PRECISION MEDICINE

original reports

SRC Family Kinase Inhibition Targets YES1 and YAP1 as Primary Drivers of Lung Cancer and as Mediators of Acquired Resistance to ALK and Epidermal Growth Factor Receptor Inhibitors

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abstract

PURPOSE The identification of novel oncogenic driver alterations and novel mechanisms of acquired resistance (AR) is the key for further development of personalized therapy. The current study investigates the potential role of *YES1* amplification as a primary driver of tumorigenesis and of *YES1/YAP1* amplifications as mediators of AR to ALK and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs).

METHODS Models of ectopic expression were established and characterized for YES1 and YAP1 in human bronchial epithelial cells and *ALK* fusion–positive (ALK+) and *EGFR*-mutant lung adenocarcinoma cell lines. MSK-IMPACT data for all lung adenocarcinoma cases and for ALK and EGFR TKI AR cases were surveyed for *YES1* and *YAP1* amplification.

RESULTS We report response to SRC family kinase (SFK) inhibition in a patient whose lung cancer exhibited *YES1* amplification, without any well-established primary driver alteration, suggesting that *YES1* amplification can also function as a primary oncogenic driver. To investigate the possibility of *YES1* as a primary driver in tumorigenesis, we established preclinical models of YES1 overexpression using human bronchial epithelial cells and normal human breast epithelial cells. We showed that YES1 overexpression conferred sensitivity to SFK TKIs and promoted EGF-independent growth in a YAP1-dependent manner. Analysis of clinical genomic sequencing data from cases of AR to EGFR and ALK inhibitors revealed acquired amplification of *YAP1* in four cases. *EGFR*-mutant and *ALK* fusion–positive cells overexpressing YES1 or YAP1 were resistant to EGFR and ALK TKIs, respectively, but were sensitive to dual inhibition of the primary driver and YES1.

CONCLUSION Our results demonstrate the therapeutic potential of SFK inhibition in primary tumorigenesis and AR driven by YES1/YAP1 signaling.

JCO Precis Oncol 6:e2200088.

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on June 7, 2022 and published at ascopubs.org/journal/po on August 11, 2022: DOI https://doi.org/10.1200/PO.22.00088

INTRODUCTION

The SRC family of kinases (SFK) is composed of nine members—SRC, YES1, FYN, FGR, LCK, HCK, BLK, LYN, and FRK. In the context of tumor progression and metastasis, SFKs cooperate with receptor tyrosine kinases to activate downstream signaling pathways and perform crucial regulatory functions in cell adhesion, migration, and invasion.[1] These characteristics of SFKs have made them appealing targets for therapeutic intervention in the treatment of cancers. Two SRC/ABL tyrosine kinase inhibitors (TKIs), dasatinib and bosutinib, have been approved by the US Food and Drug Administration (FDA) for use in the treatment of Philadelphia chromosome–positive leukemias driven by BCR-ABL fusion. For other types of cancers, however, the results of clinical trials with SFK TKIs have been largely disappointing. For example, in small studies investigating second-line therapies after acquired resistance (AR) to epidermal growth factor receptor (EGFR) inhibition in *EGFR*-mutant (*EGFR*m) lung cancers had developed, dasatinib as a single agent or in combination with the EGFR TKI afatinib had no clinical activity.[2,3] Modest activity was observed for dasatinib as first-line therapy in advanced non–small-cell lung cancer (NSCLC)[4] and for the SFK TKI saracatinib as second-line therapy in advanced, platinum-pretreated NSCLC,[5]

CONTEXT

Key Objective

We studied the role of amplification of the *YES1* gene, encoding a SRC family kinase (SFK) involved in YAP1/HIPPO pathway signaling, as a primary driver of tumorigenesis and of *YES1/YAP1* amplifications as mediators of acquired resistance (AR) to ALK and epidermal growth factor receptor kinase inhibitors.

Knowledge Generated

We document the response to SFK inhibition in a patient whose lung adenocarcinoma harbored *YES1* amplification in the absence of any established primary lung cancer driver alteration. We confirm that the biologic effects of *YES1* amplification are YAP1-dependent and demonstrate the interdependence of YES1 and YAP1 in promoting AR to epidermal growth factor receptor and ALK inhibitors in lung cancers.

Relevance

SFK inhibition can potentially be exploited to therapeutically target in primary tumorigenesis and AR driven by YES1/YAP1/HIPPO signaling.

suggesting that a subset of patients with NSCLC could potentially benefit from treatment with SFK TKIs.

Recently, we identified amplification of *YES1* as a recurrent mechanism of resistance to EGFR inhibition in *EGFR*m lung cancers,[6-8] and others have shown that amplification of *YES1* conferred resistance to human epidermal growth factor receptor 2–targeted therapy in *HER2*-amplified breast cancer cell lines.[9,10] These findings molecularly defined a subset of patients with cancer with AR whose disease might respond to SFK TKIs and raised the possibility that amplification of *YES1* could also be a targetable primary oncogenic driver.

In this study, we report a clinical response to dasatinib in a patient with metastatic lung adenocarcinoma (LUAD) that harbored amplification of *YES1* but no established primary driver alteration and use multiple cell line models to demonstrate the oncogenic potential of *YES1* and its dependence on *YAP1*. We also describe multiple cases of AR to EGFR and ALK TKIs associated with amplification of *YAP1* and demonstrate the interdependence of *YES1* and *YAP1* in mediating resistance to EGFR-directed and ALK-directed therapies, which can be effectively targeted by dual inhibition of both the primary driver and YES1.

METHODS

Detailed information about the single patient clinical data review, cell lines, reagents, and experimental methods are described in the Data Supplement.

RESULTS

A LUAD With *YES1* Amplification and No Established Primary Driver Alteration Responds to SFK Inhibition

Patient 1 was an 81-year-old man with a 50 pack-year smoking history who was diagnosed with de novo stage IV LUAD in 2016. He received standard first-line carboplatin plus pemetrexed plus bevacizumab for 5 months followed by disease progression. He was subsequently treated with second-line nivolumab and had stable disease as his best response lasting 10 months followed by disease progression. MSK-IMPACT testing revealed *YES1* amplification (5.6-fold increase) in the absence of any established primary driver alteration (Fig 1A and Data Supplement). As a result of the patient's comorbid conditions, the decision was made to forego further cytotoxic chemotherapy and to instead initiate treatment with dasatinib on the basis of the preclinical data discussed in this report. The patient began treatment with dasatinib 70 mg twice daily, which was later reduced to 70 mg once daily as a result of fatigue and edema. Computed tomography imaging performed 3 weeks after initiating dasatinib therapy showed evidence of a response, and subsequent computed tomography imaging at 6 weeks and 10 weeks confirmed a partial response by RECIST 1.1, with a 69% reduction in size of his target right lung lesion (Fig 1B). The patient continued on therapy but developed a treatment-unrelated stroke, leading to a decision to pursue hospice and to discontinue therapy.

Transforming Potential of *YES1* in Normal Lung and Breast Epithelial Cells

To assess the oncogenic potential of *YES1* amplification, we established inducible models of *YES1* overexpression in human bronchial epithelial cells (HBECs) and the normal human breast epithelial cell line MCF10A. As shown in Figure 2A, overexpression of *YES1* in HBECs resulted in increased phosphorylation of SFKs and activation of the AKT serine/threonine kinase and mitogen-activated protein kinase pathways. Because the phospho-SFK antibody does not distinguish between different SFKs, we also used a phosphokinase array that specifically measures phosphorylation of YES, SRC, FYN, and four other SFKs and confirmed that upregulation of phosphorylation was restricted to YES1 among these seven SFKs (Data Supplement). We next examined the effect of *YES1* overexpression on the growth factor dependence of lung and

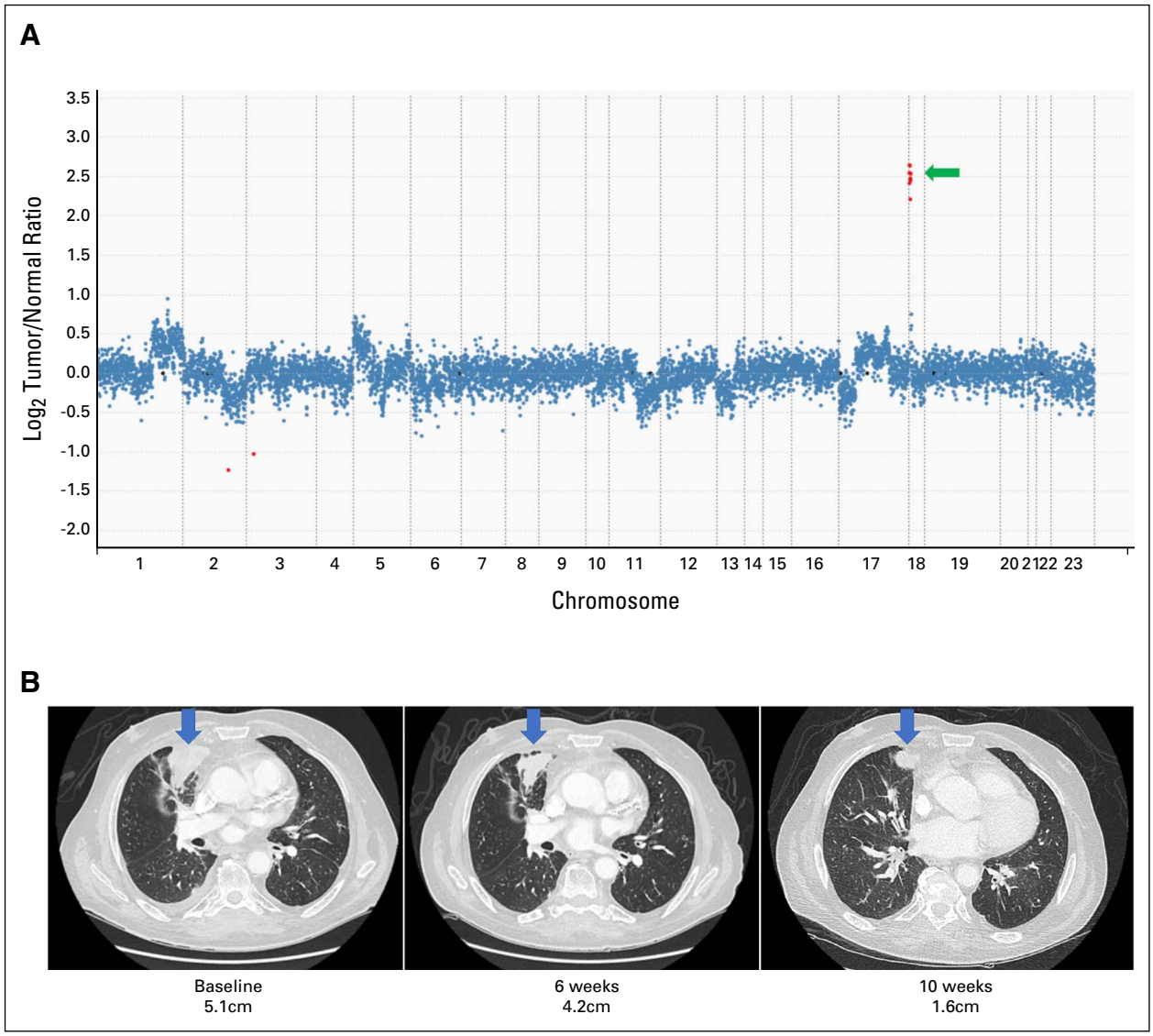

FIG 1. Clinical case of LUAD with *YES1* amplification. (A) Copy number plots for tumor samples from patient 1. Each dot represents a target region in the MSK-IMPACT–targeted capture assay. Red dots are target regions exceeding a fold change cutoff of twofold. The log ratios (*y* axis) comparing tumor versus normal coverage values are calculated across all targeted regions (*x* axis). The green arrow indicates focal amplification of *YES1* (11 coding exons targeted). (B) Radiographic response of patient 1 to treatment with dasatinib. Representative images from CT scans at baseline and after 6 and 10 weeks of treatment. Blue arrows indicate the target right lung lesion. CT, computed tomography; LUAD, lung adenocarcinoma.

breast epithelial cells. We found that overexpression of *YES1* promoted EGF-independent growth in both HBECs and MCF10A cells (Fig 2B and Data Supplement). To further test the transforming capacity of YES1, we used a focus formation assay to show that *YES1* overexpression in HBECs significantly induced foci formation (Fig 2C). In addition, we investigated the effect of *YES1* overexpression on the response of epithelial cells to SFK TKIs. Cell viability assays revealed that the induction of *YES1* overexpression in both HBECs and MCF10A cells specifically conferred increased sensitivity to the SFK TKIs dasatinib and saracatinib, but not to the EGFR TKI osimertinib or ALK TKI alectinib (Fig 2D and Data Supplement).

To gain more insight into the signaling pathways underlying the transforming potential of YES1, we focused on the role of YAP1 (YES1-associated protein 1), whose activity is known to be regulated by YES1 kinase activity.[11] Western blot analysis showed that induction of YES1 overexpression resulted in phosphorylation of tyrosine 357 (Y357) but not serine 127 (S127) on YAP1 (Fig 2E and Data Supplement). The induction of YES1 overexpression also promoted translocation of YAP1 from the cytoplasm to the nucleus (Fig 2F). To determine the functional importance of these events, we performed siRNA-mediated knockdowns of *YAP1* in *YES1*-overexpressing cells. Depletion of YAP1 had no effect on the phosphorylation status of ERKs, but completely abolished EGF-independent growth and increased sensitivity

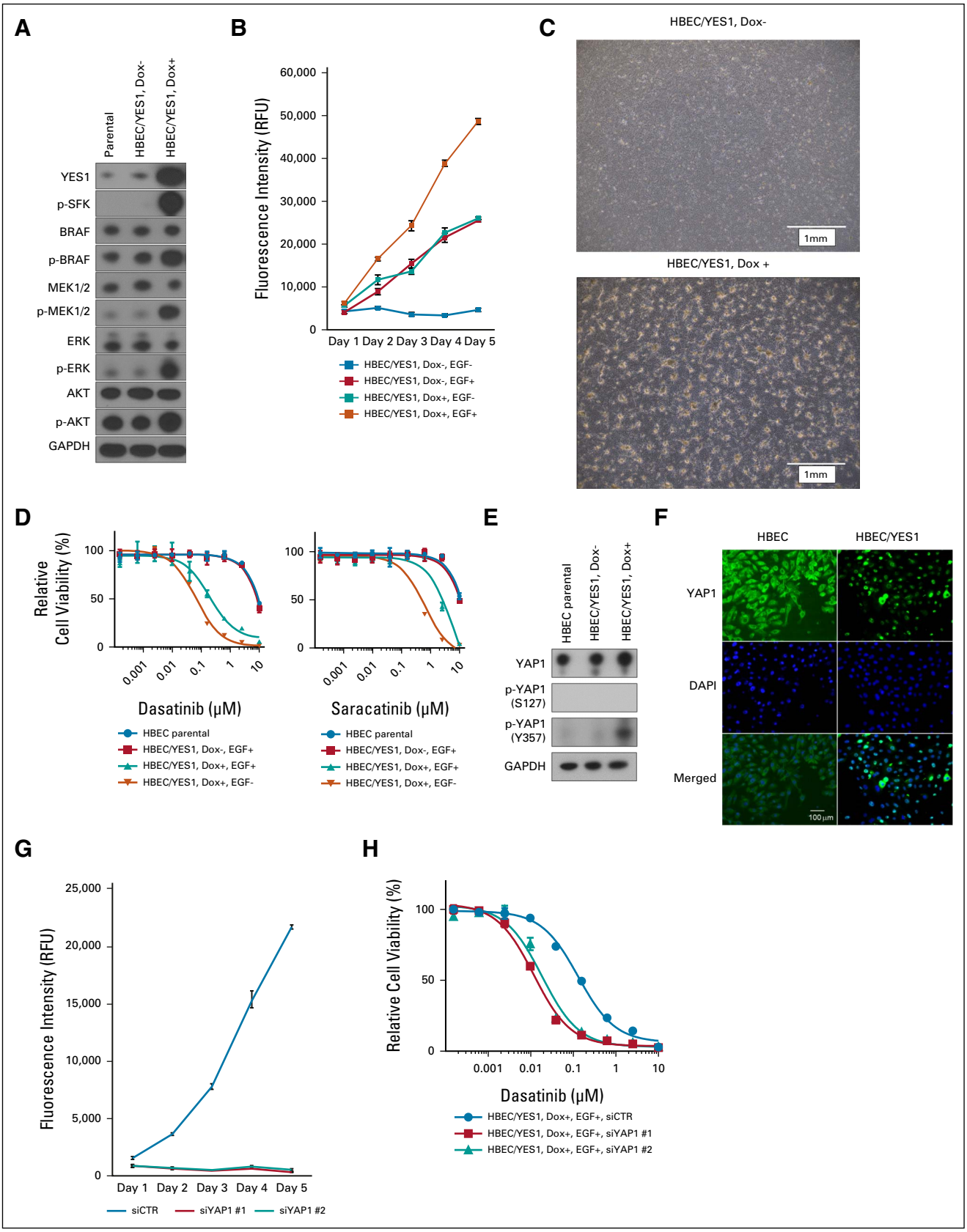

FIG 2. *YES1* amplification as a primary driver in LUAD. (A) Whole cell lysates were prepared and then subjected to western blot analysis with the indicated antibodies. (B) Cells (HBEC) were seeded onto 96-well plates at a density of 3,000 cells/well. Relative cell (continued on following page)

FIG 2. (Continued). number was assayed using alamarBlue. (C) Cells were seeded onto 6-well plates at a density of 100,000 cells/well and cultured with or without doxycycline. Focus formation was evaluated by light microscopy after 7 days (Dox+) and 14 days (Dox–). Scale bar, 1 mm. (D) Cells were treated with the indicated drugs for 96 hours, and then cell viability was assessed. Data represent the mean ± SE of four independent experiments. (E) Whole cell lysates were prepared and then subjected to western blot analysis with the indicated antibodies. (F) Immunofluorescent staining was performed to evaluate the localization of YAP1. Scale bar, 100 μm. (G) Cells were seeded onto 96-well plates at a density of 3,000 cells/well (HBEC/YES1, Dox+). Relative cell number was assayed using alamarBlue. (H) Cells were transfected with the indicated siRNAs and then seeded 24 hours later onto 96-well plates with the indicated concentrations of dasatinib. Cell viability was measured 96 hours later. Data represent the mean ± SE of four independent experiments. HBEC, human bronchial epithelial cell; LUAD, lung adenocarcinoma; RFU, relative fluorescence units.

to dasatinib in *YES1*-overexpressing HBECs and MCF10A cells (Figs 2G and 2H and Data Supplement). Taken together, these results suggest that *YES1* amplification can function in a YAP1-dependent manner as a primary driver in lung and breast tumorigenesis.

YES1 Overexpression Confers Resistance to EGFR and ALK TKIs

We previously identified *YES1* amplification as a mechanism of AR to EGFR TKIs and potentially ALK TKIs.[6] To further characterize the role of *YES1* in AR, we established inducible models of *YES1* overexpression using *EGFR*-mutant (*EGFR*m; PC9 and HCC827) and ALK fusion–positive (ALK+; H3122 and H2228) cells. Western blot and phosphokinase array analyses confirmed induction of *YES1* expression and YES1 phosphorylation and revealed an increase in ERK phosphorylation in the absence of any effect on the phosphorylation status of EGFR or ALK (Fig 3A and Data Supplement). Cell viability assays subsequently demonstrated that the overexpression of *YES1* conferred resistance to three generations of both EGFR and ALK TKIs (Figs 3B and 3C and Data Supplement). Correlative western blot analyses confirmed that the sensitivity of mutant EGFR or EML4-ALK to their respective TKIs remained unchanged in *YES1*-overexpressing cells, but SFK and ERK signaling persisted in the presence of these inhibitors (Data Supplement). Overexpression of *YES1* also resulted in increased phosphorylation of YAP1 on Y357 but not S127 (Fig 3D). To determine the functional significance of YAP1 in YES1-mediated resistance to EGFR and ALK TKIs, we performed siRNA-mediated knockdowns of *YAP1* in *YES1*-overexpressing PC9 and H3122 cells. As shown in Figures 3E and 3F, knockdowns of *YAP1* had no effect on SFK and ERK phosphorylation, but partially restored sensitivity to EGFR and ALK TKIs. These results indicate that YAP1 contributes to YES1-mediated resistance to EGFR and ALK TKIs.

YAP1 Amplification is Associated With AR to EGFR and ALK TKIs

We previously reviewed clinical genomic sequencing data for cases of AR to EGFR and ALK TKIs and found a total of seven cases associated with amplification of *YES1*.[6] In our current study, a similar analysis yielded a total of four cases of AR to EGFR (erlotinib, n = 3) and ALK TKIs (alectinib, n = 1) associated with amplification of *YAP1*. The clinical and molecular features of these four cases are summarized in the Data Supplement. Copy number profiles for two cases are shown in Figure 4A. The corresponding immunohistochemical staining of tumor samples for YAP1 showed prominent labeling of LUAD cells (Fig 4B). After the development of resistance to initial EGFR-directed or ALK-directed therapy, three patients received additional TKIs among their subsequent treatment regimens. Patient 2 had progression of disease on brigatinib and lorlatinib. Patient 5 had progression of disease on osimertinib. Patient 4 symptomatically declined on osimertinib, but the clinical course was complicated by the development of a pulmonary embolism in the absence of clear radiographic evidence of tumor progression.

To confirm that *YAP1* amplification can confer resistance to EGFR and ALK TKIs, we established *EGFR*m (PC9 and H1975) and *ALK*+ (H3122) cells stably overexpressing *YAP1*. Immunoblot analysis confirmed overexpression and phosphorylation on both Y357 and S127 of YAP1 (Data Supplement). Cell viability assays demonstrated that *EGFR*m and *ALK*+ cells overexpressing *YAP1* were more resistant to TKIs than cells stably transfected with the empty vector control (Figs 4C and 4D and Data Supplement). Correlative western blot analyses confirmed that the sensitivity of mutant EGFR or EML4-ALK to their respective TKIs remained unchanged in *YAP1*-overexpressing cells, but SFK and ERK signaling persisted in the presence of these inhibitors (Data Supplement). As expected, siRNA-mediated knockdown of *YAP1* in cells overexpressing *YAP1* fully restored sensitivity to TKIs to levels observed in parental cells (Data Supplement).

We next used two approaches to determine whether YES1 is required for YAP1-mediated resistance to EGFR and ALK TKIs. First, we performed siRNA-mediated knockdowns of *YES1* in *YAP1*-overexpressing cells. Knockdown of YES1 in *YAP1*-overexpressing cells abrogated phosphorylation of Y357 on YAP1 and fully restored sensitivity to EGFR or ALK TKIs (Fig 4E and Data Supplement). Second, we generated a Y357F mutant of YAP1, thereby eliminating the YES1 phosphorylation site. The expression of the Y357F mutant in cells resulted in a decrease in detected phosphorylation of YAP1 on Y357 (Data Supplement). *EGFR*m and *ALK*+ cell lines expressing YAP1 Y357F exhibited intermediate sensitivity to their respective TKIs, indicating that phosphorylation by YES1 of Y357 is required to confer full resistance through *YAP1* overexpression (Fig 4F). Taken together, these results support the critical regulatory role of

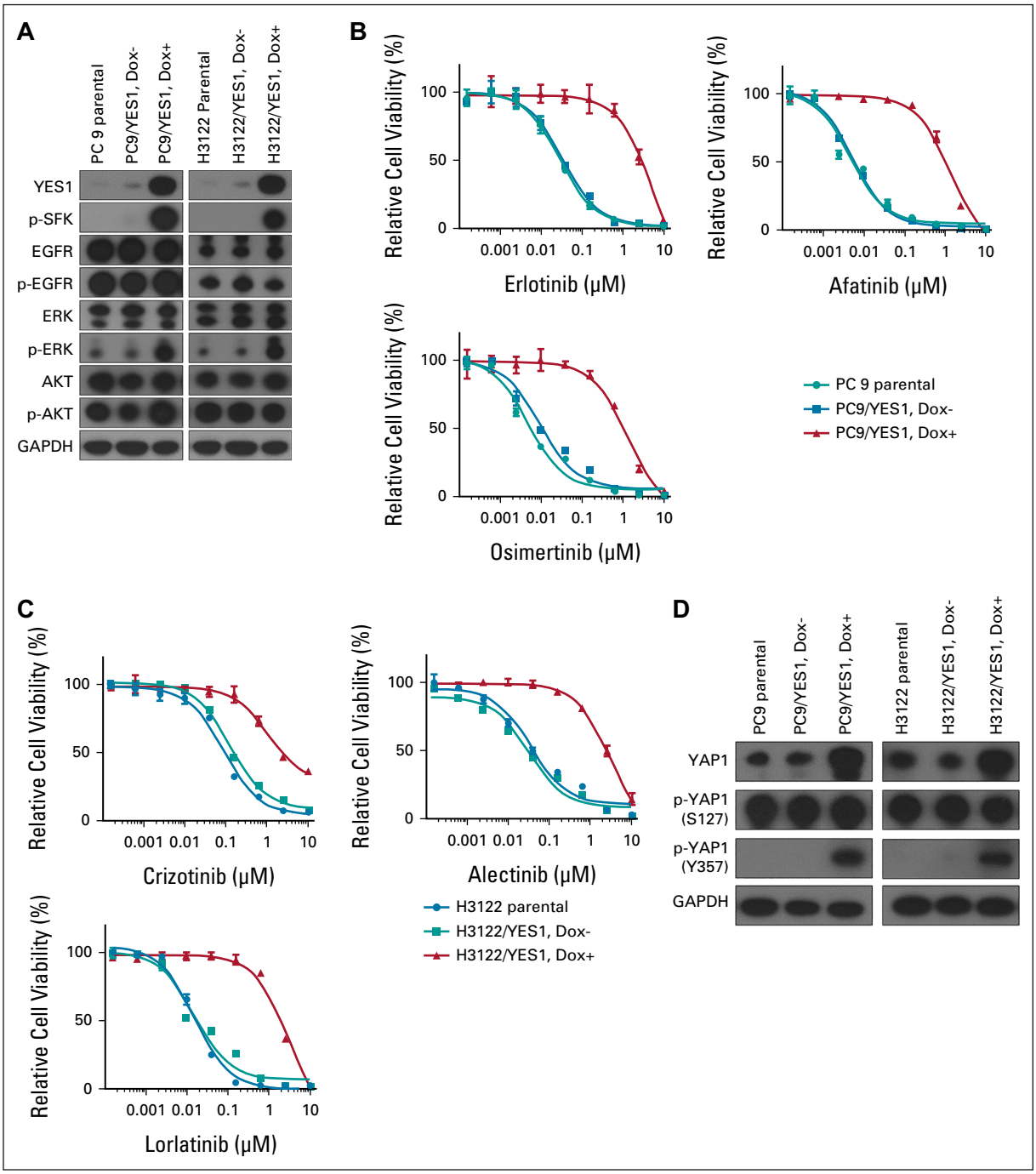

FIG 3. *YES1* overexpression confers resistance to EGFR and ALK TKIs. (A) Whole cell lysates were prepared and then subjected to western blot analysis with the indicated antibodies. (B and C) Cells were treated with each inhibitor for 96 hours, and then cell viability was determined. Data represent the mean ± SE of four independent experiments. (D) Whole cell lysates were prepared and then subjected to western blot analysis with the indicated antibodies. (E) Cells were transfected with the indicated siRNAs. Whole cell lysates were prepared 48 hours post-transfection and subjected to western blot analysis with the indicated antibodies. (F) Cells were transfected with the indicated siRNAs and then seeded 24 hours post-transfection onto 96-well plates with the indicated drug treatments. Cell viability was measured 96 hours later. Data represent the mean ± SE of four independent experiments. EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

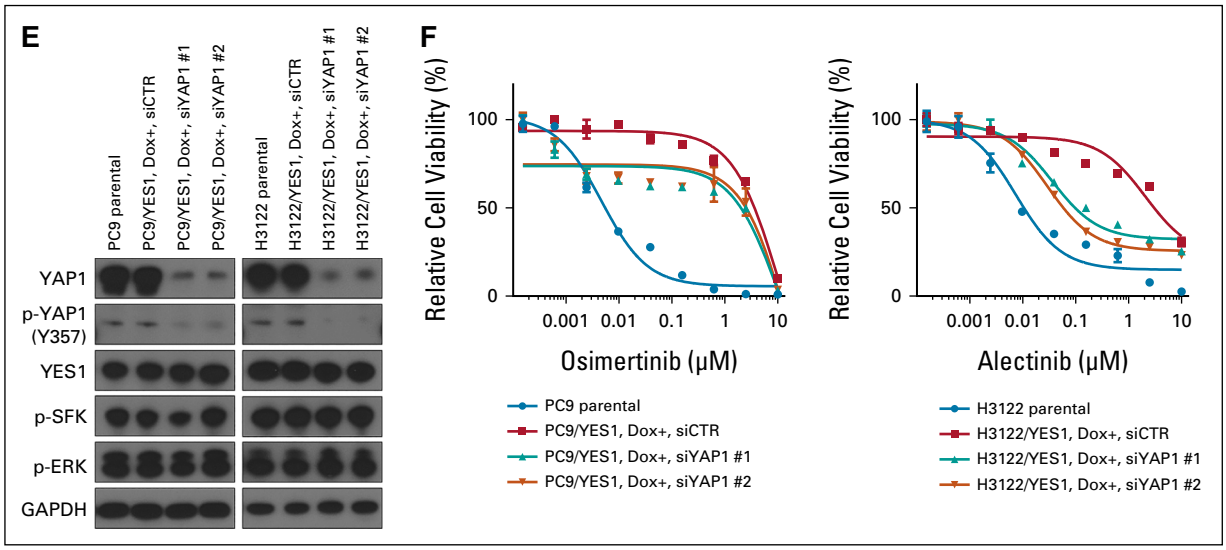

FIG 3. (Continued)

YES1 in mediating resistance conferred by overexpression of *YAP1* and suggest that the addition of SFK inhibition might be effective in overcoming both YAP1-mediated and YES1-mediated resistance to EGFR and ALK TKIs.

Dual Inhibition of the Primary Driver and SFKs Overcomes Both YES1-Mediated and YAP1-Mediated Resistance to EGFR and ALK TKIs

We next tested whether dual pharmacologic inhibition of the primary driver and SFKs could overcome resistance to EGFR and ALK TKIs conferred by overexpression of either *YES1* or *YAP1*. In selecting inhibitors for this purpose, our goal was to design treatment regimens that could be readily adapted to the clinical setting. We therefore sought inhibitors that could fulfill the following two criteria: (1) the drug is either approved or under review by the FDA and (2) the drug is a multitarget inhibitor that can block the kinase activities of both the primary driver and SFKs. For *EGFR*m cell lines, we selected dasatinib, an FDA-approved inhibitor of SRC, ABL, and c-KIT, which also has potent activity against mutant EGFR.[6] For *ALK*+ cell lines, we used repotrectinib, an inhibitor developed to overcome solvent-front mutations in ROS1, TRK, and ALK and that in addition has activity against multiple SFKs including YES1.[12] As shown in cell viability assays (Fig 5A and Data Supplement), *EGFR*m cell lines overexpressing either *YES1* or *YAP1* were equally sensitive to dasatinib as their parental cell lines. Similarly, *YES1*-overexpressing or *YAP1*-overexpressing *ALK*+ H3122 cells were also equally sensitive to alectinib as parental H3122 cells. Immunoblot analysis confirmed dose-dependent blockade of both the primary driver and SFKs and demonstrated suppression of downstream ERK signaling (Figs 5B and 5C and Data Supplement). In addition, treatment with dasatinib or repotrectinib resulted in relocalization of YAP1 from the nucleus to cytoplasm in *YES1*-overexpressing and *YAP1*-overexpressing cells (Fig 5D). In H3122 cells expressing YAP1 Y357F, localization of YAP1 was largely restricted to the cytoplasm, lending further support to phosphorylation of YES1 being required for nuclear localization of YAP1. Taken together, these results demonstrate that in *EGFRm* and *ALK+* cells overexpressing *YES1* or *YAP1*, dual inhibition of the primary driver and SFKs suppressed downstream ERK signaling, blocked nuclear import of YAP1, and overcame YES1-mediated and YAP1-mediated resistance to EGFR and ALK TKIs.

YES1 and *YAP1* Amplification Cases in the MSK Clinical Sequencing Cohort

Finally, to clinically define the subset of patients with cancers harboring *YES1*/*YAP1* amplification who could potentially benefit from treatment with SFK inhibitors, we searched for cases with *YES1*/*YAP1* amplification in the MSK Clinical Sequencing Cohort. In a consecutive cohort of 44,660 patients across all cancer types who underwent next-generation sequencing via MSK-IMPACT, we identified 463 (1%) patients with *YES1* (n = 243) or *YAP1* (n = 221) amplification. Perhaps not unexpectedly, these cases showed a pattern of mutual exclusivity, but this trend did not achieve statistical significance given the rarity of both events. Of the 463 patients, only one patient with bladder cancer had concurrent *YES1* and *YAP1* amplification. The frequency of *YES1*/*YAP1* amplification for each cancer is shown in Figures 6A and 6B. Focusing on NSCLC, 66 of 6,629 patients had *YES1*/*YAP1* amplification. The status of concurrent alterations in known driver genes for NSCLC samples with *YES1*/*YAP1* amplification is presented in Figure 6C. The details of concurrent alterations are as follows: EGFR L858R (n =

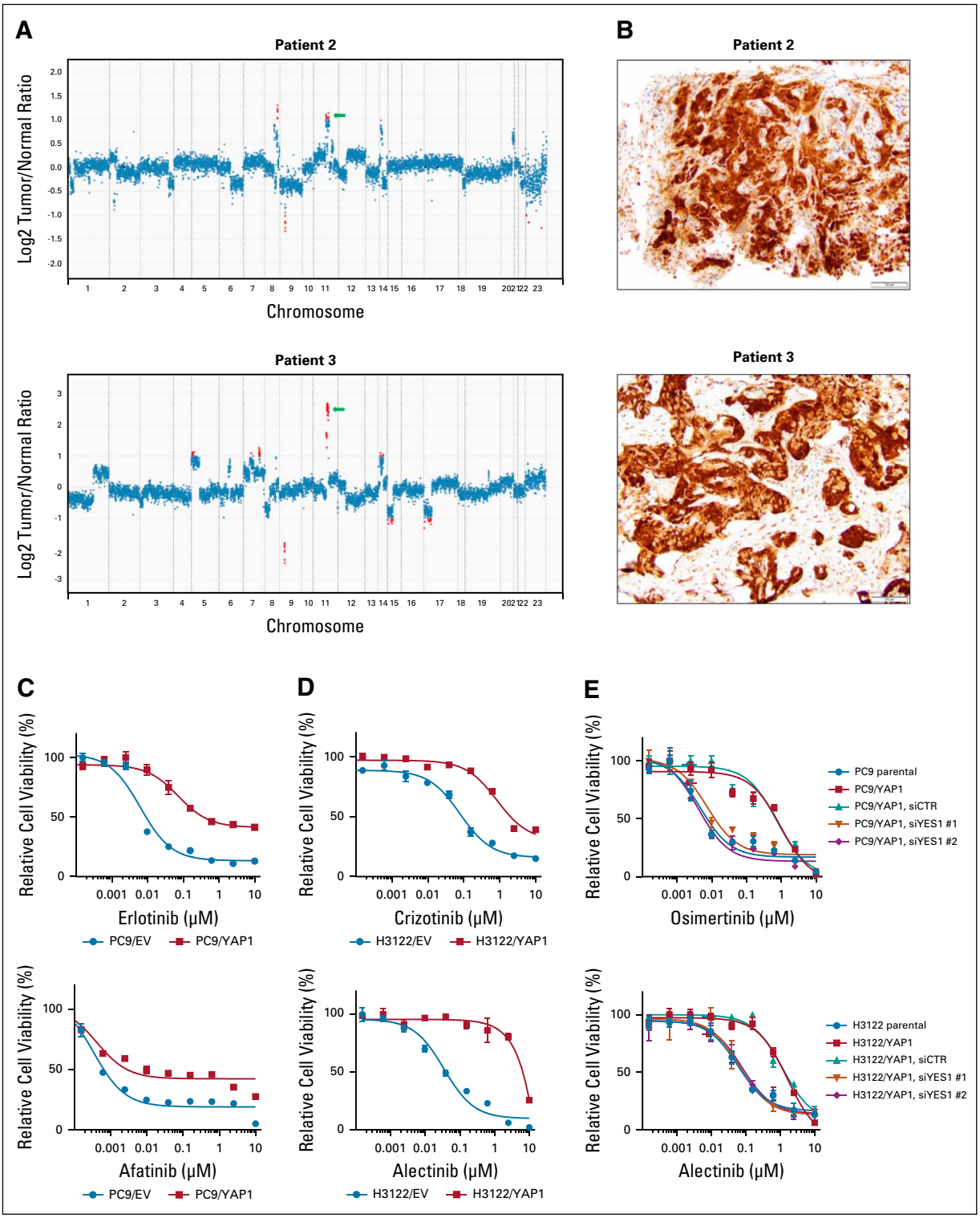

FIG 4. *YAP1* amplification is associated with AR to EGFR and ALK TKIs. (A) Copy number plots for tumor samples from patients 2 and 3. Each dot represents a target region in the MSK-IMPACT–targeted capture assay. Red dots are target regions exceeding a fold change cutoff of twofold. The log ratios (*y* axis) comparing tumor versus normal coverage values are calculated across all targeted regions (*x* axis). Green arrows indicate focal amplification of *YAP1*. (B) Immunohistochemistry for YAP1 on tumor samples from patients 2 and 3. (C and D) Cells were treated with each inhibitor for 96 hours, and then cell viability was determined. Data represent the mean ± SE of four independent experiments. (E) Cells were transfected with the indicated siRNAs and then seeded 24 hours post-transfection onto 96-well plates with the indicated drug treatments. Cell viability was measured 96 hours later. Data represent the mean ± SE of four independent experiments. (F) Cells were treated with each inhibitor for 96 hours, and then cell viability was determined. Data represent the mean ± SE of four independent experiments. AR, acquired resistance; EFGR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

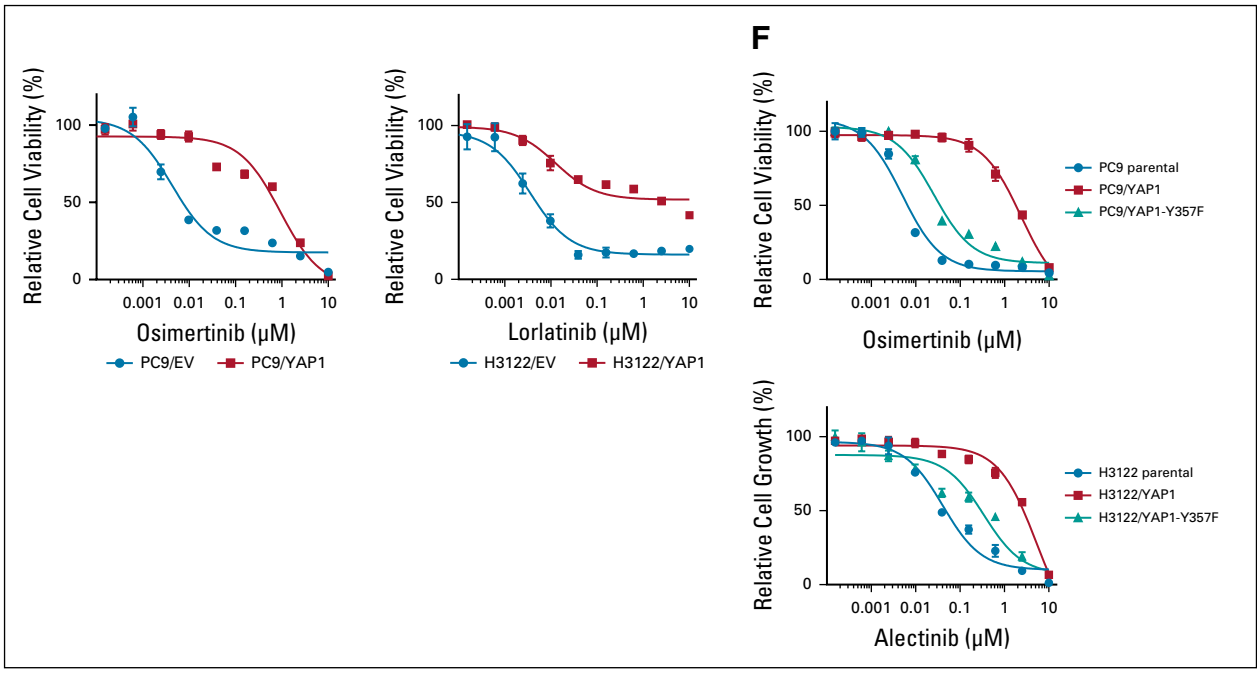

FIG 4. (Continued)

6), *EGFR* amplification (n = 7), *EGFR* in-frame deletion in ex19 (n = 7), EGFR T790M (n = 4), EGFR G719A (n = 1), *ERBB2* amplification (n = 2), *MET* amplification (n = 1), MET Y1003N (n = 1), KRAS G12 (n = 6), KRAS G13D (n = 1), *BRAF* amplification (n = 1), BRAF V600E (n = 1), ALK fusion (n = 4), and ROS1 fusion (n = 1). The remaining 31 samples harbored *YES1*/*YAP1* amplification in the absence of an established primary driver alteration. Regarding clinical outcomes, patients with *YES1*/*YAP1* amplification had shorter overall survival, compared with patients without *YES1*/*YAP1* amplification, both in all cancer types (median month survival of 19.5 months *v* 39.3 months, Fig 6D) and in the subset of NSCLC (median month survival of 13.5 months *v* 35.5 months, Fig 6E). These findings provide further evidence supporting the need for improved therapeutic options for patients with cancers that feature *YES1*/*YAP1* amplification.

DISCUSSION

To the best of our knowledge, we have reported the first documented case of a radiographic response to SFK inhibition in a patient whose malignancy exhibited amplification of an SFK gene in the absence of an established primary driver alteration. In our patient with stage IV LUAD in which amplification of *YES1* was detected, a 69% reduction in a target right lung lesion was observed after 10 weeks of treatment with dasatinib. This response highlights the therapeutic potential of targeting SFKs in molecularly defined subsets of patients with cancer. Our review of the MSK-IMPACT database revealed amplification of *YES1* across multiple tumor types, including colorectal, breast, ovarian, pancreatic, and esophagogastric cancers. Hamanaka et al,[13] who recently generated a novel YES1 inhibitor, also reported amplification of *YES1* in clinical samples from several different types of cancers. Amplifications of other SFK genes are also potentially targetable primary oncogenic drivers. For example, a recent study of 401 patients with colorectal carcinoma sequenced with MSK-IMPACT found that 7% had high level 20q amplification in the absence of RAS/RAF alterations.[14] *SRC* was the only recognized 20q oncogene with a significant inverse relationship between mRNA upregulation and *RAS*/*RAF* mutation status.

We have also shown that targeting SFKs can be effective in overcoming therapeutic resistance mediated by YES1/YAP1 signaling. In multiple *EGFR*m and *ALK+* cell lines, resistance to EGFR and ALK TKIs conferred by either *YES1* or *YAP1* overexpression was sensitive to concomitant blockade of the primary driver and *YES1*. We were able to achieve this dual inhibition by using single TKIs—dasatinib and repotrectinib—each with potent activity against both the primary driver and *YES1*. Alternatively, multiple TKIs can be combined to achieve dual inhibition. For example, early clinical trials investigating the use of combinations of EGFR and MET TKIs in *MET*-dysregulated, EGFR TKI–resistant *EGFR*m lung cancers have shown promising results that support the strategy of targeting of both primary drivers and potential bypass pathways in the setting of AR.[15,16]

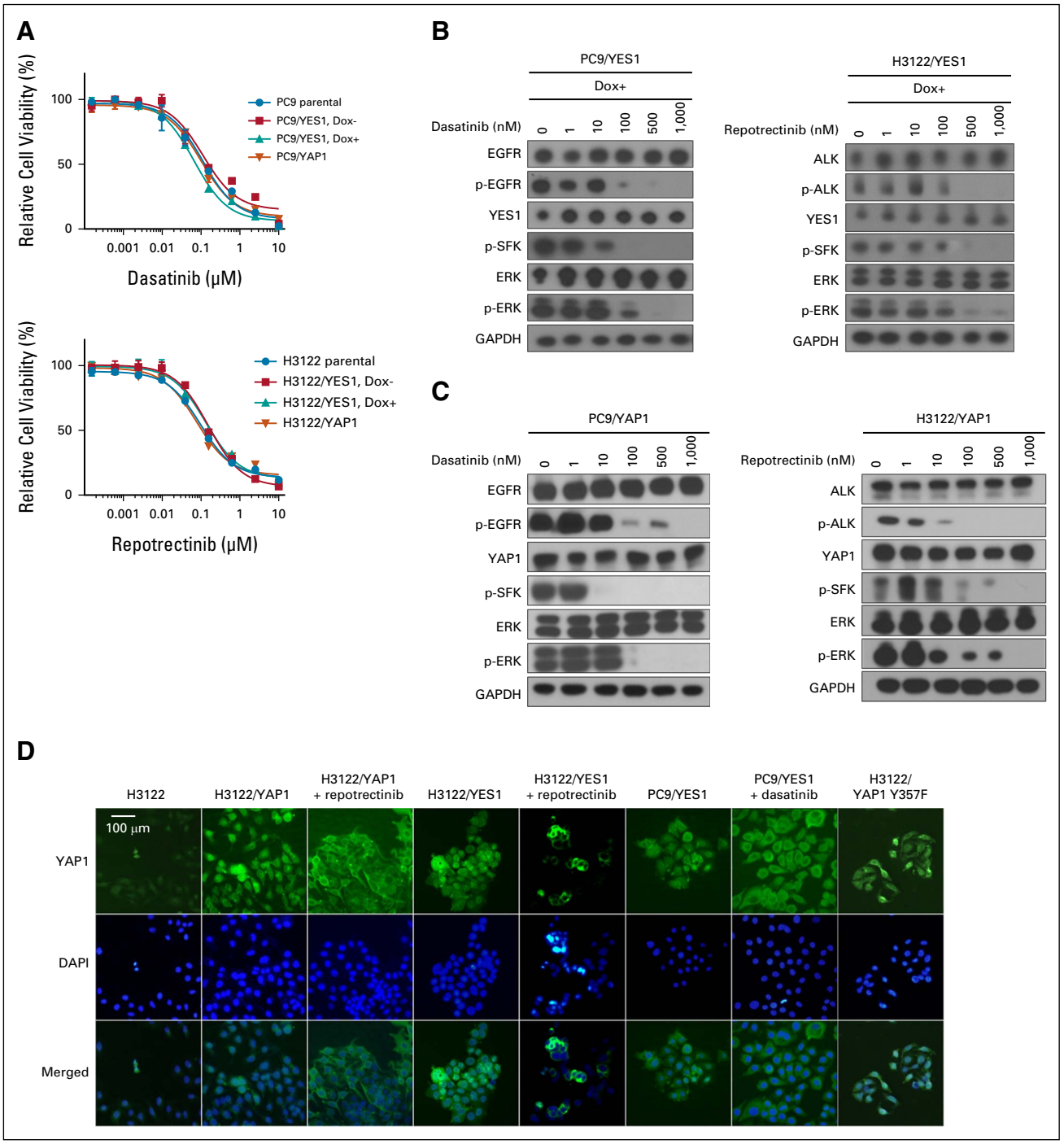

FIG 5. Dual inhibition of the primary driver and SFKs overcomes *YES1*-mediated and *YAP1*-mediated resistance. (A) Cells were treated with each inhibitor for 96 hours, and then cell viability was determined. Data represent the mean ± SE of four independent experiments. (B and C) Western blot analysis of whole cell lysates that were prepared after 4 hours of treatment with the indicated concentrations of each inhibitor. (D) Immunofluorescent staining was performed to evaluate the localization of YAP1. Scale bar, 100 μm. SFK, SRC family kinase.

Although dual inhibition of the primary driver and YES1 was able to overcome resistance mediated by YES1/YAP1 signaling in all *EGFR*m and *ALK+* cell lines that we tested, this approach was rendered ineffective by prior treatment of YES1-overexpressing or YAP1-overexpressing cells, with TKIs targeting only the primary driver. These findings suggest that *EGFRm* and *ALK+* tumors with prominent YES1/YAP1 signaling might exhibit resistance to EGFR and ALK TKIs and develop resistance to effective dual inhibition if initially treated

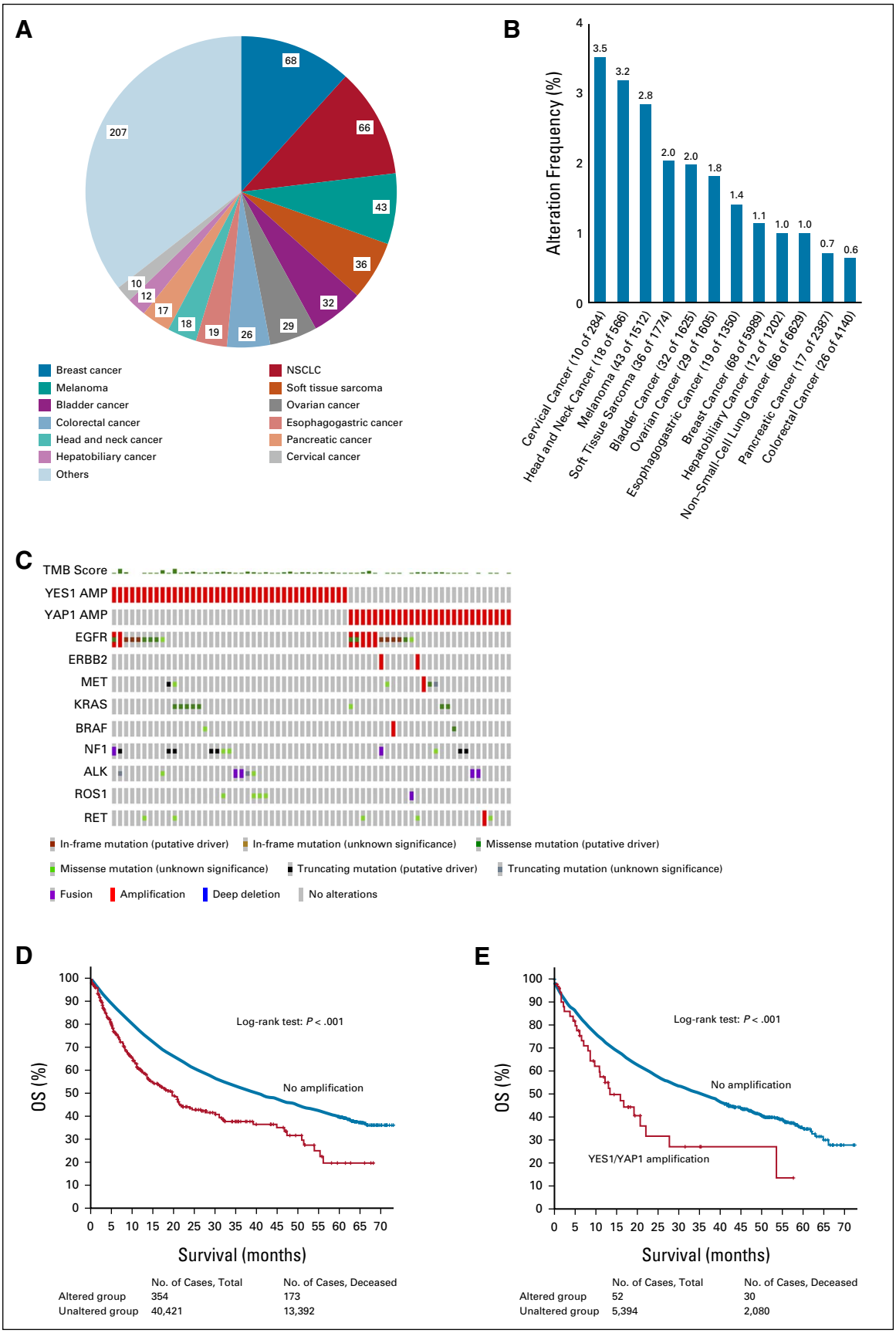
A
68
66
43
36
32
29
26
19
18
17
12
10
207
Breast cancer
NSCLC
Melanoma
Soft tissue sarcoma
Bladder cancer
Ovarian cancer
Colorectal cancer
Esophagogastric cancer
Head and neck cancer
Pancreatic cancer
Hepatobiliary cancer
Cervical cancer
Others
B
Alteration Frequency (%)
0
1
2
3
4
3.5
3.2
2.8
2.0
2.0
1.8
1.4
1.1
1.0
1.0
0.7
0.6
Cervical Cancer (10 of 284)
Head and Neck Cancer (18 of 566)
Melanoma (43 of 1512)
Soft Tissue Sarcoma (36 of 1774)
Bladder Cancer (32 of 1625)
Ovarian Cancer (29 of 1605)
Esophagogastric Cancer (19 of 1350)
Breast Cancer (68 of 5989)
Hepatobiliary Cancer (12 of 1202)
Non-Small-Cell Lung Cancer (66 of 6629)
Pancreatic Cancer (17 of 2387)
Colorectal Cancer (26 of 4140)
C
TMB Score
YES1 AMP
YAP1 AMP
EGFR
ERBB2
MET
KRAS
BRAF
NF1
ALK
ROS1
RET
In-frame mutation (putative driver)
In-frame mutation (unknown significance)
Missense mutation (putative driver)
Missense mutation (unknown significance)
Truncating mutation (putative driver)
Truncating mutation (unknown significance)
Fusion
Amplification
Deep deletion
No alterations
D
OS (%)
Log-rank test: P < .001
No amplification
Survival (months)
No. of Cases, Total
No. of Cases, Deceased
Altered group 354 173
Unaltered group 40,421 13,392
E
OS (%)
Log-rank test: P < .001
No amplification
YES1/YAP1 amplification
Survival (months)
No. of Cases, Total
No. of Cases, Deceased
Altered group 52 30
Unaltered group 5,394 2,080

FIG 6. Survey of *YES1* and *YAP1* amplification cases using cBioPortal. (A) The number of cases with *YES1*/*YAP1* amplification is shown in the pie chart graph (N = 463). (B) The frequency of *YES1*/*YAP1* amplification is shown for each cancer type. (C) OncoPrint of concurrent alterations identified in NSCLC tumor samples with *YES1*/*YAP1* amplification. (D) Kaplan-Meier curves showing the overall survival rate for patients with all cancer types stratified by *YES1*/*YAP1* amplification status. (E) Kaplan-Meier curves showing the overall survival rate for patients with NSCLC stratified by *YES1*/*YAP1* amplification status. NSCLC, non–small-cell lung cancer.

with an EGFR or ALK TKI alone. By contrast, Recondo et al[17] found that blockade of both ALK and SFKs can overcome EMT-mediated resistance to the ALK TKI lorlatinib in two patient-derived cell lines. The status of YES1/YAP1 signaling in these cell lines was not reported. Overall, our current findings warrant further investigation into the therapeutic potential of SFK inhibitors in overcoming AR driven by YES1/YAP1 signaling.

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SUPPORT

Supported by National Institutes of Health (NIH)/National Cancer Institute (NCI) Grants P01 CA129243 and P30 CA008748 and the Functional Genomics Initiative and Cycle for Survival at MSKCC.

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Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Achim Jungbluth
Patents, Royalties, Other Intellectual Property: Receive royalties and own IP for several monoclonal antibodies developed during my time at the Ludwig Institute for Cancer Research (LICR) and MSKCC. Royalties are from LICR and MSKCC, not any company.

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Research Funding: Conquer Cancer Foundation
Travel, Accommodations, Expenses: Iovance Biotherapeutics, Instil Bio Conquer Cancer Foundation
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Patents, Royalties, Other Intellectual Property: Patent application submitted covering pulsatile use of erlotinib to treat or prevent brain metastases (Inst)
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No other potential conflicts of interest were reported.

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