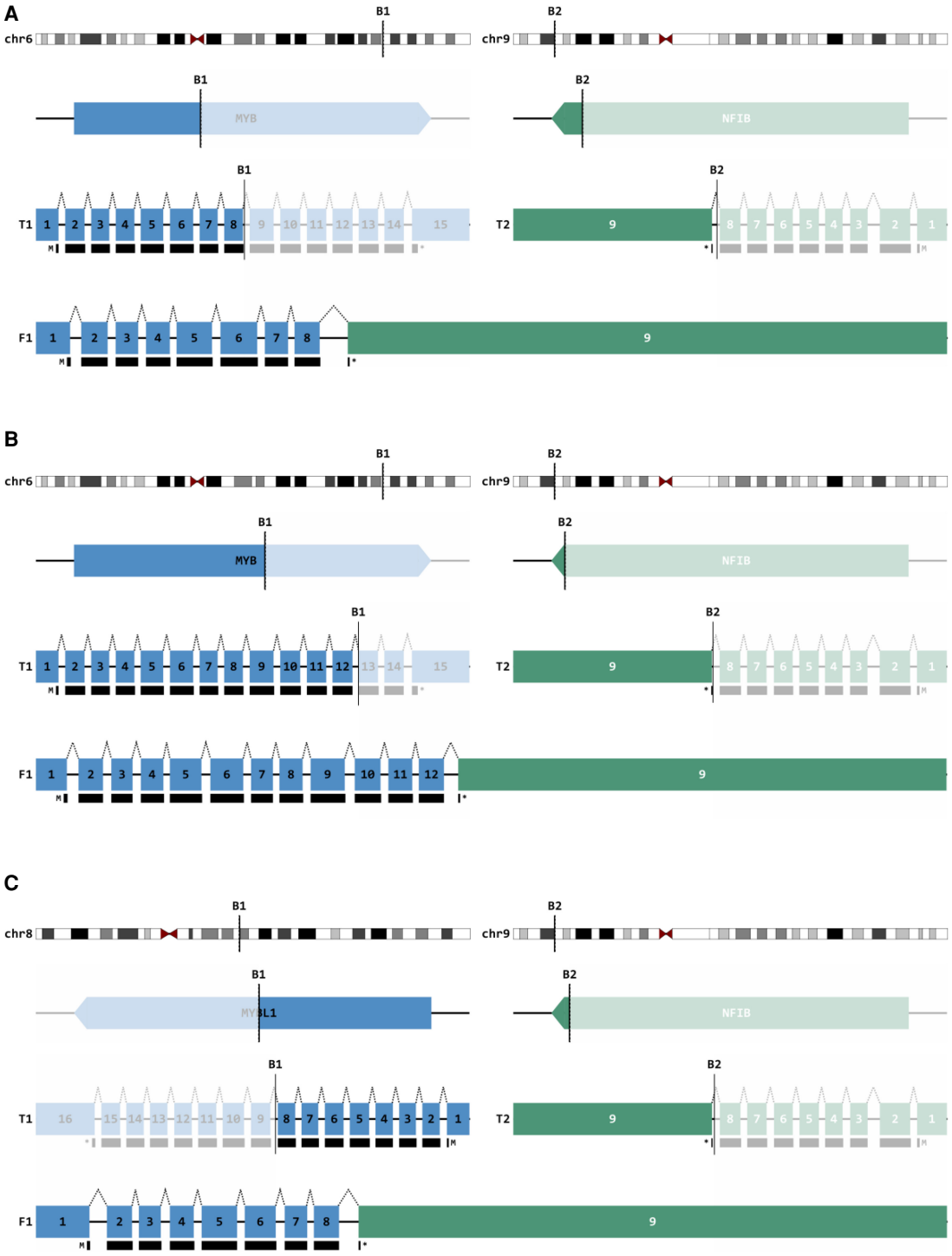

Figure 4. *MYB-NFIB* fusions. (*A*) Schematic representation of the well-characterized *MYB-NFIB* fusion (t[6;9]) identified in both the genome and transcriptome of POG 3. POG 4 and 5 created completely identical fusion products with breaks in the same introns. (*B*) Schematic representation of *MYB-NFIB* fusion in POG 2, with breakpoint at exon 12. (*C*) Schematic representation of the lesser characterized *MYBL1-NFIB* fusion (t[8;9]) identified in POG 1 involving a related, but distinct *MYB*-family transcription factor. Blue, MYB or MYBL1; green, NFIB; numbered boxes, exons; black boxes, protein coding region; F1, predicted fusion product.

Table 3. Phase II trials of targeted agents in adenoid cystic carcinoma

Agent	Molecular target	Authors	*N*	Partial response	Stable disease
Imatinib	c-KIT CD117	Hotte et al. 2005	10	0	2 (20%)
Lapatanib	HER-2, EGFR	Agulnik et al. 2007	19	0	15 (79%)
Cetuximab	EGFR	Locati et al. 2009	23	0	20 (87%)
Bortezomib	Proteosome inhibitor	Argiris et al. 2011	25	0	16 (64%)
Sunitinib	VEGFR, c-KIT, PDGFR	Chau et al. 2012	13	0	11 (62%)
Gefitinib	EGFR TKI	Jakob et al. 2015	19	0	13 (68%)
Axitinib	VEGFR, KIT, PDGFR	Ho et al. 2016	33	3 (9%)	25 (76%)
Dasatinib	c-KIT, PDGFR	Wong et al. 2016	40 (ACC)	1 (2.5%)	20 (50%)
Nintedanib	VEGFR, FGFR, PDGFR	Kim et al. 2017	20	0	15 (75%)
Vorinostat	Histone deacetylase inhibitor	Goncalves et al. 2017	30	2 (6.7%)	27 (90%)
Dovitinib	VEGFR, PDGFR, FGFR, c-KIT, CSF-1R	Dillon et al. 2017	34	2 (6%)	22 (65%)

HER-2, human epidermal receptor-2; EGFR, epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; TKI, tyrosine kinase inhibitor; FGFR, fibroblast growth factor receptor; CSF-1R, colony stimulating factor-1 receptor.

some of the targeted agents investigated in phase II trials for advanced ACC. Of note, there were no trials in which complete response was established.

POG analysis in all five of our ACC cases yielded informative and actionable results, meaning that potential targets or risk factors that could affect treatment plans were identified for all five patients (Table 4). We elucidated potential therapeutic targets by identifying mutated genes or genes that were overexpressed relative to TCGA and through subsequent literature review for drug-targeted combinations. Results were deemed "actionable" by an interdisciplinary tumor board based on options that are clinically available and relevant to the patient as determined by tumor characteristics, patient characteristics (including performance status), and drug availability. A summary was created that allowed clinicians involved to make a treatment decisions (Laskin et al. 2015). Three of the five patients have thus far received POG-informed systemic therapy.

Table 4. POG analyses in all five adenoid cystic carcinoma cases yielded informative and actionable results

Patient	Potential therapeutic targets	Actionable targets	Action taken
1	*KIT, FGFR1/2/L1, CDK1/B1/3/6, GADD45B, CCNB, PLK1, TGFA, EGFR, ERBB2, TGFA, AREG, TUBA1A/A1B/A1C, BIRC5, HMGA1*	*FGFR1/2/L1* overexpression	Dovitinib
2	*CDK4/6, CCND1, MYC, ROS1, NTRK3, EIGF1R, GFR, KIT, FGFR1/2, PDGFRA, MET, SMO, GLI3, JAG2, NOTCH1, BCL2, FZD7, BCL2*	*NOTCH1* overexpression	BMS-986115 (P1T986115)
3	*MYB, NFIB, EGFR, MET, FGFR1/2, ROS1, CDK6, MAP2K2, MMP7, FZD7*	*MET* overexpression	MGCD265 (glesatinib)
4	*ARFGEF3, CDKN2A/N2B, IGF1R, MYC, PRAME, SKP2, SCL29A1, FGFR1/2, KIT, ALK, RAS, RAF, NOTCH1, CTNNB1, WNT/FZD*	*FGFR2* gain of function	None (poor ECOG status)
5	*FOLR1, IGF2, KRAS, MDK, MYC, PRAME, PVRL4, SKP2, PDGFA/B/C, FGFR1/2, KIT, IGF2, NOTCH1, HES1/4, FZD6/7, MMP7*	Aberrations in epigenetic regulators; *FGFR1/2* overexpression	Pending; potential for HDAC or FGFR antibody therapy

Potential targets or risk factors that could affect treatment plans were identified for all five patients. Three of five patients have thus far received POG-informed systemic treatment.

Based on overexpression of *FGFR*-related genes as determined by POG analysis, and preclinical data suggesting partial response to FGFR inhibition in xenograft models of ACC (Frierson and Moskaluk 2013), Patient 1 was enrolled in a phase II clinical trial with dovitinib (TK1258), a multitargeted tyrosine kinase inhibitor with action against FGFRs 1–3, VEGFR, PDGFR, and c-KIT among other receptor kinases (Chase et al. 2007). This patient withdrew from the trial after 4 months because of intolerable side effects, despite having stable disease (as measured by <20% change in tumor size on CT scan). The patient subsequently underwent further palliative radiotherapy before transitioning to palliative care.

POG analysis of Patient 2 did not suggest any commercially available targeted therapies. However, multiple targets with potentially experimental therapies were elucidated. Analysis revealed a NOTCH1 truncating deletion at amino acid 1349, a single base mutation C942Y of unknown effect, *NOTCH1* pathway up-regulation, and up-regulation of downstream targets including *BCL2, MYC*, and *CCND1*. NOTCH1 signaling has been shown to occur frequently in ACC and may contribute to cell growth and metastasis in these tumors (Su et al. 2014; Morris et al. 2016). Accordingly, Patient 2 was enrolled in a phase I clinical trial with BMS-986115 (Bristol Myers Squibb), a γ secretase and pan-NOTCH inhibitor. Dose reduction was required early, and the patient withdrew from the trial because of toxicity after 2 months and before a response could be determined.

Patient 3 exhibited aberrations in *MET* expression and was subsequently enrolled in a phase I clinical trial with MGCD265, a multitarget and ATP-competitive inhibitor of c-MET and VEGFR1/2/3 that has recently been studied in lung cancer models (Engstrom et al. 2017). The patient remained clinically stable for several months while on the study, but was removed after 8 months because of increasing tumor size.

Potential therapeutic targets in phase 1 trials were identified for Patient 4, but the patient was not offered POG-mediated intervention because of declining functional status. POG analysis of Patient 5 revealed aberrations in multiple epigenetic modifiers including ARID2, SMARCA2, and SMARCB1 that would suggest a role for intervention with histone deacetylase inhibitors such as vorinostat, which has recently been studied in ACC in a phase II trial (Goncalves et al. 2017). This patient continues to have stable disease and therefore has not yet undergone any POG-informed therapeutic intervention.

DISCUSSION

Several recent studies have investigated the molecular landscape of ACC with a focus on primary tumors, yet there remains a gap in knowledge regarding characterization of recurrent and metastatic ACC and how to treat these tumors. Through the Personalized Oncogenomics project we have elucidated recurrent mutations among five cases of metastatic ACC and identified therapeutic targets that were implemented into clinical decisions guiding treatment for three of the five cases. It is unfortunate that the drugs in available trials were highly toxic and thus the true effect of inhibiting these active pathways remains unclear.

Approximately 55%–60% of ACCs originate in the head and neck, with the majority arising from the major salivary glands (Khafif et al. 2005; Ross et al. 2014). Accordingly, several molecular sequencing studies of ACC have focused on these subtypes (Stephens et al. 2013; Bell et al. 2016; Morris et al. 2016). Interestingly, none of our patients had cancers derived from the major salivary gland. Two cases originated from the mid respiratory tract, which account for ~20% of ACCs (Gondivkar et al. 2011). Though our sample size is insufficient to generate broad conclusions, our genomic and transcriptomic analysis help to further characterize less represented subtypes of this relatively rare cancer.

The five cases presented exhibit a low mutational burden in keeping with previous studies (Ho et al. 2013; Morris et al. 2016). Indeed, analysis of mutational signatures in our

samples did not elucidate any known mutational process driving tumor growth beyond those commonly observed in many cancer types. Interestingly, however, signature 5 is abundant despite the fact that it has not been found to correlate with age in head and neck cancers (Alexandrov et al. 2015). This, alongside the bleed between signatures 5 and 16, may suggest a yet uncharacterized mutational process or a different role of signature 5 in ACC. Despite infrequency of mutations, whole-genome and transcriptome analyses have revealed a number of alterations that may contribute to ACC tumorigenesis including mutations in *NOTCH1* (Rettig et al. 2016) and *SMARCA2* (Stephens et al. 2013; Chae et al. 2015). Our study is the first to recognize recurrent mutations in *BAP1* and *BCOR* in ACC and suggests that these alterations may play a role in the pathogenesis of metastatic ACC. *BAP1* gene mutations have been implicated in various malignancies including uveal and cutaneous melanoma, renal cell carcinoma, and mesothelioma (Carbone et al. 2013), whereas *BCOR*-inactivating mutations have been identified in various hematologic malignancies (Tanaka et al. 2017). Thus far neither have been associated with ACC. The low frequency of mutations in ACC limits the identification of potentially useful therapeutic targets, which underscores the importance of identifying novel recurrent mutations that contribute to tumor profiling.

Further highlighting the low mutational burden of ACC, four of our five cases had mutational rates below the 40th percentile relative to TCGA, which is consistent with the well-described indolent course of ACC tumor growth. Of note, mutational burden does not seem to correlate with tumor aggressiveness in our cohort. Although POG 5 had the highest mutation rate, it was not characterized by rapid clinical progression, and thus far the patient has been stably managed with localized treatment. Conversely, POG 4 exhibited the second lowest mutational burden in our samples and was characterized by high-grade solid and cribriform histology and rapid progression with distant metastases to the bone, which is known to be associated with poor outcome (Sung et al. 2003). Interestingly, comparison with tumors in TCGA also revealed similarities in the expression profiles of our cases and breast cancer tissue. This may reflect the histologic and genetic similarity among ACCs irrespective of anatomical origin site that has been previously reported (Marchio et al. 2010; Martelotto et al. 2015). ACC of the breast, which accounts for <1% of breast cancers and displays a triple negative and basal-like phenotype (Wetterskog et al. 2012), demonstrates similarity to salivary gland ACC based on the presence of the *MYB-NFIB* fusion gene (Persson et al. 2009; Fusco et al. 2016). The recurrent *MYB-NFIB* fusion demonstrated in our study may therefore rationalize the similarity with TCGA breast cancer samples.

Indeed, our analysis revealed *MYB-NFIB* fusions in four of five cases, with the fifth case exhibiting the closely related *MYBL-NFIB* fusion. Prior studies have described *MYB-NFIB* fusions to be a hallmark genetic event in ACC based on its incidence in 50%–60% of tumors (Mitani et al. 2010; Ho et al. 2013), and its presence in the majority of our cases further highlights its role as a driver mutation. Tumors with *MYB* and *MYBL1* translocation have been shown to have similar gene expression profiles, and the translocation of *MYB/MYBL* and *NFIB* activates several target genes associated with apoptosis, cell cycle control, cell adhesion, growth, differentiation, and angiogenesis (Ferrarotto et al. 2016). Among our cases, notable genes downstream from the *MYB-NFIB* fusion that were altered or overexpressed included *KIT, MYC, PRAME, FGFR2, BCL2*, and *CDK6*. The *MYB-NFIB* fusion has been associated with poorer outcome of salivary ACC (Mitani et al. 2011), and its prevalence among our cohort may suggest that it has a more significant role in the pathogenesis of rare and metastatic ACC, and can provide insight into targeted therapy for these cancers.

Although previous studies have elucidated potential therapeutic targets based on whole-genome and transcriptome sequencing of ACC (Ho et al. 2013; Morris et al. 2016), to our knowledge ours is the first study to implement such findings into direct clinical action. Among the five cases, three were treated with POG-informed therapy; specifically a multitargeted tyrosine kinase inhibitor (dovitinib), a pan-NOTCH inhibitor, and a multitarget and

ATP-competitive inhibitor of c-MET and VEGFR1/2/3, all of which were in phase I or II clinical trials at the time. In a recently published phase II clinical trial, Dillon et al. (2017) demonstrated that dovitinib had antitumor activity in patients with recently progressed ACC, but came at the expense of moderate toxicity with one in five patients having to withdraw because of intolerable side effects. Aligning with these observations, Patient 1, who underwent treatment with dovitinib based on overexpression of *FGFR*- related genes, had to withdraw from the trial. Similarly, Patient 2 chose to cease treatment with a pan-NOTCH inhibitor after requiring several dose reductions. Unfortunately both the pan-NOTCH inhibitor and dovitinib have subsequently halted development primarily because of toxicity and before efficacy could be established. Therefore, other therapeutics will need to be investigated to fulfill the need for targeting these pathways. However, despite these outcomes, our POG analysis identified possible targets within aberrant cancer-related pathways and therapeutic trials that hold potential to be effective for patients based on their genomic landscape.

In summary, our data provide insights into the genetic landscape of rare and metastatic ACC and establish that whole-genome and transcriptome sequencing can effectively yield potential therapeutic options for the treatment of these rare cancers.

METHODS

Oversight, Ethics, and Patient Sample Collection

The study was approved by the University of British Columbia Research Ethics Committee (REB# H14-00681), and written informed consent was obtained from each patient before biopsy and genomic profiling. Consent was also obtained for potential publication of findings. Patient data were de-identified within the research team and identification codes were assigned for communicating clinically relevant information to physicians. Raw sequence data and downstream analytics were maintained within a secure computing environment.

Whole-Genome and Transcriptome Sequencing

Fresh frozen biopsies were obtained and intermittent hematoxylin and eosin (H&E)-stained slides were reviewed for tumor content and cellularity. DNA and RNA extractions were performed on OCT-embedded tumor samples using either the AllPrep DNA/RNA Mini Kit (QIAGEN) or the ALine EvoPure kit (Aline Biosciences) to create genomic and transcriptomic libraries. Additionally, DNA was isolated from peripheral blood for sequencing library construction.

PCR-free libraries were constructed for tumor and normal DNA samples, and strand-specific libraries were constructed for tumor RNA samples, as described previously (Jones et al. 2017). Whole-genome sequencing (WGS) was performed to 80× redundant coverage of the tumor and 40× redundant coverage of the normal, and transcriptome sequencing (RNA-seq) was performed to a depth of 200 million reads, using Illumina HiSeq instruments with 125- or 150-bp paired-end reads (Illumina; http://www.illumina.com) (see Table 5).

Somatic Alteration Analysis

An established analytical pipeline was used for identifying somatic nucleotide and copy-number aberrations throughout the genome from tumor DNA in comparison to normal DNA from the same patient, as previously described (Jones et al. 2017). De novo assembly of genomic and transcriptomic data was carried out using ABySS (Robertson et al. 2010) and Trans-ABySS (Birol et al. 2009; Simpson et al. 2009) to detect structural rearrangements and fusions. Copy number and LOH were visualized with Circos (Krzywinski et al. 2009). Gene fusions were evaluated and visualized using MAVIS (http://mavis.bcgsc.ca/). Visualization of

Table 5. Sequencing coverage of aligned reads

Sample	Tumor DNA coverage (WGS)	Normal DNA coverage (WGS)	Tumor RNA coverage (RNA-seq)
POG 1	73×	44×	408 M reads
POG 2	88×	45×	183 M reads
POG 3	98×	50×	70 M reads
POG 4	88×	41×	161 M reads
POG 5	100×	50×	173 M reads

gene alterations by sample was through OncoPrinter and MutationMapper, and recurrence of mutations across TCGA was determined through CBioPortal (http://www.cbioportal.org/) (Cerami et al. 2012; Gao et al. 2013). Mutation signatures were computed based on genome-wide single-nucleotide variant calls and trinucleotide contexts as previously described (Alexandrov et al. 2013) and compared to COSMIC reference signatures (http://cancer.sanger.ac.uk/cosmic/signatures). Signature scores represent the fraction of mutations contributed by a particular signature. To examine the relationship between signatures, in particular signatures 5 and 16, we used a hierarchical Bayesian categorical mixture model to explore the posterior distribution of signature exposures (E Zhao, S Jones, in prep.).

Expression Analysis and Comparison with TCGA Data Sets

Expression levels of each gene were derived from aligned RNA-Seq reads as described in Butterfield et al. (2014) (Jones et al. 2017). Gene expression in the ACC samples was compared to available tumor samples from multiple cancer types in the Cancer Genome Atlas (TCGA; https://tcga-data.nci.nih.gov/tcga/) as well as normal samples from Illumina Human Body Map 2.0 to identify genes over- or underexpressed, as described in Jones et al. (2017). Similarity to TCGA cancer types was determined by a machine learning based pan-cancer classifier trained on whole-transcriptome profiles of TCGA primary cancers (J Grewal, S Jones, in prep.)

ADDITIONAL INFORMATION

Data Deposition and Access

The whole-genome sequencing and RNA-seq data for this case are available as .bam files from the European Genome-phenome Archive (EGA; www.ebi.ac.uk/ega/home) as part of the study EGAS00001001159, under accession numbers EGAD00001002032 (Patient 1), EGAD00001002627 (Patient 2), EGAD00001003065 (Patient 3), EGAD00001003640 (Patient 4), and EGAD00001003679 (Patient 5). Somatic variants were deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under accession numbers SCV000693656 (*BCOR*, patient 1), SCV000693657 (*MECOM*, patient 1), SCV000693671 (*MYBL1-NFIB* fusion, patient 1), SCV000693658 (*BCOR*, patient 2), SCV000693659 (*NOTCH1*, patient 2), SCV000693660 (*NOTCH1*, patient 2), SCV000693672 (*MYB-NFIB* fusion, patient 2), SCV000693673 (*MYB-NFIB* fusion, patient 3), SCV000693661 (*BAP1*, patient 4), SCV000693662 (*FGFR2*, patient 4), SCV000693674 (*MYB-NFIB* fusion, patient 4), SCV000693663 (*ARID2*, patient 5), SCV000693664 (*BAP1*, patient 5), SCV000693665 (*BCOR*, patient 5), SCV000693666 (*JAK3*, patient 5), SCV000693667 (*KRAS*, patient 5), SCV000693668 (*SMARCA2*, patient 5), SCV000693669 (*SMARCB1*, patient 5), SCV000693670 (*STAT3*, patient 5), and SCV000693675 (*MYB-NFIB* fusion, patient 5).

Ethics Statement

The study was approved by the University of British Columbia Research Ethics Committee (REB# H14-00681). Written informed consent was obtained from each patient prior to genomic profiling. Patient identity was anonymized within the research team and an identification code was assigned to the case for communicating clinically relevant information to physicians. The patients consented to potential publication of findings. Raw sequence data and downstream analytics were maintained within a secure computing environment.

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Competing Interest Statement

The authors have declared no competing interest.

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Author Contributions

M.C. and J.L. devised the study. M.C. and E.P. wrote the manuscript with additional text and feedback from all authors. M.C., E.P., J.G., E.Z., M.R.J., Y.S., K.L.M., M.B., and G.A.T. performed the data analysis. Y.M., A.J.M., and R.A.M. supervised the data generation. T.N., E.C., and S.Y. performed the pathology analysis. H.L., D.R., and J.L. were involved in clinical management. S.J.M.J., M.A.M., and J.L. supervised the project.

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