

Clinically Integrated Molecular Diagnostics in Adenoid Cystic Carcinoma

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ABSTRACT

Background. Adenoid cystic carcinoma (ACC) is an aggressive salivary gland malignancy without effective systemic therapies. Delineation of molecular profiles in ACC has led to an increased number of biomarker-stratified clinical trials; however, the clinical utility and U.S.-centric financial sustainability of integrated next-generation sequencing (NGS) in routine practice has, to our knowledge, not been assessed.

Materials and Methods. In our practice, NGS genotyping was implemented at the discretion of the primary clinician. We combined NGS-based mutation and fusion detection, with MYB break-apart fluorescent in situ hybridization (FISH) and MYB immunohistochemistry. Utility was defined as the fraction of patients with tumors harboring alterations that are potentially amenable to targeted therapies. Financial sustainability was assessed using the fraction of global reimbursement.

Results. Among 181 consecutive ACC cases (2011–2018), prospective genotyping was performed in 11% ($n = 20/181$;

$n = 8$ nonresectable). Testing identified 5/20 (25%) NOTCH1 aberrations, 6/20 (30%) MYB-NFIB fusions (all confirmed by FISH), and 2/20 (10%) MYBL1-NFIB fusions. Overall, these three alterations (MYB/MYBL1/NOTCH1) made up 65% of patients, and this subset had a more aggressive course with significantly shorter progression-free survival. In 75% ($n = 6/8$) of nonresectable patients, we detected potentially actionable alterations. Financial analysis of the global charges, including NGS codes, indicated 63% reimbursement, which is in line with national (U.S.-based) and international levels of reimbursement.

Conclusion. Prospective routine clinical genotyping in ACC can identify clinically relevant subsets of patients and is approaching financial sustainability. Demonstrating clinical utility and financial sustainability in an orphan disease (ACC) requires a multiyear and multidimensional program. *The Oncologist* 2019;24:1356–1367

Implications for Practice: Delineation of molecular profiles in adenoid cystic carcinoma (ACC) has been accomplished in the research setting; however, the ability to identify relevant patient subsets in clinical practice has not been assessed. This work presents an approach to perform integrated molecular genotyping of patients with ACC with nonresectable, recurrent, or systemic disease. It was determined that 75% of nonresectable patients harbor potentially actionable alterations and that 63% of charges are reimbursed. This report outlines that orphan diseases such as ACC require a multiyear, multi-dimensional program to demonstrate utility in clinical practice.

INTRODUCTION

Adenoid cystic carcinoma (ACC) is, despite its deceptively low-grade histological appearance, an aggressive type of

salivary gland malignancy arising in major and minor salivary glands. In the U.S., there are ~15,000 patients with ACC

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alive today, and ACC thereby fulfills the criteria of the “rare (orphan) disease act” of 2002 (affecting <200,000 people in the U.S.). ACC grows slowly, and the disease has a seemingly indolent, yet relentless, course. Disease-specific survival at 5 years is 89% and drops to 40% at 15 years [1]. Complete surgical resection of ACC is, whenever possible, the treatment of choice [2]; however, ACC frequently recurs, and treatment failure is often characterized by distant metastasis [1]. Radiation therapy can lower the rate of local recurrence (so-called “local control”) but does not improve overall survival [3]. Currently, there is no effective chemotherapy [4], clinical trial data are sparse [5, 6], and standard off-label use of cytotoxic chemotherapy is so ineffective that current guidelines state that enrollment in clinical trials is preferred [7].

Numerous studies have delineated key molecular profiles of ACC [8–13] and collectively indicate pathways for genotype-stratified therapies [14–18]. The concept, to match each patient according to the tumor pathway profile to the most effective (biologically sound) and least toxic treatment, rests on the prevalence of actionable alterations [14, 19–21]. Assessment of established alterations in clinical practice requires comprehensive genotyping efforts, for example, accomplished via next-generation sequencing (NGS) technologies. For routine patient management in the U.S., tests are required to be clinical grade (i.e., derived from a Clinical Laboratory Improvements Amendments (CLIA)-certified laboratory) and, because of the increased quality standards and documentation efforts, are typically 2–3 times more expensive than the same test in the research setting [22, 23]. Although drug costs are a major burden in precision medicine, test cost coverage and financial sustainability are substantial challenges to clinical genomics—in particular for orphan diseases.

Through institutional prioritization, we have initiated pan-cancer genetic testing in 2008, transitioned to NGS-based testing in 2011, and adopted specific NGS billing codes in 2015. In common cancers, the identification of rare subsets of actionable targets has been established (e.g., National Comprehensive Cancer Network [NCCN], College of American Pathologists guidelines); however, the need for more effective, biomarker-informed therapies in a disease as rare as ACC poses unique challenges to demonstrate clinical utility and U.S.-centric financial sustainability [14, 19, 21, 23].

Here we assessed clinical utility by determining the prevalence and relevance of molecular alterations. In addition, we assessed financial sustainability by determining reimbursement rates in comparison with local, national, and international rates. By combining clinical utility and financial sustainability, we outline an integrated approach to identify clinically relevant subsets of patients with ACC in an economically sustainable manner.

MATERIALS AND METHODS

Study Design

Samples were identified retrospectively (2011–2018), and we included only patients with a histologically confirmed diagnosis of ACC. We obtained demographics, anatomic location, date of diagnosis, time to progression, and/or last follow-up by review of the electronic medical record (Qpid

Health, Boston, MA). Appropriate institutional review board approval was obtained, and the research was performed in accordance with the Declaration of Helsinki. We defined “relevant” as having diagnostic and/or prognostic value and “actionable” as having potential therapeutic relevance [24]—assessed via review of relevant trials (Table 1).

Morphology

Pathological TNM staging followed the American Joint Committee on Cancer (AJCC) staging system, 8th edition [25]. At least three board-certified pathologists reviewed and followed the diagnostic criteria proposed by the World Health Organization [26]. ACC was subclassified by growth pattern into cribriform, tubular, solid, or mixed (defined as containing a solid component). Mixed tumors containing several growth patterns, but with one predominant (>60%), were classified according to the predominant pattern.

Next-Generation Sequencing

Genotyping at our institution is performed in a clinical (CLIA-certified) molecular diagnostics laboratory and includes NGS-based testing and reporting of results into the medical record. Briefly, isolated nucleic acids from tumor specimens were analyzed using anchored multiplex polymerase chain reaction (AMP) to detect single-nucleotide variants and insertions/deletions in a target set of cancer-related genes. To detect fusion transcripts, we also applied the AMP technology in a separate RNA-based NGS assay [27]. We sequenced on Illumina NextSeq (for details, see supplemental online data).

FISH Assay and Immunohistochemistry

MYB gene rearrangements were analyzed using a break-apart probe strategy (supplemental online data), and Myb protein expression was assessed using immunohistochemistry. A detailed description of both methods is provided in supplemental online material and methods.

Economic Analysis

We reviewed reimbursement (defined as amount refunded from payors) by current procedural terminology (CPT) code and claim adjustment codes (if applicable). We standardized across payors using the national values provided via the clinical laboratory fee schedules/physician fee schedule from the Center for Medicare and Medicaid Services (CMS). In 2015, the American Medical Association introduced specific NGS panel codes, and we calculated the overall reimbursement rate by dividing the total sum of obtained payments through the total sum of charges using CMS values as a reference. For comparison of our reimbursement rates with local, national, and international rates, we reviewed reimbursement data from selected publications [28–36].

RESULTS

Clinically Integrated Molecular-Genetic Testing in ACC

In total, 181 patients with the diagnosis of an ACC were identified between 2011 and 2018. Twenty of these ACC cases were genotyped clinically (Fig. 1A) by combining NGS-based

Table 1. Clinical trials for patients with adenoid cystic carcinoma by molecular target

Targets	Compound	Name	Sponsor	Locations	Phase	NCT number
NOTCH ^a	OMP-52M51	Brontictuzumab	MD Anderson Cancer Center	Houston, TX	NA	NCT02662608
NOTCH ^a	BMS-906024	AL101	Ayala Pharmaceuticals	Multiple locations in U.S.	II	NCT03691207
NOTCH ^a PIK3CA ^a	Multiple drugs	Drug therapies for salivary gland cancers based on testing of genes	University Health Network, Toronto	University Health Network, Toronto	NA	NCT02069730
MYB ^a	AG-013736	Axitinib	Pfizer Oncology NCCN/NIH/NCI	MSKCC, U.S.	II	NCT01558661
MYB ^{a,b}	ReposMZB	Mebendazol	Repos Pharma	Uppsala University	Ila/ preclinical	NCT03628079
MYB ^a and PD-1	TetMYB Vaccine and BGB-A317	MYB vaccine and tislelizumab	Peter MacCallum Cancer Centre	Melbourne, Australia	I	NCT03287427
MDM2 ^a	APG-115	APG-115	Ascentage	San Antonio, TX	I	NCT02935907
MDM2 ^a	DS-3032b	DS-3032b	Daiichi Sankyo	Multiple locations in U.S.	I	NCT01877382
PIK3CA ^a BRCA1 ^a (multiple targets)	Taselisib (PIK3CA) Adavosertib (BRCA1) (multiple drugs)	NCI-MATCH	National Cancer Institute	More than 1,000 locations in U.S.	II	NCT02465060
BRCA1 ^a (multiple targets)	Olaparib (BRCA1) (multiple drugs)	ASCO TAPUR	American Society of Clinical Oncology	More than 20 locations in U.S.	II	NCT02693535
VEGFR	Apatinib	Apatinib and proton radiation	Shanghai Proton and Heavy Ion Center	Shanghai, China	II	NCT02942693
VEGFR FGFR RET c-KIT PDGFR	Lenvatinib	Lenvima	Fondazione IRCCS Istituto Nazionale dei Tumori	Milan, Italy	II	NCT02860936
HDAC	Chidamide	Epidaza	Chinese Academy of Medical Sciences	Beijing, China	II	NCT02883374
HDAC	Chidamide and cisplatin	Epidaza and cisplatin	Fudan University	Shanghai, China	II	NCT03639168
PRMT5	GSK3326595	GSK3326595	GlaxoSmithKline	Multiple locations in U.S.	I	NCT02783300
PD-1	Pembrolizumab and docetaxel	Keytruda and docetaxel	University of Chicago	Chicago, IL	II	NCT03360890
PD-1/CTLA-4	Nivolumab and ipilimumab	Opdivo and Yervoy	Northwestern University	Chicago, IL	II	NCT03146650
PD-1/CTLA-4	Nivolumab and ipilimumab	NCI-DART Opdivo and Yervoy	National Cancer Institute	More than 600 locations in U.S.	II	NCT02834013

^aIndicates targets identified in our cohort of 20 patients with adenoid cystic carcinoma.

^bThe trial listed is for gastrointestinal cancers, and evidence for activity of mebendazole against MYB is preclinical.

Abbreviations: BRCA1, breast cancer type 1 susceptibility DNA repair associated; c-kit, KIT proto-oncogene receptor tyrosine kinase; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; FGFR, fibroblast growth factor receptor; HDAC, histone deacetylase; MDM2, proto-oncogene encoding E3 Ubiquitin Ligase; MSKCC, Memorial Sloan Kettering Cancer Center; MYB, proto-oncogene/transcription factor; NA, not applicable; NCCN, National Comprehensive Cancer Network; NCI, National Cancer Institute; PD-1, programmed cell death protein 1; PDGFR, platelet-derived growth factor receptor; PIK3CA, phosphatidylinositol-3-OH kinase; PRMT5, protein arginine methyltransferase 5; RET, proto-oncogene rearranged during transfection; VEGFR, vascular endothelial growth factor receptor.

DNA mutation assessment, RNA-based fusion detection, MYB immunohistochemistry, and MYB break-apart fluorescent in situ hybridization (FISH). The clinicopathological characteristics of the study cohort are summarized in Table 2. We refer to this approach as clinically integrated molecular-genetic testing, and an overview is provided in Figure 1B. In our clinical practice, providers order molecular testing in patients with recurrent, progressive, or nonresectable tumors (see below) in order to identify clinically relevant and potentially

actionable alterations (i.e., genetic alterations expected to change management or treatment; Table 1) [24].

Computational Landscape of ACC in Clinical Practice

NGS identified 27 alterations in 12/20 (60%) patients in our study cohort (Fig. 2), and 8/20 patients (40%) were fusion positive. Fusions for MYB-NFIB or MYBL1-NFIB (40%) and NOTCH1 mutations (25%) were the most common alterations. Across all cancer types tested in our institution (n = 2,701 cases were

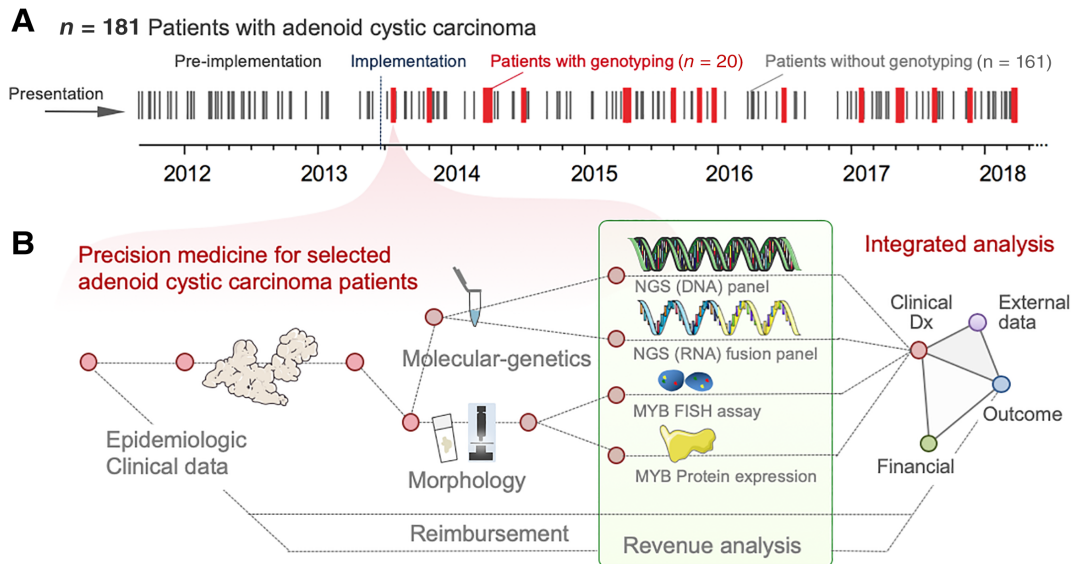


Figure 1. Clinically integrated diagnostics and analytical workflow. **(A):** Timeline in days, indicating the diagnosis of adenoid cystic carcinoma (ACC) between 2011 and 2018 by black lines (patients without genotyping) as well as 20 ACC cases, that were sequenced within that timeframe (red lines, patients with genotyping). **(B):** Analysis of 20 clinically performed formalin-fixed paraffin-embedded tissue samples and corresponding clinical data. We integrated five separate diagnostic components including (a) histomorphology, (b) MYB immunohistochemistry, (c) MYB break-apart FISH, (d) an NGS DNA-based gene panel, and (e) an RNA-based NGS panel for fusion detection. For outcome assessment, we employed 201 publicly available (external) data. Abbreviations: Clinical Dx, clinical diagnostics; FISH, fluorescent in situ hybridization; NGS, next-generation sequencing.

genotyped between 2013 and 2018) [27, 37], we have only seen MYB and MYBL1 fusions in adenoid cystic carcinomas (specificity: 100%) [38]. We modeled that even if a non-ACC would harbor one MYB/MYBL1 fusion, it would take ~14 cases of non-ACC for the specificity to drop below 99.5%. Therefore, ACC should be the top differential diagnosis when encountering an MYB/MYBL1 fusion (= diagnostic relevance).

Anatomical distribution and gender were not correlated with fusion- or mutational status (Fig. 2A). However, as previously noted [16], NOTCH1 mutations were solely found in solid and cribriform subtypes (Fig. 2B) and occurred predominantly in older patients (i.e., ≥ 55 years; $p = .17$; Fisher's exact). Additional (and in part recurrent) mutations were present in ARID1A, BRCA1, phosphatidylinositol-3-OH kinase (PIK3CA), TGFBR2, CTNNB1, ATM, TERT, MDM2, FBXW7, and MYC; for details, see supplemental online Table 1. Notably, in case 13, we identified coamplification of NOTCH1 and MYC genes (supplemental online Fig. 1A), whereas case 12 was remarkable for a canonical TERT promoter mutation at position -124 (supplemental online Fig. 1B) [39]. Importantly, these additionally detected genetic aberrations occurred in tumors with MYB/MYBL1/NOTCH1 alterations, whereas the remaining "wild-type" tumors showed no additional changes by our targeted gene panel ($p = .002$, Fisher's exact).

MYB-Fusion Status and Correlation with Protein Expression

Interestingly, the occurrence of MYB/MYBL1/NOTCH1 gene alterations was, with one exception (case 10), mutually exclusive [40]. Review of this computed case 10 showed a geographically distinct MYB protein expression pattern (supplemental online Fig. 2A), possibly reflecting intratumoral heterogeneity [41] with respect to the underlying MYB-NFIB and NOTCH1 gene alterations. Unless limited by tissue availability,

we tested NGS-positive MYB-NFIB cases by MYB break-apart FISH and confirmed all cases as MYB rearranged (Fig. 2B). Correlation of the three methods identified (a) NGS-concordant protein expression (supplemental online Fig. 3) [10, 17], (b) strong overexpression of MYB protein (supplemental online Fig. 2B) despite being fusion- and FISH negative (supplemental online Fig. 2C), or (c) unusual "green-only" MYB probe pattern by FISH (supplemental online Fig. 3E, circles) with MYB protein expression (supplemental online Fig. 3F). These findings are compatible with loss of the corresponding exons (supplemental online Fig. 4A–C) and/or a locus disruption outside the MYB coding region affecting the "red" probe binding site. One way to improve sensitivity of the FISH assay would be to modify FISH probe position (supplemental online Fig. 4A–C). In other words, relying on a single method may be inferior because our findings underscore the complementary nature of various testing modalities [38].

Outcome Analysis and Clinical Utility

To assess prognostic relevance in our setting, we first integrated our 20 (internal) samples/patients with 201 publicly available (external; www.cbioportal.org) data (total case number = 221). Notably, when comparing progression-free survival periods, we found significantly shorter progression-free survival in our internal population as compared with publicly available data (supplemental online Fig. 5; $p = .0003$, log-rank). The significant difference is likely due to specific order-practice in our setting, where providers use genotyping in patients with locally advanced or systemic/palliative disease settings (Table 2; 87.5% pT3/4 internal vs. 55% external). A striking 40% of the tested patients in our setting experienced immediate tumor progression (supplemental online Fig. 5). Comparing the prevalence of NOTCH1 mutations and MYB/MYBL1 fusions revealed a similar frequency in

Table 2. Clinicopathological characteristics of the study cohort according to mutational status of MYB, MYBL1, and/or NOTCH1

Characteristics	Patients, <i>n</i> (%) <i>n</i> = 20	MYB/MYBL1/NOTCH1 Positive Patients, <i>n</i> (%) <i>n</i> = 12	Wild-type Patients, <i>n</i> (%) <i>n</i> = 8	<i>p</i> value
Age, years				
Average	56	61	48	.06
Median (range)	56 (28–90)	60 (28–90)	47 (34–59)	
Gender				
Male	13 (65)	7 (58)	6 (75)	.44
Female	7 (35)	5 (42)	2 (25)	
Localization				
Salivary gland	6 (30)	5 (42)	1 (12.5)	.19
Tonsil/tongue	4 (20)	2 (17)	2 (25)	
Paranasal sinuses	3 (15)	2 (17)	1 (12.5)	
Trachea	3 (15)		3 (37.5)	
Orbit	2 (10)	1 (8)	1 (12.5)	
Lung	2 (10)	2 (17)		
Tumor size ^a				
T1/T2	2 (12.5)	2 (18)	1 (20)	.07
T3/T4	14 (87.5)	9 (82)	4 (80)	
Nodal status ^a				
N0	12 (71)	8 (67)	4 (80)	.58
N+	5 (29)	4 (33)	1 (20)	
Metastasis ^a				
M0	15 (79)	8 (67)	7 (100)	.09
M1	4 (21)	4 (33)		
Histology				
Solid	3 (15)	3 (25)		.12
Cribriform	6 (30)	5 (42)	1 (12.5)	
Tubular	3 (15)	1 (8)	2 (25)	
Mixed	8 (40)	3 (25)	5 (62.5)	
Resectability				
Resectable	12 (60)	6 (50)	6 (75)	.26
Nonresectable	8 (40)	6 (50)	2 (25)	

^aTotal (*n*) of clinicopathological details differs due to missing data.

external and internal data sets (not shown), and both cohorts were pooled for further analysis (*n* = 221). The group of cases with NOTCH1, MYB, and MYBL1 alterations makes up half of all ACC (Fig. 3A), and when compared with the wild-type group, the NOTCH1/MYB/MBL1 group (= signature) does not demonstrate significantly different clinicopathological features (Table 2). Although the median progression-free survival in the “signature” group was only 2.6 years (vs. 4.3 years in the wild-type group), the difference did not reach statistical significance (*p* = .16, log-rank; Fig. 3B). Prior studies have reported a more aggressive clinical course of NOTCH1/MYB/MYBL1 patients [10, 15, 16]; however, the direct clinical application as a prognosticator remains to be explored [42, 43]. For assessment of clinical utility, we considered two key clinical management questions regarding (a) identification of treatment options (= actionability) and (b) prognostication. First, we examined the subset

of patients with initially nonresectable or systemic tumors (*n* = 8/20, 40% of the cohort) and determined the fraction of identified potentially actionable molecular alterations. When taking into account the shifting landscape of clinical trials (including “basket trials” and those listed in Table 1), our integrated molecular workup revealed potentially actionable alterations in 6 of 8 patients (75%; Fig. 3C). Second, when initial complete surgical resection can be achieved, we examined whether the NOTCH1/MYB/MYBL1 signature can serve as a prognosticator. We found that median progression-free survival in the signature group was 1.6 years versus 4.3 years in the wild-type group (*p* = .04, log-rank; Fig. 3D). Although conclusions will inevitably suffer from the very small sample size, considering that ACC is an orphan disease, these data outline clinical utility by distinguishing relevant subgroups of patients with ACC in clinical practice.

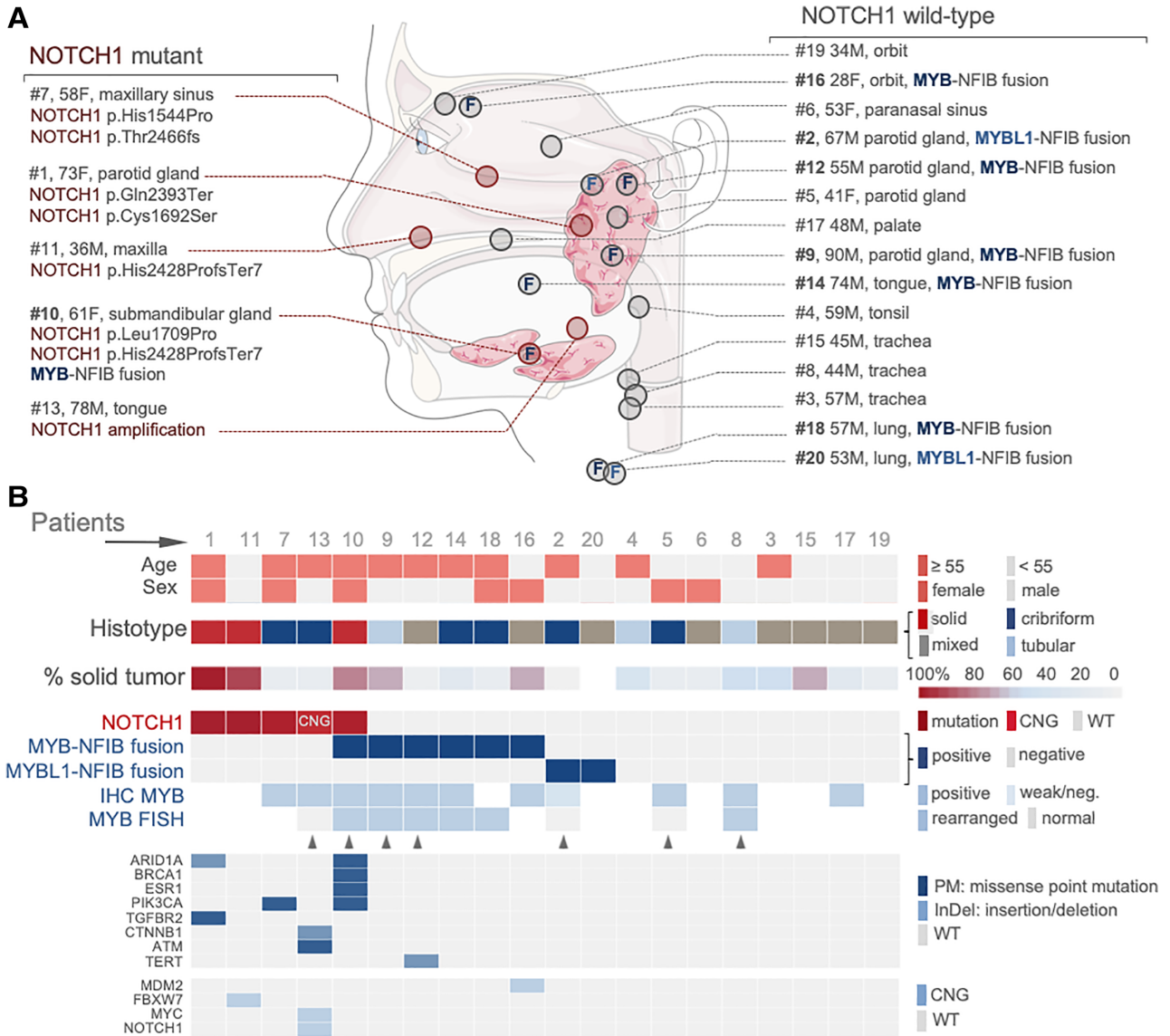


Figure 2. Anatomic location and mutational landscape. **(A):** Anatomic location of each case shown with salient clinical and key mutational findings (i.e., NOTCH1/MYB/MYBL1 findings). **(B):** Mutational landscape provided for each sample (column) along with clinical and molecular features (rows). Triangles mark cases with additional details provided in the Supplement. Abbreviations: CNG, copy number gain; F, female; InDel, insertion/deletion; M, male; NGS, next-generation sequencing; weak/neg., weak/negative; PM, point (missense) mutation; WT, wild-type.

Assessment of Financial Sustainability

A key factor in maintaining an integrated genotyping program in clinical practice is achieving financial sustainability. In contrast to cancers with higher prevalence, the rarity in orphan diseases poses additional hurdles [14, 21, 44], particularly when genotyping is applied only to the meaningful subsets of patients. During the initial phase (2013–2015; Fig. 4A), we applied the miscellaneous CPT code 81479 “Unlisted molecular pathology procedure” for NGS testing because specific NGS codes were not available at the time. We excluded data from this time frame because of the nonspecific nature of the CPT code and high variability in payments. Of the 12 cases charged after NGS code introduction, we excluded 2 cases because of payment through alternative sources. Review of the remaining 10 cases included in the analysis (Fig. 4A) showed that 72% of the

total amount charged derived from NGS panel codes whereas 28% derived from non-NGS codes. Reimbursement analysis indicates that 29% of payments derive from NGS codes whereas 34% of payments derive from non-NGS codes. In summary, the fraction of reimbursement for NGS and non-NGS tests sums up to a total of 63% (Fig. 4B). When comparing this fraction of reimbursement with published local, national, and international reimbursement rates, the overall fraction is similar (Fig. 4C). Notably, these comparison data are obtained in nonorphan disease settings and include multisite studies including large community centers or even national initiatives [28–36]. Nonetheless, obtaining reimbursement requires carefully designed workflows to account for prior authorization, policy tailoring, and appeals processes [45–47]. Review of the claim adjustment codes in our cohort indicates “charge exceeds maximum allowable”

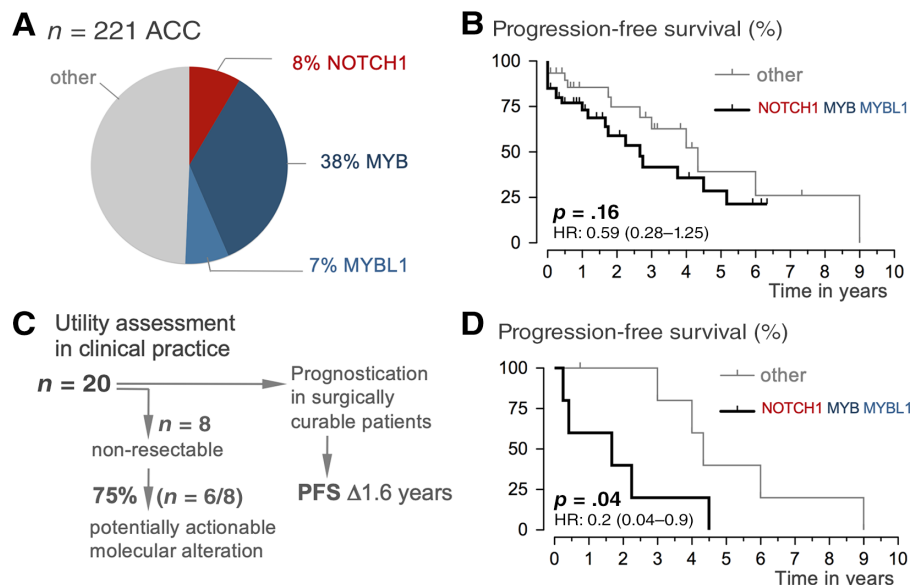


Figure 3. Outcome analysis and clinical utility. **(A):** Distribution of NOTCH1, MYB, and MYBL1 alterations in a cohort of 221 cases. **(B):** Progression-free survival of patients by NOTCH1/MYB/MYBL1 status (altered vs. nonaltered). Note: The grouping is based on prognostic impact, and we acknowledge that MYB/MYBL1 and NOTCH1 signaling is functionally distinct. Kaplan-Meier survival plots demonstrate shorter time to metastasis or local tumor progression for patients whose tumors showed NOTCH1/MYB/MYBL alterations versus those who did not; the difference did not reach statistical significance (p , log-rank test). **(C):** Utility assessment of molecular findings is twofold: (a) identification of potential actionable alterations in patients with nonresectable tumors (see also Table 1) and (b) prognostication in surgically curable patients. **(D):** Progression-free survival of patients by NOTCH1/MYB/MYBL1 status (altered vs. nonaltered). Kaplan-Meier plots show shorter progression-free survival time for patients with surgically resectable tumors in the subgroup with NOTCH1/MYB/MYBL alterations (p , log-rank test). Abbreviation: ACC, adenoid cystic carcinoma; HR, hazard ratio; PFS, progression-free survival.

($n = 6$ of 10) as the most frequent reason for adjusted payments.

Therefore, our data indicate that integrated genotyping of selected patients with ACC in our practice is approaching financial sustainability. In conjunction with the ability to identify potentially (therapeutically) actionable subsets of patients, our data indicate that achieving financial sustainability requires a multiyear and multidimensional program.

DISCUSSION

Here we report that clinically integrated molecular-genetic diagnostics can identify a significant subset of potentially actionable molecular alterations in patients with nonresectable ACC. Identification of MYB/MYBL1 fusions is 100% specific for ACC and can serve as a diagnostic biomarker. Molecular genotyping can furthermore serve as a prognostication tool for progression-free survival in initially surgically curable patients with ACC. Finally, we present reimbursement data, indicating that approaching financial sustainability requires a multiyear and multidimensional program.

Generally, ACC is being portrayed as an indolent disease; however, it has become clear that this is not always the case [48]. We fully acknowledge that the rarity and the small number of patients represent a significant limitation of our study. However, the demand for larger cohorts and studies does not solve day-to-day clinical management questions: for example, how to identify the subset of patients with ACC with a more aggressive clinical course. Notably, despite various studies outlining the prognostic relevance of

molecular markers [5, 9, 15, 16, 42, 43, 48–54], clinical guidelines currently rely on staging, margin status [55], perineural invasion [56], and resectability for treatment stratification (NCCN guidelines Version 2/2018; www.nccn.org, last accessed 12/22/2018).

Molecular genotyping in ACC is primarily performed in research settings, and numerous studies have outlined relevant mutational profiles [17, 57–59]. In addition, various biological [12, 17, 60], diagnostic [60–64], prognostic [16, 54, 64], and therapeutic advances [14, 15, 59] have been proposed. It is important to note that our study did *not* focus on identification or description of novel biomarkers. In contrast, our aim was to specifically address clinical utility of established alterations in clinical practice. In other words, our integrated precision medicine program explicitly prioritizes the individualization of care and focuses attention on unique characteristics of a particular patient subset. The approach thereby differs from traditional evidence-based medicine, which seeks to determine the best course of action for a patient with an appeal to generalizable knowledge gained from population-based studies [24, 65]. In the case of the orphan disease ACC, this specifically means to demonstrate utility in the small subset of meaningful patients. Demonstrating utility of previously described molecular alterations in the relevant subset of patients in clinical practice is the next logical and important step toward identifying when expanded or integrated genotyping should be considered standard of care.

The MYB translocation t(6;9)(q21-24;p13-23) results in a fusion protein of two transcription factors (MYB and NFIB). The MYB-NFIB fusion results in an increased expression of

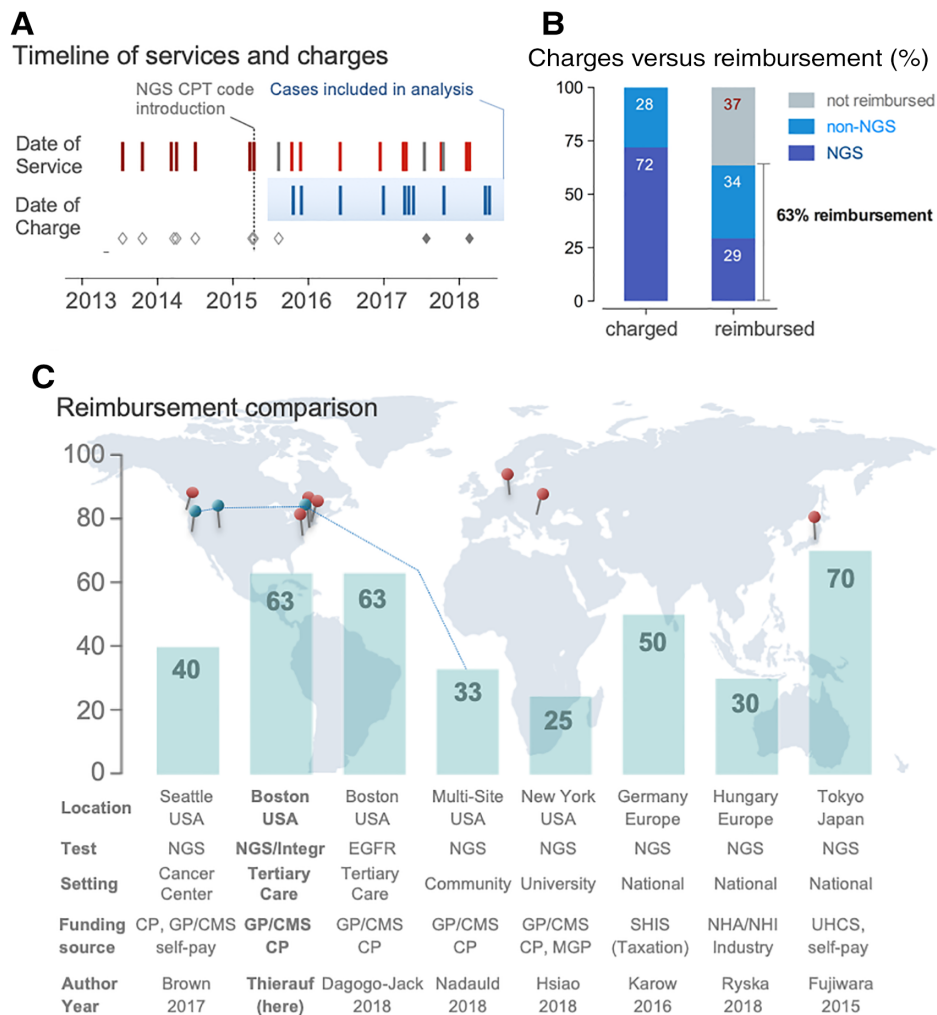


Figure 4. Financial sustainability of adenoid cystic carcinoma testing in clinical practice. **(A):** Timeline shows dates of service and dates of charges. In 2015, CPT codes for NGS-based panel testing were available and introduced (vertical line). We excluded cases with miscellaneous CPT codes (applied before availability of NGS codes indicated by open gray diamonds) and those paid for from alternative sources (indicated by filled gray diamonds). The cases included in the reimbursement analysis span 2.5 years. **(B):** Bar graphs comparing charged (left) and reimbursed amounts (right) separated by NGS- and non-NGS related fractions. Note: The lower rate in the fraction of reimbursement of NGS codes when compared with non-NGS codes is likely related to a combination of (a) lower rates of payments, (b) denials, and/or (c) a relative larger fraction of payments related to non-NGS codes. **(C):** Bar graphs indicating reimbursement in percent; the location of the eight references (see Results) is provided on the world map. Abbreviations: Author, first author of the publication; CP, commercial plans; CPT, current procedural terminology; EGFR, epidermal growth factor receptor (single gene testing); GP/CMS, government plans/Center for Medicare and Medicaid services; MGP, Managed government plans (i.e., Medicaid health maintenance organizations and managed Medicaid/Medicare plans); NGS, next-generation sequencing; NHA/NHI, national health authority/national health insurance; SHIS, Germany's statutory health insurance system; UHCS, Japanese universal health care system.

the protein Myb [43]. Myb is important for the growth of multiple solid tumors, and MYB protein overexpression in ACC is associated with a more aggressive clinical course [33, 47–49]. For example, Mitani et al. [40] reported that the level of MYB expression in identical histological subtypes of ACC is associated with a shorter survival time. Recently, xenografting [66] and the first MYB-NFIB-positive cell line (UM-HACC-2A) have been established and represent interesting tools to explore novel therapeutic possibilities [67]. Therapeutically, MYB-targeted antisense oligodeoxynucleotides (G4460; INX-3001; LR3001) [68] have not been effective in clinical trials [69]; however, several lines of evidence suggest that MYB is a promising therapeutic target [70–73]. Currently, the first clinical trial of a therapy that directly

targets MYB, in combination with an anti-PD-1 immune checkpoint inhibitor, opened (MYPHISMO, NCT03287427), and the first patient with ACC received her first infusion of the MYB DNA vaccine in September 2018 [74]. Collectively, these data suggest that identification of MYB alterations represents a promising approach to identify a subset of patients with ACC for molecularly targeted therapies.

NOTCH1 alterations also identify a subset of patients with an aggressive disease course [50] that are potential candidates for clinical trial enrollment. Importantly, NOTCH1 knockdown results in effective suppression of metastasis in vivo [51], and the efficacy of gamma-secretase inhibitors targeting the canonical NOTCH1 signaling pathway [19] is currently being explored (NCT03422679). Ferrarotto et al. [75]

described activating NOTCH1 mutations that 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 in a patient-derived xenograft model. Furthermore, an index patient with NOTCH1-mutant ACC showed partial response to brontictuzumab [16]. Those results led to a clinical trial for patients with ACC treated with brontictuzumab (OMP-52 M51), a humanized monoclonal antibody directed against the Notch-1 receptor with potential antineoplastic activity (NCT02662608). Moreover, CB-103, a small molecule inhibitor, is being tested in a phase IIA trial in up to 140 patients with preselected cancer indications with tumor cells characterized by NOTCH overactivation (NCT03422679). In addition, the first phase II clinical trial of a NOTCH inhibitor recently opened (NCT03691207). These data collectively indicate NOTCH1 and MYB genes as promising therapeutic targets. Most recently a clinical trial for NOTCH-mutant ACCs has started at our institution (ACCURACY trial; NCT03691207).

Additional cancer-relevant alterations co-occurred within the set of MYB/MYBL1/NOTCH1 aberrations that may contribute to the more aggressive clinical course in this group of ACC. Several other aberrations may lead to new therapeutic strategies (Table 1). For example, PARP inhibitors such as rucaparib and other agents such as trabectedin are actively tested in tumors with BRCA1 mutations (NCT01989546). PIK3CA are responsible for coordinating a diverse range of cell functions including proliferation and survival. Novel therapeutics targeting different components of the PI3K pathway showed preclinical efficacy in an array of human cancer, and several compounds as well as dual PI3K-mTOR inhibitors, PI3K inhibitors (that do not inhibit mTOR), AKT inhibitors, and mTOR catalytic site inhibitors are moving toward clinical trials (Table 1) [76–78]. Interestingly, ESR1 has also been linked to PIK3CA variants in metastatic breast cancer [53, 79], and one of our ACC cases with a PIK3CA mutation (number 10) also harbored an ESR1 aberration. Strategies to block estrogen signaling have shown extraordinary success in the prevention and treatment of breast cancer, and the development of therapeutic approaches that directly target ESR1-mutated clones is an appealing concept.

Although primarily designed to identify therapeutically actionable alterations (Table 1), our testing platform also showed prognostically relevant signatures (Fig. 3). Many head and neck cancer centers will not have local access to molecular testing that integrates DNA and RNA NGS, FISH, and IHC data. Furthermore, establishing such testing practices is challenging when cost-coverage for molecular tests is restricted to settings that have been shown to directly influence therapeutic decision-making. In some diagnostically challenging cases, there is value in detecting a fusion that is highly specific for ACC; however, our testing approach was not specifically built for ACC. We combined and repurposed several existing infrastructures from former initiatives [80–83] to illustrate clinical utility in ACC. At first glance, this cross-functionality seems straightforward; however, we want to underscore that this platform is *not* a “one-time research project” but represents a fully integrated clinical-grade workflow. Two components are noteworthy: first, a dedicated clinical data science team that maintains the

computational infrastructure; and second, adopting up-to-date U.S.-billing practices to approach financial sustainability. With only 10 cases over multiple years, any economic argument can only be anecdotal and numerous limitations apply (e.g., variance by payor, coinsurances, copayments, deductibles, etc.). Furthermore, some may argue that CMS charges can only be regarded as an imperfect reference cost and do not account for additional indirect cost components, laboratory maintenance cost, etc. Importantly, when taking into account that our approach requires navigation of at least four distinct CPT codes from various payors, different negotiated hospital contracts (that changed over time), and the various components and tools of the U.S.-centric revenue cycle, it becomes clear that achieving financial sustainability in an orphan disease is a multiyear endeavor and that the integrated testing approach has to entail a highly specialized and policy-tailored billing process [45–47], an arguably overlooked aspect of personalized medicine. Demonstrating financial sustainability is important; however, in other settings, the implementation of integrated NGS testing in an orphan cancer will require modification(s). Despite these limitations, our comparison with other molecular testing initiatives (Fig. 4C) indicates largely compatible rates, and it is critical to understand the underlying motivation of the reimbursement or funding source. In our setting—a U.S.-based, CLIA-certified diagnostic laboratory in a tertiary care (academic) medical center—we were able to align the goals of private payors for medically meaningful testing (and reduction of unnecessary testing) with the needs of our providers and the aims of the health care organization. When considering other variables, for example, single-payer systems with national quality improvement initiatives, or initiatives that aim to tackle multiple diseases or even disease groups, financial sustainability can only be achieved through meticulous planning and the combination of mural and extramural funding strategies. Simply put, the (financial) sustainability of a novel initiative is a direct function of how well the core value-proposition of the program is aligned with the aims of the funding source (supplemental online Fig. 6). For orphan cancers, there is currently no generalizable “cost-benefit approach” that can serve as a one-size-fits-all approach for the plethora of possible settings. Therefore, managing the variable economic burden of NGS testing programs remains context dependent, and we have summarized the paradigm for achieving financial sustainability in modern medicine by funding source (supplemental online Fig. 6). Interestingly, several centers recently established rare tumor clinics [14, 21, 44], and we wanted to share our financial data because these additional layers of complexity in sustaining clinical genotyping are largely ignored, particularly when relevant subsets of patients with recurrent or metastatic ACC only present 2–3 times per year.

CONCLUSION

Clinically integrated workup of patients with ACC can identify a significant subset of potentially actionable molecular alterations in nonresectable tumors and serves as a prognostication tool for progression-free survival in initially surgically curable patients. The full potential of molecularly informed, personalized medicine relies on the availability of testing, access to

molecularly matched therapeutic strategies, and ultimately demonstration of outcome differences. In our practice, a significant fraction of patients could benefit from genotype-stratified and targeted therapies—which matches the experience by others. However, clinical implementation requires at least initial investment until financial sustainability is achieved, especially at times of intense economic pressure and demand to manage rapidly escalating health care costs.

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DISCLOSURES

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For Further Reading:

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Implications for Practice:

The Notch family is a highly conserved gene group that regulates cell-cell interaction, embryogenesis, and tissue commitment. This review summarizes the existing data on the third subtype of the Notch family, Notch3. The role of Notch3 in different types of cancers is discussed, as well as implications of its modification and new strategies to affect Notch3 signaling activity.