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# A phase II study of axitinib (AG-013736) in patients with incurable adenoid cystic carcinoma

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**Background:** Recurrent/metastatic adenoid cystic carcinoma (ACC) is an incurable disease with no standard treatments. The majority of ACCs express the oncogenic transcription factor MYB (also c-myb), often in the context of a *MYB* gene rearrangement. This phase II trial of the tyrosine kinase inhibitor (TKI) axitinib (Pfizer) tested the hypothesis that targeting pathways activated by MYB can be therapeutically effective for ACC.

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**Patients and methods:** This is a minimax two-stage, phase II trial that enrolled patients with incurable ACC of any primary site. Progressive or symptomatic disease was required. Patients were treated with axitinib 5 mg oral twice daily; dose escalation was allowed. The primary end point was best overall response (BOR). An exploratory analysis correlating biomarkers to drug benefit was conducted, including next-generation sequencing (NGS) in 11 patients.

**Results:** Thirty-three patients were registered and evaluable for response. Fifteen patients had the axitinib dose increased. Tumor shrinkage was achieved in 22 (66.7%); 3 (9.1%) had confirmed partial responses. Twenty-five (75.8%) patients had stable disease, 10 of whom had disease stability for >6 months. The median progression-free survival (PFS) was 5.7 months (range 0.92–21.8 months). Grade 3 axitinib-related toxicities included hypertension, oral pain and fatigue. A trend toward superior PFS was noted with the *MYB/NFIB* rearrangement, although this was not statistically significant. NGS revealed three tumors with 4q12 amplification, producing increased copies of axitinib-targeted genes *PDGFR/KDR/KIT*. Two 4q12 amplified patients achieved stable disease for >6 months, including one with significant tumor reduction and the longest PFS on study (21.8 months).

**Conclusions:** Although the primary end point was not met, axitinib exhibited clinical activity with tumor shrinkage achieved in the majority of patients with progressive disease before trial enrollment. Analysis of MYB biomarkers and genomic profiling suggests the hypothesis that 4q12 amplified ACCs are a disease subset that benefit from TKI therapy.

**Key words:** axitinib, adenoid cystic carcinoma, MYB

## introduction

Adenoid cystic carcinoma (ACC) is a malignant neoplasm that commonly arises from minor or major salivary glands, and more rarely from other sites. There are no standard treatments for incurable, recurrent/metastatic (R/M) ACC, as cytotoxic chemotherapy provides limited benefit. Axitinib (AG-013736) is a receptor tyrosine kinase inhibitor (TKI) of vascular endothelial growth factor receptors (VEFGRs) 1–3, KIT and platelet-derived growth factor receptors (PDGFRs) A/B, each of which may be critical for ACC pathogenesis. Multivariate analyses have shown that high VEGF expression in ACCs is an independent prognostic factor for survival [1]. KIT is highly expressed in >90% of ACCs, and is a hallmark of ACC histology [2]. Copy number analysis has uncovered recurrent gains at *PDGF* and *PDGFR* gene loci in ACC tumors [3, 4]. In the axitinib phase I trial, one of the three confirmed partial responses (cPRs) observed was in an ACC patient [5].

ACCs can be characterized by a unique t(6;9) translocation that creates a gene fusion of the *MYB* (also c-myb) and *NFIB* transcription factors, resulting in increased *MYB* expression [6, 7]. *MYB* is a bona fide oncogene in T-cell acute leukemia and is overexpressed in breast and colorectal cancers. In ACC, increased MYB transcriptional activity presumably drives overexpression of *MYB*-regulated genes, including *VEGFA* and *KIT* [7]. While ~50% or more of ACCs are fusion-positive, ~60%–70% of fusion-negative tumors also have elevated MYB, suggesting alternative mechanisms of activation [6]. Given MYB-dependent and -independent mechanisms of VEGFR/KIT/PDGFR activation and phase I evidence of clinical activity, we conducted a phase II trial to evaluate the efficacy of axitinib in patients with progressive, incurable ACC.

## methods

### study patients

Patients were required to have pathologically confirmed, incurable ACC (salivary or non-salivary primaries). RECIST version 1.1 measurable disease and evidence of disease progression (the presence of a new or progressive lesion on imaging carried out within 6 months of study enrollment and/or worsening disease-related symptoms) were required. All patients were treated at Memorial Sloan Kettering Cancer Center (MSKCC). The protocol (NCT01558661) was approved by the MSKCC Institutional Review Board (IRB). Written informed consent was obtained from all patients. See supplementary Figure S1, available at *Annals of Oncology* online, for complete protocol eligibility criteria.

### study treatment

Patients were started on axitinib 5 mg oral twice daily (b.i.d.) (1 cycle = 4 weeks). Those without drug-related adverse events > grade 2 (CTCAE v4.0) for 2 weeks and blood pressure of ≤150/90 without antihypertensive medications were eligible for non-mandatory dose escalation to 7 mg b.i.d., and then 10 mg b.i.d. Dose reductions to 3 mg b.i.d. and 2 mg b.i.d. were allowed for toxicity. RECIST v1.1 tumor assessments were done at baseline and then every two cycles. After 10 months, assessments were done every three cycles. Patients remained on study until disease progression, unacceptable toxicity or withdraw of consent.

### MYB immunohistochemistry

Paraffin sections were analyzed with the MYB antibody from Abcam (EP769Y). MYB quantification was assessed as previously published [8]: 2+ for strong staining in >50% of cancer cells, 1+ for weak or strong staining in <50% of the cells and 0 for <5% staining.

### fluorescence *in situ* hybridization for MYB and NFIB rearrangements

Fluorescence *in situ* hybridization (FISH) was carried out on paraffin-embedded 5 μm sections utilizing custom probes developed from bacterial artificial chromosomes (BACs) covering and flanking the *MYB* and *NFIB* genes (see supplementary Table S1, available at *Annals of Oncology* online). Two hundred successive nuclei were examined. Detection of a sufficient break-apart signal was interpreted as a positive score.

### next-generation sequencing

Ten cases were evaluated using the next-generation sequencing (NGS) assay MSK-IMPACT (Memorial Sloan Kettering-Integrated Mutation Profiling

of Actionable Cancer Targets) after informed written consent to an IRB-approved study (NCT01775072). This assay is optimized for DNA from formalin-fixed, paraffin-embedded samples, and targets single-nucleotide variants (SNVs), indels and structural variants in 341 cancer-related genes, in addition to genome-wide copy number [9, 10]. One case was analyzed on the FoundationOne platform. Gene level copy number changes were calculated using segmented log-ratio values (Circular Binary Segmentation) of the tumor and normal sample. A test for significance was carried out on distance of the segment to zero log ratio. Log ratio of 1 and −1 were the thresholds for amplification and deletion, respectively.

### statistical analysis

This was a single-arm, minimax two-stage phase II trial. The primary end point was best overall response (BOR) rate per RECIST version 1.1 criteria. In order to detect a difference between an unacceptable BOR rate of 5% and a desirable rate of 20% with a one-sided type I error of 10% and power of 90%, at least 1 response was required among the first 18 patients in the first stage within 10 cycles of treatment. If these criteria were met, then the study would accrue an additional 14 patients in the second stage. If $\geq$4 patients had a response out of a total of 32 enrolled, the regimen would be considered worthy of further investigation. Patients who received at least one dose of medication were included in the primary end point analysis. Patients who discontinued treatment without tumor assessment were classified as non-responders. The secondary end point was progression-free survival (PFS) estimated using the Kaplan–Meier methodology, with time origin at the start of the treatment, followed until progression of disease (PD) or death. Seven patients were censored up to the date of the last tumor assessment: five for withdrawal of consent, one for removal due to toxicity and one for development of inevaluable disease. The association between PFS and MYB biomarkers was evaluated by log-rank tests in an exploratory fashion. The 95% confidence intervals for proportions were calculated by the Clopper and Pearson method.

## results

### patient and disease characteristics

Between March 2012 and May 2013, 33 patients were enrolled: 18 in the first stage, 15 in the second. One patient enrolled in the second stage was determined to be ineligible and was replaced after two doses. This patient was considered evaluable for BOR, but not PFS. Patient characteristics are summarized in Table 1. Nearly all patients had distant metastatic disease (32/33 patients), and the majority had previously received systemic therapy [19/33 (57.6%)]. Six patients had previously been treated with antiangiogenesis agents. All patients had evidence of disease progression before study participation. All patients were started with axitinib at 5 mg orally b.i.d. Of 19 patients eligible for dose escalation, 15 had the dose increased: 6 patients to 7 mg b.i.d. and 9 increased two levels to 10 mg b.i.d.

**Table 1.** Patient characteristics

| Patient characteristic | No. of patients ($n$ = 33) |
|---|---|
| Age | |
| Median | 56 (range 39–78) |
| Sex | |
| Male | 18 (54.5%) |
| Female | 15 (45.5%) |
| ECOG performance status | |
| 0 | 3 (9.1%) |
| 1 | 22 (66.7%) |
| 2 | 8 (24.2%) |
| Primary tumor site | |
| Major salivary gland | 9 (27.2%) |
| Parotid | 1 |
| Submandibular | 7 |
| Sublingual | 1 |
| Minor salivary gland | 17 (51.5%) |
| Floor of mouth | 2 |
| Base of tongue | 6 |
| Hard palate | 5 |
| Paranasal sinus | 2 |
| Oral tongue | 1 |
| Oral cavity | 1 |
| Other | 7 (21.2%) |
| Lacrimal gland | 1 |
| Lung | 2 |
| Breast | 2 |
| Trachea | 1 |
| Unknown primary | 1 |
| Disease distribution | |
| Locoregional disease only | 1 (3.0%) |
| Distant metastases | 32 (97.0%) |
| Lung only | 9 |
| Liver | 8 |
| Peritoneum | 2 |
| Bone | 7 |
| Skin | 1 |
| Brain | 2 |
| Leptomeninges | 1 |
| Prior therapy | |
| Systemic therapy | 19 (57.6%) |
| For recurrent/metastatic ACC | 17 |
| As adjuvant therapy (w/o RT) | 1 |
| With radiation | 7 |
| Radiation | 31 (93.9%) |
| Axitinib dose | |
| Eligible for dose escalation | 19 (57.6%) |
| Dose escalated | 15 (45.5%) |
| 7 mg b.i.d. | 6 (18.2%) |
| 10 mg b.i.d. | 9 (27.3%) |
| Dose reduced | 11 (33.3%) |
| Without prior dose escalation ($n$ = 18) | 7 (63.6%) |
| After dose escalation ($n$ = 15) | 4 (26.7%) |

### efficacy

BOR outcomes are summarized in Figure 1A. The requirement that $\geq$1 confirmed responses be observed within 10 months of therapy among the first 18 patients was met, triggering enrollment of an additional 15. The majority of patients on trial experienced tumor shrinkage [22/33 (66.7%)] (Figure 1B), including three (9.1%) cPRs with durable benefit for more than 9 months (two with a PFS >11 months). Twenty-five (75.8%) patients had stable disease (SD), 10 for >6 months. Thirteen (40.63%) patients remained on axitinib for >6 months; two patients remained on axitinib for >1 year (14.5, 21.8 months) (supplementary Figure S2, available at *Annals of Oncology* online). Only four patients had PD as best response. The median PFS among 32 assessable patients was 5.7 months (95% CI: 5.3–9.1 months) (Figure 1C; median time on study was 5.3 months).

Of the three cPR patients, one was dose escalated, only later to be dose reduced for toxicity. All nine patients who underwent two dose escalations to 10 mg b.i.d. had SD as the best response. Two of the cPR patients had not previously received systemic therapy, while the other had been treated with a RAF inhibitor on a phase I trial.

### toxicity and reasons for study removal

Treatment was well tolerated without any grade 4/5 toxicities attributable to axitinib. The most frequently reported axitinib-related toxicities were hypertension, fatigue, diarrhea, weight loss, anorexia, hand–foot syndrome, nausea, oral pain, myalgia, oral mucositis and liver function test elevations (supplementary Table S2, available at *Annals of Oncology* online). Grade 3 toxicities included hypertension (11), oral pain (4) and fatigue (2). Eleven (33.3%) patients required dose reduction, four of whom had the dose reduced after it had been escalated beyond 5 mg b.i.d. The most common reason for study discontinuation was radiographic PD [21 (63.6%)] (Figure 1A). Five (15.2%) were removed for clinical progression and one for toxicity (intolerable grade 2 fatigue). Three of the five patients who withdrew consent cited drug side-effects and poor quality of life as the reason.

### analysis of MYB, NFIB status and clinical efficacy

MYB immunohistochemistry (IHC) was carried out on tumors from 26 patients; half (13) had detectable MYB expression (Figure 2A). FISH for *MYB* and *NFIB* rearrangements carried out in tumors from 24 patients showed 14 (58.3%) harbored both *MYB* and *NFIB* rearrangements, while 3 were negative for rearrangement in either gene. Four had only the *MYB* break-apart signal, and three had only the *NFIB* break-apart signal. Of the 18 tumors with a *MYB* rearrangement (14 *MYB+/NFIB+* and 4 *MYB+/NFIB−* tumors), 9 had detectable MYB protein by IHC (1+ or 2+). Among the three *MYB−/NFIB−* tumors, MYB IHC expression was absent in two and detectable in one.

No relationship between MYB expression by IHC and PFS was detected (mPFS: MYB+ 7.4 months, MYB− 7.2 months) (supplementary Figure S3, available at *Annals of Oncology* online). A longer mPFS [11.6 months (95% CI: 9.0–NA) versus 5.7 months (95% CI: 5.3–NA)] was observed in patients with *MYB+/NFIB+* tumors compared with those with other FISH patterns (*MYB+/NFIB−*, *MYB−/NFIB+* and *MYB−/NFIB−*), although the difference was not statistically significant (Figure 2B).

### genomic analysis

NGS was carried out in 11 cases, including for the three patients with cPR (Figure 2C). The assessments were carried out on tissues obtained pre-axitinib in six cases (patients 3, 5, 6, 7, 9 and 10) and post-axitinib in five (patients 1, 2, 4, 8 and 11). The number of alterations discovered ranged from zero to eight. The most commonly detected were *NOTCH1* alterations and 4q12 amplification (3 cases each; 27.3%) (Figure 2C). The 4q12 amplicon increases gene copy number for three molecular targets of axitinib: *PDGFRA*, *KDR* (*VEGFR2*) and *KIT* (2-, 9- and 14-fold gains). Two of the 4q12 amplified patients (4 and 5) were treated with axitinib for >1 day and achieved SD for >6 months, including a patient with lung ACC who experienced significant regression of the primary tumor and the longest PFS on study (21.8 months) (patient 5; Figure 2D). Patient 4 achieved a PFS of 7.2 months (12% regression), despite dose reduction to 3 mg b.i.d. after cycle 2. The twofold 4q12 amplification for this patient was detected in a post-axitinib tumor sample obtained after progression. Patient 4's tumor also harbored both *telomerase reverse transcriptase* (*TERT*) gene amplification and a novel t(20;5) translocation that produced a *PRNP-TERT* gene fusion in which the *PRioN Protein* (*PRNP*) gene promoter/5′UTR replaces the *TERT* promoter, suggesting that inappropriate *TERT* expression may also be critical to the oncogenic phenotype in this case.

## discussion

The challenge of ACC trial design is reliably measuring drug activity in a patient population with a broad spectrum of disease aggressiveness. This challenge was addressed here by requiring disease progression before study entry and designating BOR as the primary end point. While this ACC trial successfully met the early efficacy signal for moving to the second stage, the 3 cPRs observed out of 33 total patients (9.1% response rate) fell just short of the pre-specified goal of at least 4 responders. Still, this trial was conducted in a progressive disease population, over half (57.6%) of whom had been previously treated with systemic therapy, and yet tumor shrinkage was still achieved in 22 of 33 (66.7%) and PR/SD for >6 months was observed in 13 (39.4%) (2 with cPR stayed on drug for >11 months). The 9.1% response rate reported here is comparable with the 0%–11% rates observed in several phase II trials of other multi-targeted TKIs tested in ACC, including sorafenib [11, 12] (VEGFR, PDGFR inhibitor), dovitinib [13, 14] (VEGFR, PDGFR, FGFR1-3, KIT inhibitor) and sunitinib (VEGFR, PDGFR, KIT, RET, FLT3 inhibitor) [15]; two of these studies were deemed positive for meeting a PFS primary end point [12, 14].

Identifying clinical and molecular markers that correlate to benefit is one strategy that would enhance the clinical utility of axitinib for ACC. This study concept was in part developed with the rationale that MYB is a central oncogenic driver that activates a number of signaling pathways targeted by axitinib. However, no association between clinical outcome and MYB expression by IHC was found. We did observe a longer mPFS among *MYB+/NFIB+* patients relative to those with tumors harboring other FISH patterns (*MYB+/NFIB−*, *MYB-/NFIB+* and *MYB−/NFIB−*), although the mechanistic basis for this remains unclear and the difference was not statistically significant. More recently, two groups published the observation that over one-third of t(6;9)-negative or MYB-negative ACCs harbor t(8;9) rearrangements resulting in high expression of another MYB family gene, *MYBL1*, producing a gene expression signature similar to that observed in *MYB* fusion tumors [16–18]. *MYB* status alone may be insufficient for delineating meaningful clinical subsets.

NGS of 11 cases identified 3 cases of 4q12 amplification, resulting in increased gene copy number of the axitinib targets *PDGFRA/KDR*/KIT. This 4q12 amplicon has been described in glioblastomas, malignant peripheral nerve sheath tumors and non-small-cell lung cancer with preclinical evidence linking it to susceptibility to TKIs [19]. Axitinib for two 4q12 amplified patients did produce tumor regression and SD for >6 months. While the twofold copy number increase for patient 4 was

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| | No. of patients ($n$ = 33) | Percentage (95% CI) |
|---|---|---|
| Best overall response | | |
| CR | 0 | 0.0% (0.0–10.6%) |
| PR | 3 | 9.1% (1.9–24.3%) |
| SD | 25 | 75.8% (57.7–88.9%) |
| PD | 4 | 12.1% (3.4–28.2%) |
| PR or SD >6 months | 13 | 39.4% (22.9–57.9%) |
| SD <6 months | 15 | 45.5% (28.1–63.6%) |
| Reasons for discontinuation | | |
| Progression of disease (radiographic) | 21 | 63.6% |
| Progression of disease (clinical) | 5 | 15.2% |
| Withdrawal of consent | 5 | 15.2% |
| Toxicity | 1 | 3.0% |
| Protocol violation | 1 | 3.0% |

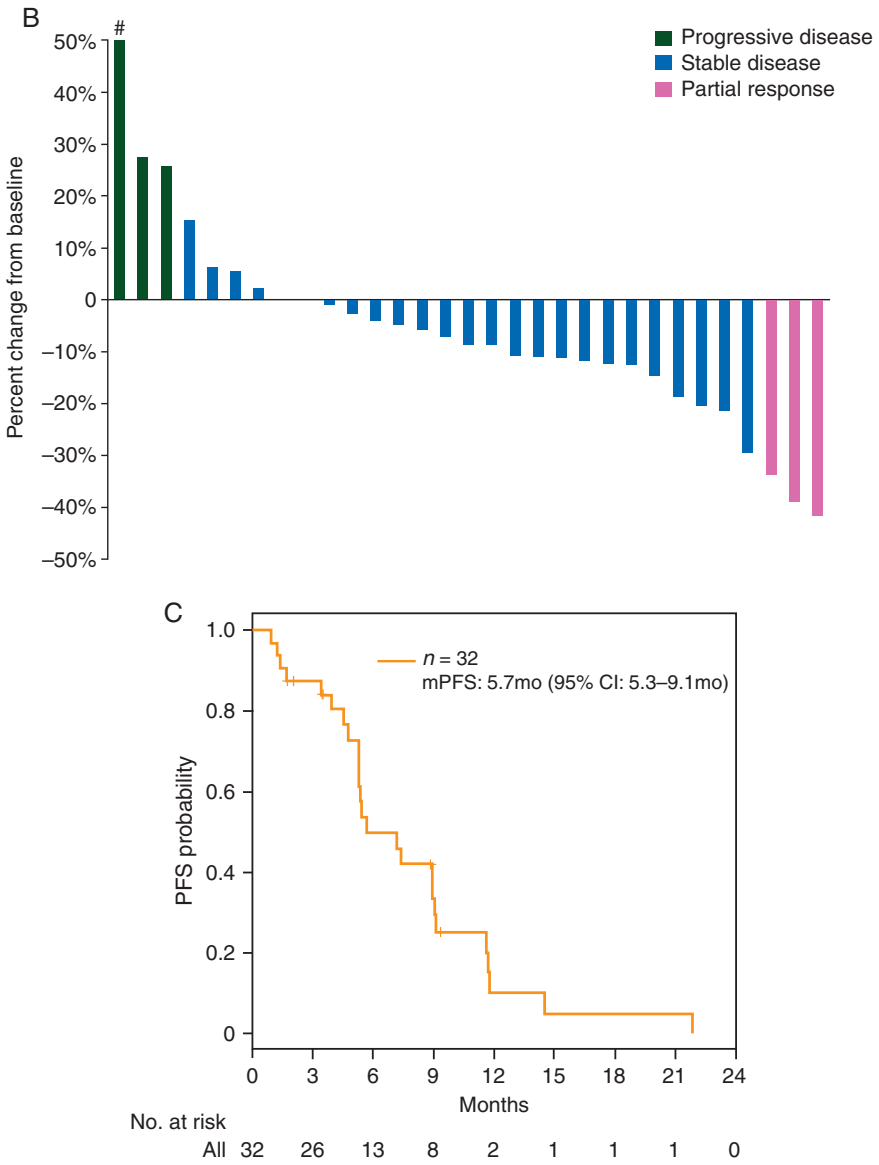


**Figure 1.** Axitinib efficacy in incurable adenoid cystic carcinoma patients. (A) Summary of efficacy data. (B) Waterfall plot of maximum tumor reduction. Hash denotes that RECIST progression was due to the appearance of a new site of disease. (C) Kaplan–Meier curve for progression-free survival (PFS).

A

| MYB IHC | No. of patients ($n$ = 26) |
|---|---|
| Score 0 | 13 |
| Score +1 | 8 |
| Score +2 | 5 |
| **MYB/NFIB FISH** | **No. of patients ($n$ = 24)** |
| MYB (–)/NFIB (–) | 3 |
| MYB (+)/NFIB (–) | 4 |
| MYB (–)/NFIB (+) | 3 |
| MYB (+)/NFIB (+) | 14 |

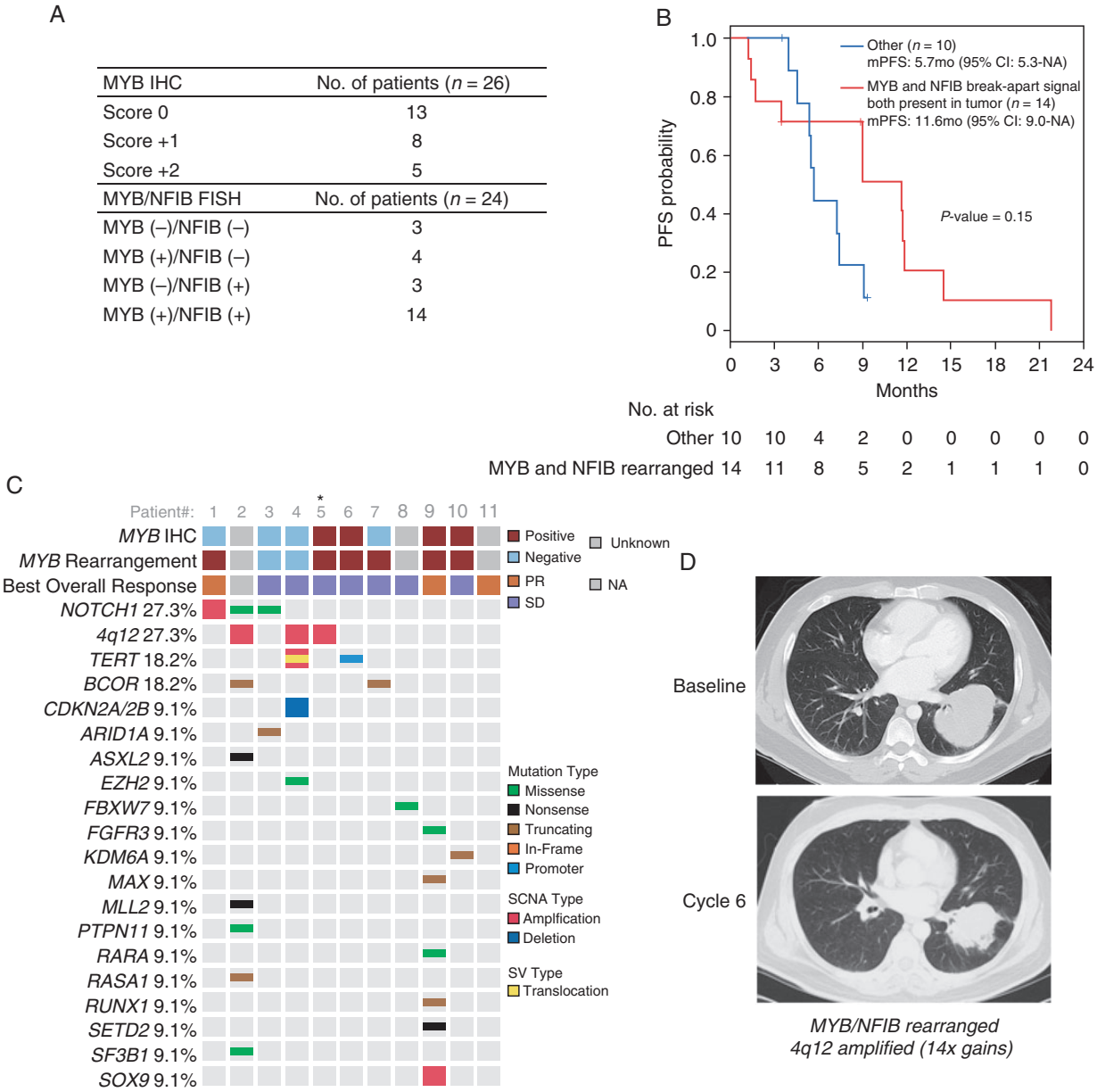


**Figure 2.** Adenoid cystic carcinoma (ACC) biomarkers/genomics and axitinib efficacy. (A) MYB immunohistochemistry and FISH for *MYB* and *NFIB* rearrangements. (B) Progression-free survival for the FISH detected *MYB*+/*NFIB*+ rearrangements and other FISH patterns (*MYB*+/*NFIB*–, *MYB*–/*NFIB*+, and *MYB*–/*NFIB*–). (C) Genetic alterations detected in ACC tumors from 11 enrolled study patients. Asterisk denotes the case that was profiled on the FoundationOne platform. (D) Significant response in a primary lung ACC tumor harboring both *MYB/NFIB* rearrangements and 4q12 amplification (14-fold amplification in *PDGFRA/KDR/KIT*).

detected in a post-axitinib sample, the impressive degree of tumor regression and PFS (21.8 months) achieved in patient 5 (14-fold copy number increase) suggests the hypothesis that in both cases, 4q12 amplification denotes oncogenic dependence upon PDGFRA/KDR/KIT signaling and susceptibility to axitinib. Genomic analysis of primary ACC cases revealed this amplification was present in only 1 out of 55 (1.8%) tumors analyzed [20], raising the possibility that observing it in 3 of 11 (27.3%) cases here is an enrichment in more advanced disease. Ideally, future trials will enrich for 4q12 amplified patients to further evaluate TKI efficacy for this ACC subset and address how the degree of amplification may correlate to drug benefit. It bears highlighting that our study demonstrates that the presence of 4q12 amplification is not requisite for axitinib benefit as durable tumor regressions were achieved among those without this alteration. Additionally, the genomic analysis here is limited to a small number of patients, and there is a need to comprehensively investigate the utility of using profiling to identify predictors of benefit for axitinib in ACC patients.

*NOTCH1* alterations in ACC are of increasing interest, and these were among the most common detected by NGS in this study. There is growing evidence that activating NOTCH pathway alterations are enriched in patients with more aggressive disease (higher grade tumors, liver/bone metastases, shorter

survival) [21–23] and can be therapeutically targeted [24]. *NOTCH1* amplification in patient 1 did correlate with atypically aggressive disease that included peritoneal metastases, and this patient experienced a cPR with a PFS of nearly 1 year (supplementary Figure S4, available at *Annals of Oncology* online), although the connection between *NOTCH1* activation and axitinib susceptibility is not clear, since this alteration was detected in a post-axitinib specimen. The biologic significance of the other two cases of *NOTCH1* alterations is unknown, given that both were located outside of the C-terminal heterodimerization and PEST domains in which activating mutations typically arise.

In conclusion, axitinib possesses activity against ACC, achieving tumor reductions in the majority of a clinically challenging recurrent/metastatic disease population. Biomarker and genomic analysis provided unique insights into the biologic landscape for a small cohort of incurable ACCs. There remains a need to more comprehensively incorporate molecular analyses in ACC investigations to inform how novel therapeutic approaches may be effectively developed.

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## disclosure

MGF is currently employed at Regeneron Pharmaceuticals. CSS is currently employed at Genentech/Roche. All remaining authors have declared no conflicts of interest.

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