ORIGINAL ARTICLE

Solid Predominant Histology and High Podoplanin Expression in Cancer-Associated Fibroblast Predict Primary Resistance to Osimertinib in *EGFR*-Mutated Lung Adenocarcinoma

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ABSTRACT

Introduction: Resistance to EGFR tyrosine kinase inhibitors is influenced by tumor-intrinsic and -extrinsic factors. We investigated the impact of tumor cell histology and tumor microenvironment on the efficacy of osimertinib.

Methods: We evaluated surgically resected adenocarcinoma from patients treated with first-line osimertinib at the National Cancer Center Hospital East (2016–2023), evaluating clinicopathologic characteristics, tumor cell histology, podoplanin expression in cancer-associated fibroblasts (CAFs) identified by immunohistochemistry, and outcomes. We also investigated HGF mRNA expression levels, using The Cancer Genome Atlas Program and Singapore Oncology Data Portal cohorts.

Results: The study included 93 patients. Solid (n = 19) versus non-solid predominant (n = 74) histology was not associated with worse disease-free survival after surgery ($p = 0.12$), but was significantly associated with worse progression-free survival (PFS) and overall survival following osimertinib treatment ($p = 0.026$, $p = 0.004$). Similarly, high-podoplanin (n = 31) versus low-podoplanin (n = 62) expression in CAFs was not associated with worse disease-free survival after surgery ($p = 0.65$), but was significantly associated with worse PFS and showed a trend towards worse overall survival following osimertinib treatment ($p < 0.001$, $p = 0.11$). In the multivariable analysis, solid predominant histology and high-podoplanin expression in CAFs were independently associated with worse PFS. In the cohorts of The Cancer Genome Atlas Program and Singapore Oncology Data Portal, *EGFR*-mutated lung adenocarcinoma with solid predominant histology or high-podoplanin expression exhibited significantly higher HGF expression.

Conclusions: Solid predominant histology and high-podoplanin expression in CAFs predicted osimertinib resistance, potentially guiding the selection of patients for

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more intensive treatments beyond osimertinib monotherapy.

Keywords: Solid predominant subtype; Lung adenocarcinoma; EGFR mutation; Cancer associated fibroblasts (CAFs); Podoplanin

Introduction

EGFR-activating mutations are among the most frequent druggable driver mutations in patients with NSCLC. These mutations render the cancer sensitive to EGFR tyrosine kinase inhibitors (TKIs), leading to a marked improvement in both progression-free survival (PFS) and overall survival (OS) for patients with metastatic or resected *EGFR*-mutated NSCLC. In the FLAURA trial, first-line (1L) osimertinib markedly extended PFS and OS compared with first-generation EGFR TKIs in patients with *EGFR*-mutated NSCLC.[1,2] More recently, in the ADAURA trial, adjuvant osimertinib was found to provide a notable benefit in PFS and OS among patients with resected *EGFR*-mutated NSCLC.[3,4] Osimertinib, a third-generation EGFR TKI, has become the standard of care for patients with *EGFR*-mutated NSCLC in both the metastatic and adjuvant settings. Nevertheless, despite initial responses, tumor recurrence is inevitable with osimertinib treatment owing to primary and acquired resistance.[5–7]

In June 2020, the Pathology Committee of the International Association for the Study of Lung Cancer (IASLC) proposed a histologic grading system for invasive lung adenocarcinoma.[8] This new histologic grading system, which is based on a combination of the most predominant subtype and any high-grade histologic pattern, has been proven to be a prognostic factor for lung adenocarcinoma.[8,9] Our previous study indicated that the solid predominant histology was associated with worse outcomes in patients who underwent surgical resection and were treated with 1L first-generation EGFR TKIs.[10]

Cancer-associated fibroblasts (CAFs) have been found to play a crucial role in tumor progression and therapeutic responses.[11] We previously identified a specific subpopulation of CAFs, podoplanin-expressing CAFs, which exhibit a tumor-promoting phenotype in lung adenocarcinoma.[12–15] Notably, podoplanin-expressing CAFs are more prevalent in the tumor microenvironment of poorly differentiated adenocarcinomas.[12,13] On direct contact with cancer cells, these CAFs induced resistance to first-generation EGFR TKIs in cancer cells by up-regulating p-ERK levels in vitro.[16] In addition, these CAFs were associated with poorer responses and worse PFS in patients with recurrence treated with first-generation EGFR TKIs.[16] Soluble factor-mediated underlying mechanism involves higher levels of HGF secreted by CAFs, which bypass EGFR dependence by activating tyrosine kinase receptor-mediated signaling through the HGF-MET axis, contributing to resistance against EGFR TKIs.[17–22]

In this study, we investigated the impact of solid predominant histology and podoplanin-expressing CAFs on survival outcomes of recurrence patients with *EGFR*-mutated lung adenocarcinoma treated with osimertinib. We also provided evidence of a potential association between solid predominant histology and podoplanin-expressing CAFs and HGF mRNA expression levels, using The Cancer Genome Atlas Program (TCGA) and the Singapore Oncology Data Portal (OncoSG) cohorts.

Methods

Study Population

Between June 2016 and March 2023, 1881 consecutive patients with surgically resected adenocarcinoma were enrolled in this study. We evaluated *EGFR* mutation using the Cobas EGFR mutation test versions 1 and 2, Therascreen EGFR RGQ PCR Kit, or the PCR-Invader method. A total of 150 patients experienced postoperative recurrence and received osimertinib treatment. Among them, 102 patients received 1L osimertinib. Nine patients with uncommon *EGFR* mutations were excluded from this study. Ultimately, 93 patients with postoperative recurrence of *EGFR*-mutant NSCLC, treated with 1L osimertinib at our institution were eligible for analysis (Supplementary Fig. 1). We obtained informed consent from all patients before enrollment in this research (National Cancer Center Hospital IRB approval number: 2023-218).

Histologic Diagnosis

Surgical samples were fixed in 10% formalin and then embedded in paraffin. The paraffin-embedded tumor blocks were sliced at approximately 5 to 10 mm in thickness, and consecutive 4-μm sections underwent staining with hematoxylin and eosin (HE). Pathologic diagnoses were made according to the guidelines of the fifth edition of the WHO's classification of lung tumors. Five predominant histologic subtypes of adenocarcinoma were identified, as follows: lepidic predominant, acinar predominant, papillary predominant (Fig. 1*A*), solid predominant (Fig. 1*B*), and micropapillary predominant. Pathologic staging adhered to the criteria of the eighth edition of the TNM classification system. In addition, histologic grading (grade 1, well differentiated;

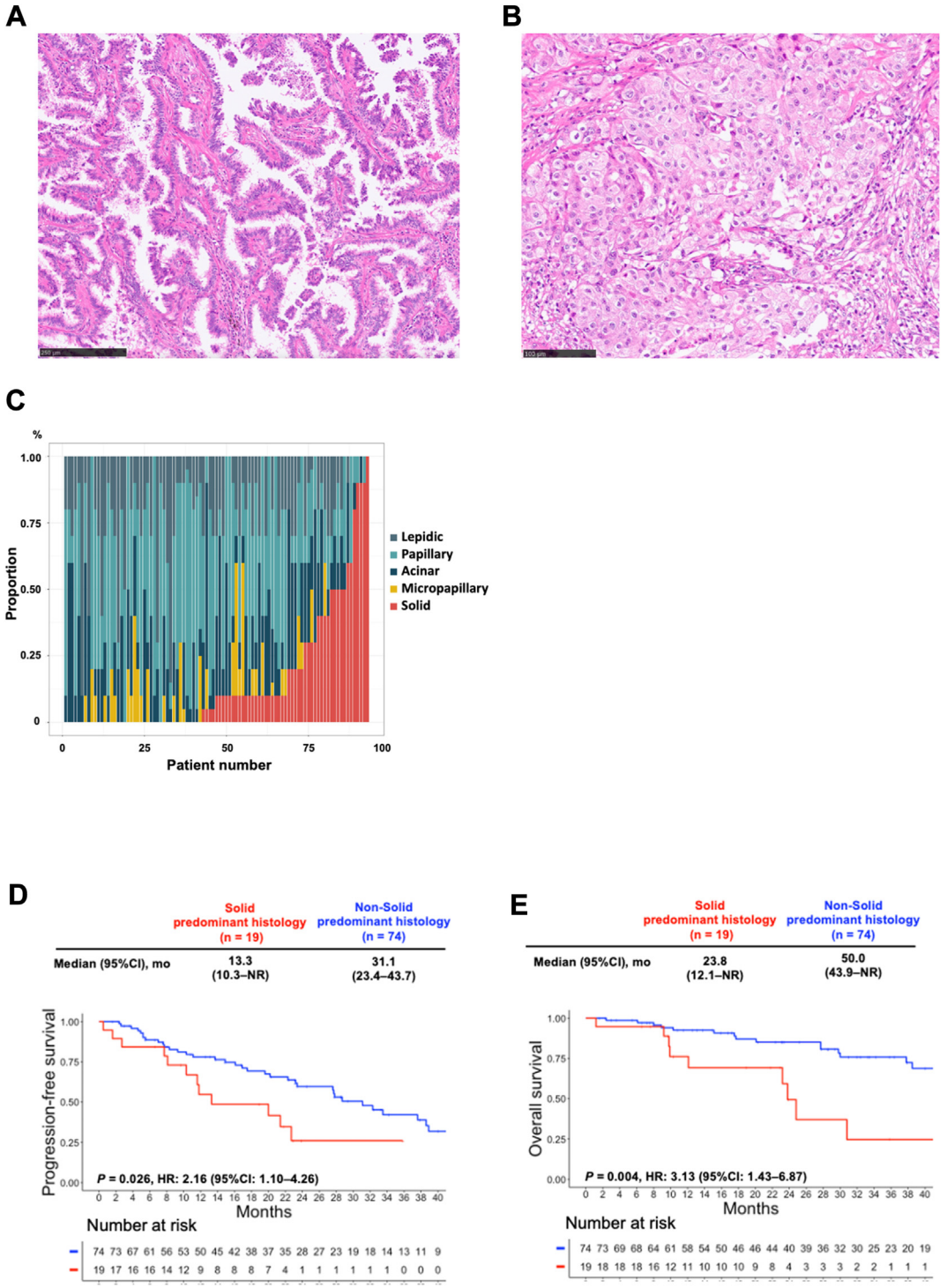

Figure 1. Impact of predominant histology (solid versus non-solid) on the outcome of osimertinib. Microscopic imaging of (*A*) non-solid predominant histology (papillary predominant subtype with a minor micropapillary subtype) and (*B*) solid predominant histology. (*C*) Proportion of each patient's histologic subtype. (*D*) PFS and (*E*) OS after osimertinib treatment stratified by predominant histology (solid versus non-solid). CI, confidence interval; HR, hazard ratio; OS overall survival; PFS, progression-free survival.

grade 2, moderately differentiated; grade 3, poorly differentiated, any tumors with 20% or more of high-grade patterns; solid, micropapillary, or complex glandular patterns) was performed in accordance with the latest grading system proposed by the IASLC.[8] The proportion of histologic subtypes and histologic grading

was previously described in all pathologic reports (TT and GI). For the current research, all HE slides were re-reviewed and classified by three pathologists (YU, TT, and GI), who were blinded to patient clinical outcomes, based on the slides or whole slide images. In cases of disagreement, the predominant subtype and histologic grading were defined by majority or consensus.

Evaluation of Clinicopathologic Features

We retrospectively analyzed patients' clinicopathologic details through their electronic medical records, including age, sex, smoking history, Eastern Cooperative Oncology Group performance status (ECOG PS), *EGFR* mutation status, pathologic stage, presence of lymphovascular and pleural invasion, pulmonary metastasis, predominant histologic subtype, use of adjuvant chemotherapy, response rate, and survival.

Immunohistochemistry

As a marker for tumor-promoting CAFs, we used podoplanin. Anti-podoplanin (mouse monoclonal antibody, diluted at 1:2000, 1D9F3, Proteintech Group, Inc., Rosemont, IL) served as the tumor-promoting CAF marker. The selection of the representative slide for each case was based on the presence of the largest amount of tumor tissue. We used NanoZoomer 2.0-HT digital pathology microscope (Hamamatsu Photonics, Hamamatsu, Japan) for scanning each tumor slide. To quantify the area in the immunohistochemistry sections, digital image analysis was performed using HALO Imaging Analysis Software (Indica Labs, Corrales, NM). We overlaid HE staining (Supplementary Fig. 2B and F) with immunohistochemistry (Supplementary Fig. 2C and G) to exclude podoplanin that overlaps with the cancer cells (Supplementary Fig. 2D and H). The percentage of positive green pixels within the podoplanin-positive area among the red pixels in the invasive tumor area was calculated (Supplementary Fig. 2D and H).

Evaluation of HGF mRNA Expression

To analyze the association between the predominant histologic subtype and podoplanin expression and HGF mRNA expression, we extracted corresponding HGF mRNA expression data (RNAseq V2, RSEM) from TCGA and OncoSG tumor samples through cBioPortal (https://www.cbioportal.org/).[23–25] A total of 66 *EGFR*-mutated TCGA lung adenocarcinoma samples, mainly from white and European patients and available for both predominant histologic subtype and mRNA expression, were included in this study.[24,25] Pathology reports and HE-stained slides, digitized in the TCGA archives, were used to identify predominant histologic subtype. In addition, 93 OncoSG *EGFR*-mutated lung adenocarcinoma samples, consisting of Asian patients and available for RNA expression analysis, were assessed.[23] The mRNA expression levels were categorized as high or low groups based on the median value.

Statistical Analysis

Descriptive statistics were used to summarize baseline characteristics, with the chi-square test applied to categorical variables and the Wilcoxon ranked sum test to continuous variables. The 95% confidence interval (CI) for the overall response rate (ORR) according to Response Evaluation Criteria in Solid Tumors 1.1 criteria was calculated using the exact binomial distribution. Disease-free survival (DFS) was defined as the time of surgery to disease progression, local or distant recurrence, or death from any cause, regardless of which occurred first. PFS was defined as the time from the initiation of 1L osimertinib treatment to disease progression or death from any cause, with censoring occurring at the last follow-up before disease progression or death. OS was defined as the time from the initiation of 1L osimertinib treatment to death from any cause, with censoring at the last follow-up before death. Survival curves were estimated using the Kaplan-Meier method, and comparisons were made using the log-rank test. The hazard ratio (HR) was calculated using Cox regression analysis. On the basis of previous research,[1,2,7,10,26] we included sex, age, ECOG PS, brain metastasis, *EGFR* mutation status, pathologic stage, histologic pattern (solid versus non-solid), and podoplanin expression in CAFs (high versus low) in the multivariable analysis for PFS and OS. For DFS, we included sex, age, *EGFR* mutation status, pathologic stage, use of adjuvant chemotherapy, presence of lymphovascular and pleural invasion, histologic pattern (solid versus non-solid), and podoplanin expression in CAFs (high versus low) in the multivariable analysis.

A cutoff point of podoplanin-positive CAF area (high versus low) was established to investigate the independent prognostic impact of podoplanin-positive CAF area, using the minimum p value method.[27] This method selects a cutpoint based on the maximum chi-square statistic as the optimal point when outcomes are binary. The association between the percentage of podoplanin-positive area and PFS was evaluated using the minimum p value approach in the log-rank test.

All statistical analyses were conducted using R version 4.3.2 (R Project for Statistical Computing). Significance was determined at a two-sided p value of less than 0.05.

Results

Clinicopathologic Characteristics

Table 1 illustrates the clinical-pathologic characteristics of the cohort. The mean age at the time of initiating 1L osimertinib treatment was 70 (range, 40–90) years, with

Table 1. Patient Baseline Characteristics Stratified by Predominant Histology (Solid Versus Non-Solid)

Characteristics	Total (n = 93)	Solid Predominant (n = 19)	Non-Solid Predominant (n = 74)	*p* Value
Median age (range), y	70 (40-90)	74 (51-90)	70 (40-85)	0.10
Sex, n (%)				
Women	66 (71)	12 (63)	54 (73)	0.58
Men	27 (29)	7 (37)	20 (27)	
Smoking history, n (%)				
Never	66 (71)	14 (64)	52 (70)	0.99
Former	27 (29)	5 (26)	22 (30)	
ECOG PS, n (%)				
0	32 (34)	6 (32)	26 (35)	0.32
1	57 (61)	11 (58)	46 (62)	
≥2	4 (4)	2 (11)	2 (3)	
EGFR mutation status, n (%)				
Exon 19 deletion	36 (39)	5 (26)	31 (42)	0.33
Exon 21 L858R	57 (61)	14 (74)	43 (58)	
Pathologic stage, n (%)				
IA	14 (15)	1 (5)	13 (18)	0.62
IB	22 (24)	3 (16)	19 (26)	
IIA	7 (8)	2 (11)	5 (7)	
IIB	10 (11)	3 (16)	7 (10)	
IIIA	28 (30)	7 (37)	21 (28)	
IIIB	12 (13)	3 (16)	9 (12)	
Adjuvant chemotherapy, n (%)				
Yes	44 (47)	11 (58)	33 (45)	0.44
No	49 (53)	8 (42)	41 (55)	
Histologic subtype, n (%)				
Lepidic predominant	11 (12)	0	11 (15)	<0.001
Acinar predominant	13 (14)	0	13 (18)	
Papillary predominant	47 (51)	0	47 (64)	
Solid predominant	19 (20)	19 (100)	0	
Micropapillary predominant	3 (3)	0	3 (4)	
Grading, n (%)				
Grade 1 (well differentiated)	11 (12)	0	11 (15)	<0.001
Grade 2 (moderately differentiated)	51 (55)	0	51 (69)	
Grade 3 (poor differentiated)	31 (33)	19 (100)	12 (16)	
Lymphatic permeation, n (%)				
Yes	41 (44)	12 (63)	29 (39)	0.11
No	52 (56)	7 (37)	45 (61)	
Vascular invasion, n (%)				
Yes	61 (66)	16 (84)	45 (61)	0.10
No	32 (34)	3 (16)	29 (39)	
Pleural invasion, n (%)				
Yes	39 (42)	6 (32)	33 (45)	0.44
No	54 (58)	13 (68)	41 (55)	
Pulmonary metastases[a], n (%)				
Yes	8 (9)	1 (5)	7 (10)	0.90
No	85 (91)	18 (95)	67 (90)	
Brain metastases,[b] n (%)				
Yes	11 (12)	4 (21)	7 (10)	0.32
No	82 (88)	15 (79)	67 (90)	

[a]At the time of pathologic diagnosis.
[b]At the time of metastatic disease.
ECOG, Eastern Cooperative Oncology Group; PS, performance status.

66 female patients (71%). Among these patients, 66 patients (71%) had never smoked and most (96%) had ECOG PS of either 0 or 1. Furthermore, 36 patients (39%) and 57 patients (61%) had tumors with exons with 19 deletions and L858R mutations, respectively. Most histologic subtypes were the papillary predominant subtype

(51%; Fig. 1*A*, note: papillary predominant subtype with a minor micropapillary subtype), followed by the solid (20%; Fig. 1*B*), acinar (14%), lepidic (12%), and micropapillary (3%) predominant subtypes. Figure 1*C* illustrates the proportion of each patient's histologic subtype within the cohort. Histologic grading was classified as grade 1 (12%), grade 2 (55%), and grade 3 (33%).

Solid Predominant Histology Was Not Associated With Worse DFS but Was Significantly Associated With Worse PFS and OS After Osimertinib Treatment

To investigate the impact of baseline histologic subtypes on the efficacy of osimertinib, survival analysis after osimertinib treatment was conducted. Patient baseline characteristics stratified by solid predominant histology and non-solid predominant histology are found in Table 1. These characteristics were generally balanced between the two groups. Patients with solid predominant histology were not associated with worse DFS (median DFS, 17.9 versus 20.0 mo; HR, 1.51; 95% CI: 0.90–2.55; $p = 0.12$; Supplementary Fig. 3A) but were significantly associated with worse PFS and OS after osimertinib treatment compared with patients without the solid predominant histology (median PFS, 13.3 versus 31.1 mo; HR, 2.16; 95% CI: 1.10–4.26; $p = 0.026$: median OS, 23.8 versus 50.0 mo; HR, 3.13; 95% CI: 1.43–6.87; $p = 0.004$) (Fig. 1*D* and *E*). The ORR was 37% (95% CI: 16–62) in the solid predominant histology and 69% (95% CI, 57–79) in the non-solid predominant histology ($p = 0.021$) (Supplementary Table 1).

Histologic Grading Was Not Associated With DFS, PFS, or OS After Osimertinib Treatment

We found no significant difference in DFS among patients with grade 1, grade 2, and grade 3 (median DFS: 15.5 versus 22.0 versus 17.9 mo; grade 3 versus grade 1, $p = 0.13$; grade 3 versus grade 2, $p = 0.61$; Supplementary Fig. 3B). Similarly, we found no significant difference in PFS and OS after osimertinib treatment among patients with grade 1, grade 2, and grade 3 (median PFS: 38.9 versus 28.7 versus 21.4 mo; grade 3 versus grade 1, $p = 0.28$; grade 3 versus grade 2, $p = 0.25$; Supplementary Fig. 4A; median OS: not reached [NR] versus 43.9 versus 30.8 mo; grade 3 versus grade 1, $p = 0.40$; grade 3 versus grade 2, $p = 0.58$; Supplementary Fig. 4B).

High Podoplanin Expression in CAFs Was Not Associated With Worse DFS but Was Significantly Associated With Worse PFS and Had a Trend Toward Worse OS After Osimertinib Treatment

We previously revealed that podoplanin-positive CAFs induced primary resistance to first-generation EGFR TKIs.[16] To investigate the impact of podoplanin expression levels in CAFs on the outcomes of patients treated with osimertinib, we divided the patients into groups with high and low podoplanin expression in CAFs. The cutoff value for podoplanin expression in CAFs was set at 0.7%, using the minimum p value method (Supplementary Fig. 5), classifying patients into the podoplanin-low CAF group ($\leq$0.7%; 62 patients, 67%) and the podoplanin-high CAF group (>0.7%; 31 patients, 33%) (Fig. 2*A* and *B*). The distribution of cases by podoplanin-positive CAF area (%) is found in Figure 2*C*. Patient baseline characteristics stratified by podoplanin expression in CAFs are described in Table 2. Although the characteristics were generally balanced between the two groups, a higher proportion of patients with predominant solid histology, grade 3 tumors, and vascular invasion was found in the podoplanin-high CAF group. Patients with high podoplanin expression in CAFs were not associated with worse DFS (median DFS, 21.4 versus 19.4 mo; HR, 0.90; 95% CI: 0.58–1.40; $p = 0.65$; Supplementary Fig. 3C) but were significantly associated with worse PFS compared with those with low podoplanin expression in CAFs (median PFS, 11.6 versus 38.6 mo; HR, 6.14; 95% CI: 3.19–11.8; $p < 0.001$; Fig. 2*D*). OS after osimertinib treatment was numerically worse with podoplanin-high CAF group (median: 45.4 mo) versus podoplanin-low CAF group (median: 53.1 mo); however, the difference was not statistically significant (HR = 0.11; HR, 1.83; 95% CI: 0.87–3.83; $p = 0.11$; Fig. 2*E*). The ORR was 58% (95% CI: 39–75) in the podoplanin-high CAF group and 65% (95% CI: 51–76) in the podoplanin-low CAF group ($p = 0.71$), respectively (Supplementary Table 2).

We further validated the association between podoplanin expression in CAFs and survival outcomes by comparing the podoplanin-negative CAF group (0%) with the podoplanin-positive CAF group (>0%). Patients in the podoplanin-positive CAF group had worse PFS compared with those in the podoplanin-negative CAF group (median PFS, 22.3 versus 38.6 mo; HR, 2.16; 95% CI: 1.14–4.10; $p = 0.018$; Fig. 2*F*). There was no significant difference in OS after osimertinib treatment between the two groups (median OS, 45.4 versus 43.9 mo; HR, 1.09; 95% CI: 0.52–2.23; $p = 0.83$; Fig. 2*G*).

Solid Predominant Histology and High Podoplanin Expression in CAFs Are Independently Worse Survival Outcomes After Osimertinib Treatment

Using multivariable Cox regression analysis, neither solid predominant histology nor high podoplanin expression in CAFs was associated with inferior DFS (Supplementary Table 3). Nevertheless, both solid

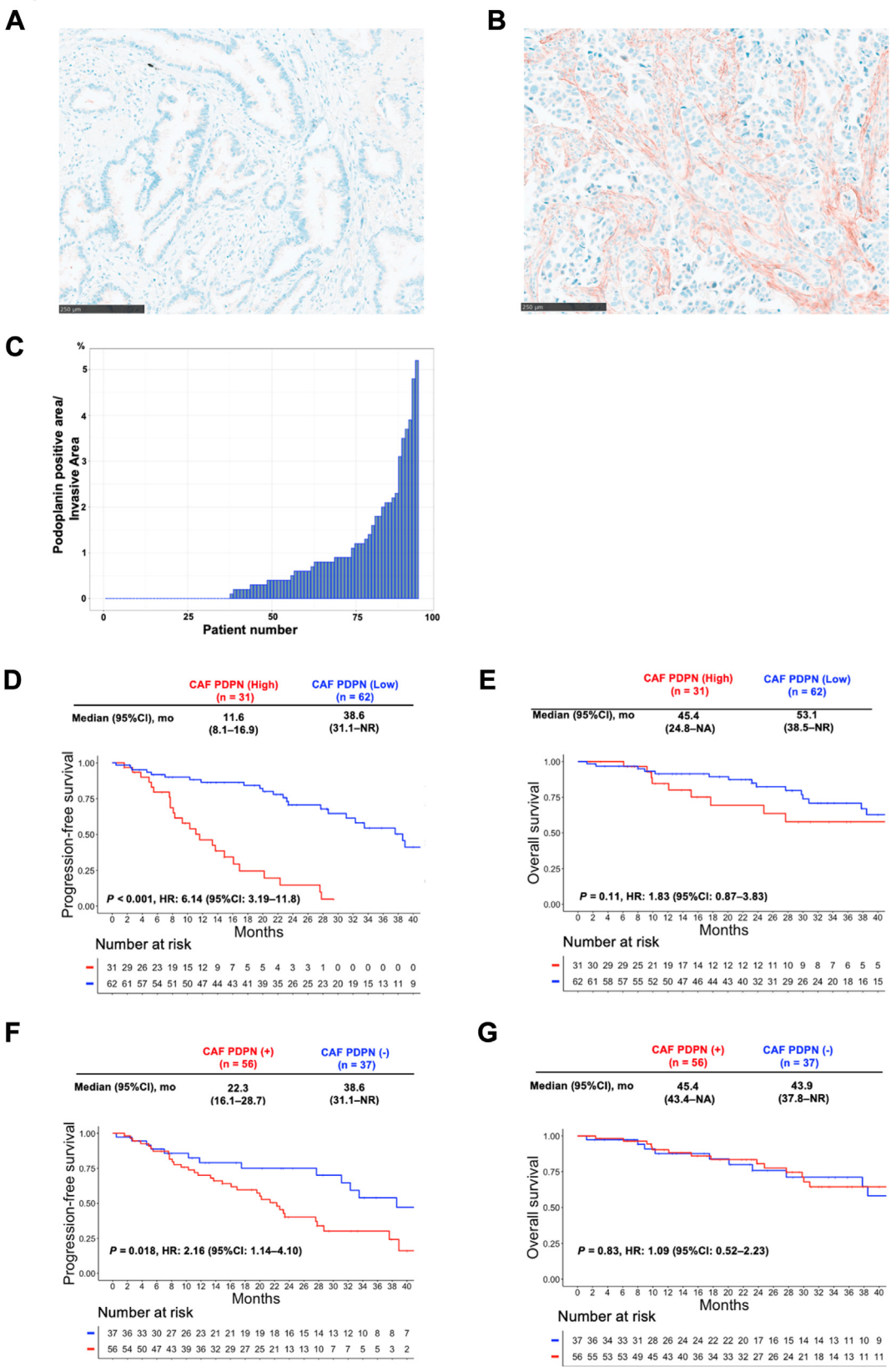

Figure 2. Impact of podoplanin-expressing CAFs on the outcome of osimertinib. Case with (*A*) podoplanin-negative CAFs and (*B*) podoplanin-positive CAFs. (*C*) Distribution of cases by podoplanin-positive CAF area (%). (*D*) PFS and (*E*) OS after osimertinib treatment stratified by high and low podoplanin expression in CAFs. (*F*) PFS and (*G*) OS after osimertinib treatment stratified by positive and negative cases with podoplanin expression in CAFs. CAFs, cancer-associated fibroblasts; CI, confidence interval; HR, hazard ratio; OS overall survival; PFS, progression-free survival.

Table 2. Patient Baseline Characteristics Stratified by Podoplanin Expression in CAFs

Characteristics	Total (n = 93)	Podoplanin-High CAF (n = 31)	Podoplanin-Low CAF (n = 62)	*p* Value
Median age (range), y	70 (40-90)	71 (45-77)	70 (40-90)	0.60
Sex, n (%)				
Women	66 (71)	21 (68)	45 (73)	0.81
Men	27 (29)	10 (32)	17 (27)	
Smoking history, n (%)				
Never	66 (71)	20 (64)	46 (74)	0.47
Former	27 (29)	11 (36)	16 (26)	
ECOG PS, n (%)				
0	32 (34)	6 (19)	26 (42)	0.090
1	57 (61)	23 (74)	34 (55)	
≥2	4 (4)	2 (7)	2 (3)	
EGFR mutation status, n (%)				
Exon 19 deletion	36 (39)	8 (26)	28 (45)	0.11
Exon 21 L858R	57 (61)	23 (74)	34 (55)	
Pathologic stage, n (%)				
IA	14 (15)	3 (10)	11 (18)	0.91
IB	22 (24)	8 (26)	14 (23)	
IIA	7 (8)	2 (7)	5 (8)	
IIB	10 (11)	3 (10)	7 (11)	
IIIA	28 (30)	11 (36)	17 (27)	
IIIB	12 (13)	4 (13)	8 (12)	
Adjuvant chemotherapy, n (%)				
Yes	44 (47)	17 (55)	27 (44)	0.42
No	49 (53)	14 (45)	35 (56)	
Histologic subtype, n (%)				
Lepidic predominant	11 (12)	0 (0)	11 (18)	0.055
Acinar predominant	13 (14)	4 (13)	9 (15)	
Papillary predominant	47 (51)	18 (58)	29 (47)	
Solid predominant	19 (20)	9 (29)	10 (16)	
Micropapillary predominant	3 (3)	0 (0)	3 (5)	
Grading, n (%)				
Grade 1 (well differentiated)	11 (12)	0 (0)	11 (18)	0.044
Grade 2 (moderately differentiated)	51 (55)	19 (61)	32 (52)	
Grade 3 (poor differentiated)	31 (33)	12 (39)	19 (31)	
Lymphatic permeation, n (%)				
Yes	41 (44)	16 (52)	25 (40)	0.42
No	52 (56)	15 (48)	37 (60)	
Vascular invasion, n (%)				
Yes	61 (66)	25 (81)	36 (58)	0.054
No	32 (34)	6 (19)	26 (42)	
Pleural invasion, n (%)				
Yes	39 (42)	13 (42)	26 (42)	1.0
No	54 (58)	18 (58)	36 (58)	
Pulmonary metastases,[a] n (%)				
Yes	8 (9)	3 (10)	5 (8)	1.0
No	85 (91)	28 (90)	57 (92)	
Brain metastases,[b] n (%)				
Yes	11 (12)	6 (19)	5 (8)	0.21
No	82 (88)	25 (81)	57 (92)	

[a]At the time of pathologic diagnosis.
[b]At the time of metastatic disease.
CAFs, cancer-associated fibroblasts; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

predominant histology and high podoplanin expression in CAFs were independently and significantly associated with inferior PFS (HR for solid predominant histology, 2.40; 95% CI: 1.20–5.28, $p = 0.015$; HR for high podoplanin expression in CAFs, 4.81; 95% CI: 2.31–10.0, $p < 0.001$; Table 3). Solid predominant histology was

Table 3. Univariable and Multivariable Analysis of Progression-Free Survival After Osimertinib Treatment

	Univariable Analysis			Multivariable Analysis		
Characteristics	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Sex (women vs. men)	1.34	0.77-2.48	0.35	1.09	0.55-2.17	0.81
Age (≥75 vs. <75)	1.02	0.99-1.05	0.56	1.16	0.60-2.23	0.66
ECOG PS (≥1 vs. <1)	4.00	1.70-9.40	0.002	3.25	1.32-8.04	0.011
Brain metastasis (yes vs. no)	2.26	1.05-4.85	0.037	1.35	0.58-3.16	0.48
EGFR mutation status (exon 21 L858R vs. exon 19 deletion)	2.00	1.07-3.66	0.029	1.17	0.60-2.27	0.64
Pathologic stage II vs. I	1.77	0.72-4.14	0.23	1.43	0.58-3.55	0.44
Pathologic stage III vs. I	1.04	0.56-1.92	0.90	1.03	0.53-2.00	0.93
Histologic pattern (solid vs. non-solid)	2.16	1.10-4.26	0.026	2.40	1.20-5.28	0.015
Podoplanin expression in CAFs (high vs. low)	6.14	3.19-11.8	<0.001	4.81	2.31-10.0	<0.001

CAFs, cancer-associated fibroblasts; CI, confidence interval; HR, hazard ratio; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

significantly associated with inferior OS after osimertinib treatment, but high podoplanin expression in CAFs was not (HR for solid predominant histology, 3.09; 95% CI: 1.33–7.17, $p = 0.008$; HR for high podoplanin expression in CAFs, 1.36; 95% CI: 0.57–3.20, $p = 0.49$; Supplementary Table 4).

To investigate how combining these two biomarkers affected survival outcomes, we stratified patients into the following three groups: group A, with solid predominant histology and high podoplanin expression in CAFs; group B, with solid predominant histology and low podoplanin expression in CAFs or with non-solid predominant histology and high podoplanin expression in CAFs; and group C, with non-solid predominant histology and low podoplanin expression in CAFs. Group A had significantly worse PFS than group B (median PFS, 10.3 versus 16.9 mo; HR, 2.86; 95% CI: 1.14–7.16, $p = 0.025$) or group C (median PFS, 10.3 versus 38.6 mo; HR, 12.9; 95% CI: 4.62–36.1, $p < 0.001$) (Fig. 3*A*). Furthermore, group A had had significantly worse OS than group B (median OS 12.1 versus 50.0 mo; HR, 6.50; 95% CI: 2.01–21.0, $p = 0.002$) or group C (median OS 12.1 mo versus NR; HR, 10.5; 95% CI: 3.25–33.7, $p < 0.001$) (Fig. 3*B*).

HGF Expression Is Higher in Patients With EGFR-Mutated Lung Adenocarcinoma With Solid Predominant Histology or High Podoplanin Expression

Previous research has revealed that exogenous HGF, secreted by CAFs in *EGFR*-mutated NSCLC, could activate the MET receptor and its downstream signaling pathways, despite the EGFR pathway blockade by EGFR TKIs, leading to primary resistance to EGFR TKI treatment.[17–21] To explore the biological basis of the inferior survival outcomes after osimertinib treatment in patients with solid predominant histology or high podoplanin expression in CAFs, we evaluated HGF mRNA expression in *EGFR*-mutated lung adenocarcinoma within the TCGA and OncoSG cohorts.[23,24,28] In the TCGA cohort, HGF expression was significantly higher in patients with solid predominant histology compared with those with non-solid predominant histology (Fig. 3*C*). Across the TCGA and OncoSG cohorts, HGF expression was significantly higher in patients with high podoplanin expression than in those with low podoplanin expression (Fig. 3*D* and *E*). To investigate how combining these two biomarkers affects HGF expression, we stratified patients in the TCGA cohort into the following three groups: group A, with solid predominant histology and high podoplanin expression; group B, with solid predominant histology and low podoplanin expression or with non-solid predominant histology and high podoplanin expression; and group C, with non-solid predominant histology and low podoplanin expression. HGF expression was significantly higher in group A than group C ($p = 0.004$) (Fig. 3*F*).

Discussion

In this study, we found that *EGFR*-mutated lung adenocarcinoma with either solid predominant histology or high podoplanin expression in CAFs was significantly associated with worse PFS after osimertinib treatment compared with those with non-solid predominant histology or low podoplanin expression in CAFs; however, in this cohort, neither factor was significantly associated with worse DFS. In addition, both factors were independently associated with worse PFS in the multivariable analysis, and patients with both solid predominant histology and high podoplanin expression in CAFs had the worst survival outcomes after osimertinib treatment. These findings suggest that both solid predominant histology and high podoplanin expression in CAFs could

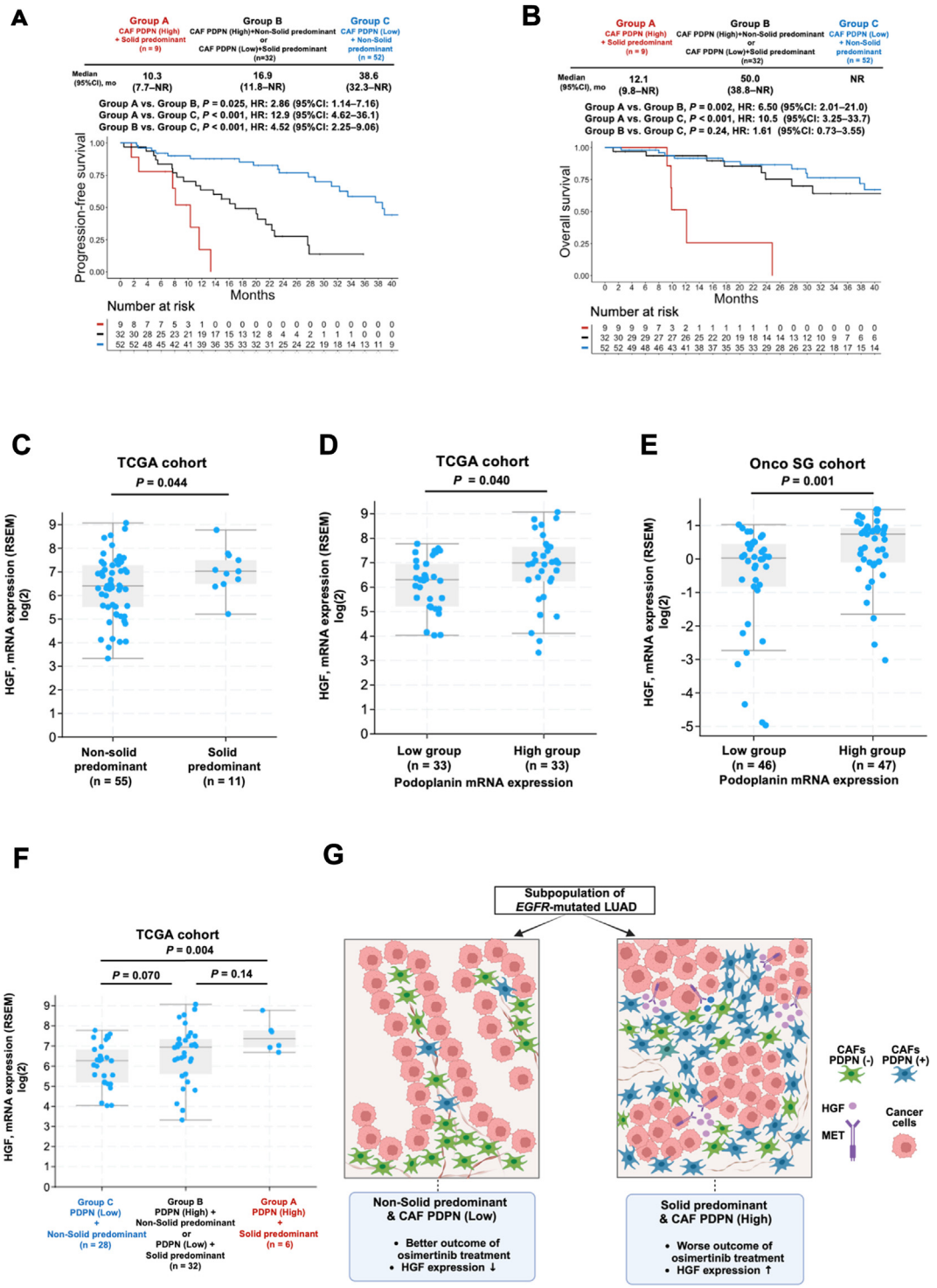

Figure 3. Impact of combining predominant histology (solid versus non-solid) and podoplanin-expressing CAFs on the outcome of osimertinib treatment and HGF expression. (*A*) PFS and (*B*) OS stratified by predominant histology (solid versus non-solid) and podoplanin expression in CAFs (high versus low). (*C*) HGF expression in solid versus non-solid predominant histology in the TCGA cohort. (*D*) HGF expression in high versus low podoplanin expression in the TCGA cohort. (*E*) HGF expression in high versus low podoplanin expression in the OncoSG cohort. (*F*) HGF expression stratified by predominant histology (solid versus non-solid) and podoplanin expression (high versus low) in the TCGA cohort. (*G*) Graphic summary of the current study. CAFs, cancer-associated fibroblasts; CI, confidence interval; HR, hazard ratio; LUAD, lung adenocarcinoma; OncoSG, Singapore Oncology Data Portal; OS overall survival; PFS, progression-free survival; TCGA, The Cancer Genome Atlas Program.

predict primary resistance to osimertinib. Moreover, in the TCGA and OncoSG cohorts, *EGFR*-mutated lung adenocarcinoma with solid predominant histology or high podoplanin expression exhibited higher HGF expression.

This study revealed that the solid predominant histology was significantly associated with worse survival outcomes of osimertinib treatment, which is consistent with our previous study on patients with postoperative recurrence of lung adenocarcinoma treated with first-generation EGFR TKIs.[10] Interestingly, histologic grading was not associated with survival outcomes after osimertinib treatment in our study. Grade 3 tumors are defined as any tumor with 20% or more of high-grade patterns (solid, micropapillary, and complex glandular patterns).[8] Previous research has revealed that patients with micropapillary predominant histology tended to respond better to EGFR TKIs than those with solid predominant histology.[29] The difference in predictive factor for the efficacy of EGFR TKI treatment between solid and micropapillary predominant histology in *EGFR*-mutated lung adenocarcinoma may account for the discrepancies observed between predominant histology and histologic grading in our study. Moreover, tumors with micropapillary predominant histology had a higher frequency of *EGFR* mutations, whereas those with solid predominant histology had the opposite trend.[29–32] Although the prognostic value of the histologic grading system proposed by the IASLC has been validated in numerous reports on lung adenocarcinoma overall,[9,33] our findings suggest that the predominant histologic subtype is a more accurate predictive factor for the efficacy of EGFR TKI treatment in *EGFR*-mutated lung adenocarcinoma.

Podoplanin is a transmembrane receptor glycoprotein that is sometimes up-regulated on CAFs, leading to cancer progression.[13,14,34,35] We previously revealed that the knockdown of podoplanin expression on CAFs negated resistance to EGFR TKIs in cancer cells and that podoplanin-expressing CAFs affected intracellular signaling pathways, including MAPK pathway, in cancer cells.[16] In this study, high podoplanin expression in CAFs was significantly associated with worse PFS after osimertinib treatment in comparison with low podoplanin expression in CAFs, consistent with our previous findings in patients treated with first-generation EGFR TKIs. Considering that high podoplanin expression in CAFs was not significantly associated with worse DFS, these findings suggest that high podoplanin expression in CAFs may predict the efficacy of osimertinib treatment.

Reactivation of downstream signaling pathways of EGFR, such as PI3K-AKT and MAPK, represents a significant resistance mechanism in *EGFR*-mutated NSCLC.[5,36] Previous research has revealed that exogenous HGF, especially when secreted by CAFs in *EGFR*-mutated NSCLC, could activate MET receptor and its downstream signaling pathways, despite the blockade of EGFR pathway by EGFR TKIs.[17–21] These findings suggest that HGF-expressing CAFs protect cancer cells by activating tyrosine kinase receptor-mediated signaling through HGF-MET axis, bypassing the dependence on EGFR pathway. Notably, both in vitro and in vivo studies have revealed that MET inhibitors abrogated the rescue effect provided by HGF in patient-derived CAFs.[17,18] In our study, solid predominant histology and high podoplanin expression were associated with higher HGF expression than non-solid predominant histology and low podoplanin expression. These findings indicate that HGF in solid predominant histology and podoplanin-expressing CAFs could lead to resistance against EGFR TKI treatment by circumventing the EGFR pathway, resulting in worse survival outcomes with osimertinib treatment (Fig. 3*G*).

Recently, the MARIPOSA phase III study revealed that the combination of amivantamab (an EGFR-MET bispecific antibody) and lazertinib (a third-generation EGFR TKI) improved PFS compared with lazertinib alone.[37,38] Furthermore, ongoing trials are evaluating whether MET inhibitor and EGFR TKI combination therapy can overcome acquired MET-mediated osimertinib resistance.[39–43] Considering *EGFR*-mutated lung adenocarcinoma with solid predominant histology or high podoplanin expression exhibited higher HGF expression, a strategy targeting both EGFR and MET seems promising for overcoming the primary resistance associated with solid predominant histology and high podoplanin expression by inhibiting HGF-MET signaling. With the upcoming decision on the 1L treatment for *EGFR*-mutated NSCLC—choosing between osimertinib monotherapy and treatments targeting both EGFR and MET—identifying solid predominant histology and high podoplanin expression in CAFs might serve as valuable predictors in clinical practice.

Previous studies, including our own, reported that high podoplanin expression in CAFs and solid predominant histology were associated with worse DFS in stage I lung adenocarcinoma.[12,15,44,45] Nevertheless, these findings were not observed in this study. One potential reason for the difference is that the current cohort focuses specifically on recurrent *EGFR*-mutated lung adenocarcinoma, with pathologic staging varying from stage I to stage III.

Our study has several limitations, including its retrospective design and the relatively small sample size. In addition, because all patients in our study received osimertinib treatment, solid predominant histology and podoplanin-expressing CAFs as predictive factors for the efficacy of osimertinib treatment compared with chemotherapy could not be evaluated. Furthermore, we

did not evaluate co-occurring alterations, such as *TP53* mutations, which could influence the efficacy of osimertinib treatment.[46] Notably, tumors with solid predominant histology or high podoplanin expression exhibited a higher incidence of *EGFR* L858R mutations, a major mutation associated with worse outcomes of osimertinib treatment.[1,2] Given that some tumor-suppressor genes such as *RB1*, *PTEN*, or *ARID1A* mutations are linked to poor outcomes on EGFR TKIs,[47] further comprehensive genomic profiling is needed to elucidate the background genome of tumors with solid predominant histology and high podoplanin expression.

In conclusion, solid predominant histology or high podoplanin expression in CAFs was significantly associated with worse PFS after osimertinib treatment, along with higher HGF expression, in patients with *EGFR*-mutated lung adenocarcinoma. This suggests that both factors have the potential to predict primary resistance to osimertinib. These findings may provide insights into the biological reasons behind the varying responses to osimertinib treatment and could potentially guide the selection of patients for more intensified interventions beyond 1L osimertinib monotherapy, including third-generation EGFR TKIs in combination with MET inhibitors targeting the HGF-MET axis.

CRediT Authorship Contribution Statement

Yuji Uehara: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing - original draft.

Hiroki Izumi: Conceptualization, Resources, Investigation, Writing - review & editing.

Tetsuro Taki: Resources, Writing - review & editing.

Tetsuya Sakai: Resources, Writing - review & editing.

Hibiki Udagawa: Resources, Writing - review & editing.

Eri Sugiyama: Resources, Writing - review & editing.

Shigeki Umemura: Resources, Writing - review & editing,

Yoshitaka Zenke: Resources, Writing - review & editing.

Shingo Matsumoto: Resources, Writing - review & editing.

Kiyotaka Yoh: Resources, Writing - review & editing.

Shoko Kubota: Investigation.

Keiju Aokage: Resources, Writing - review & editing.

Naoya Sakamoto: Writing - review & editing.

Shingo Sakashita: Writing - review & editing.

Motohiro Kojima: Writing - review & editing.

Michiko Nagamine: Writing - review & editing.

Yukio Hosomi: Writing - review & editing.

Masahiro Tsuboi: Resources, Writing - review & editing.

Koichi Goto: Resources, Writing - review & editing.

Genichiro Ishii: Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100779.

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