Real-World Validation of the Purity Independent Subtyping of Tumors Classifier for Informing Therapy Selection in Pancreatic Ductal Adenocarcinoma

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ABSTRACT

PURPOSE FOLFIRINOX (FFX) and gemcitabine + nab-paclitaxel (GnP) are the most commonly administered first-line (1L) regimens for advanced, nonresectable, pancreatic ductal adenocarcinoma (PDAC). In the absence of biomarkers to predict response, clinical covariates such as age and performance status are often used by clinicians to select optimal treatment regimens. Purity independent subtyping of tumors (PurIST) is a molecular subtyping algorithm that classifies tumors as classical or basal. The current study was designed to validate PurIST as a prognostic biomarker for patients receiving 1L FFX and as a predictive biomarker for patients more likely to benefit from FFX versus GnP.

PATIENTS AND METHODS This is a prospectively designed, retrospective study using a real-world data set of 931 patients with advanced PDAC, treated with either 1L FFX or GnP, and designed to demonstrate associations of PurIST subtypes with clinical outcomes. The primary end point was overall survival (OS) in classical versus basal patients treated with 1L FFX, while the secondary end point was OS in classical patients—with an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1—to compare 1L FFX versus GnP.

RESULTS Within the cohort of patients receiving 1L FFX (n = 536), basal subtype patients had a median OS of 7 months compared with classical subtype patients with a median OS of 11.8 months (hazard ratio [HR], 1.86 [95% CI, 1.49 to 2.33]; $P < .001$). In an analysis restricted to patients with classical subtype and ECOG PS of 0 or 1 (n = 311), there was a 33% relative risk reduction of death in patients treated with FFX compared with GnP, adjusting for age and ECOG PS (HR, 0.67 [95% CI, 0.48 to 0.94]; $P < .009$), with no comparable risk reduction in basal patients (subtype-treatment interaction, $P = .002$).

CONCLUSION Patients with PDAC of the PurIST classical subtype showed a significant OS benefit when treated with FFX as 1L versus GnP.

ACCOMPANYING CONTENT

Data Supplement

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INTRODUCTION

Pancreatic cancer represents approximately 3% of all new cancer diagnoses but is responsible for 9% of cancer-associated deaths.[1] The disease has a dismal 5-year survival of approximately 12%, owing to it commonly presenting in the metastatic setting in addition to its infiltrative growth pattern, which makes surgical cure extremely challenging. Histologically, ductal adenocarcinoma makes up 90% of all pancreatic cancer cases, which is a genomically homogeneous disease defined by alterations in *KRAS* (93%) and *TP53* (72%), and less frequently by *SMAD4* (32%) and *CDKN2A* (30%).[2] Molecular targetable alterations in pancreatic cancer are rare and largely consisting of the subset of patients with *BRCA1/2* alterations (approximately 5%) who are eligible for poly (ADP-ribose) polymerase inhibitor therapy,[3] and microsatellite instability-high tumors (approximately 1%),[4] where there is emerging evidence supporting treatment with

CONTEXT

Key Objective

Can the purity independent subtyping of tumors (PurIST) molecular subtyping algorithm be validated as a prognostic and predictive biomarker to inform first-line (1L) therapy selection in advanced pancreatic ductal adenocarcinoma (PDAC)? This study uniquely applies PurIST in a large, real-world cohort to inform clinical management in the 1L setting between FOLFIRINOX (FFX) and gemcitabine + nab-paclitaxel (GnP).

Knowledge Generated

PurIST basal subtype patients treated with FFX had significantly shorter overall survival than classical subtype patients. Among classical subtype patients with Eastern Cooperative Oncology Group 0-1, FFX conferred a substantial survival benefit over GnP.

Relevance

These findings support the clinical use of PurIST subtyping to inform 1L therapy in patients with advanced PDAC, enabling more effective, biomarker-driven treatment selection and potentially improving patient outcomes.

immune checkpoint therapy[5] and *KRAS* wild-type tumors (10%), which are enriched for various targetable alterations.

Given the rarity of molecular targets and immunotherapy success, cytotoxic therapy has remained the standard of care for most patients. In the (neo)adjuvant and advanced settings, cytotoxic treatment consists of a fluorouracil and oxaliplatin backbone (FFX/NALIRIFOX/FOLFOX), or gemcitabine with the addition of nab-paclitaxel (GnP) in the advanced setting. In the advanced or unresectable setting, recent randomized controlled trials evaluating FFX versus GnP (JCOG1611-GENERATE[6] and PASS-01[7]) or NALIRIFOX versus GnP (NAPOLI-3[8]) in addition to previous retrospective studies have demonstrated mixed or variable efficacy of the two regimens.[9-14] Specifically, in JCOG1611-GENERATE and PASS-01, GnP had a longer median overall survival (mOS) than FFX, while in NAPOLI-3, NALIRIFOX had a longer mOS versus GnP. In terms of treatment toxicity, the regimens have also shown different side-effect profiles.[9,14,15] These conflicting findings and current clinical practice heterogeneity speak to the need for a biomarker-informed strategy in advanced-stage pancreatic cancer.

Molecular subtypes of PDAC have been previously described with strong prognostic associations.[16-19] The Moffitt et al[19] subtyping schema described two molecular subtypes,[2,20] a basal-like (hereafter basal) and a classical subtype, which remained stable and distinct regardless of patient treatment. Rashid et al[20] formalized the Moffitt schema with purity independent subtyping of tumors (PurIST), a single-sample classifier optimized for clinical use. PurIST recapitulated Moffitt basal and classical subtype calls with high fidelity across multiple platforms (microarray, NanoString, and Illumina Next Generation Sequencing [NGS]) and sample types (flash-frozen, formalin-fixed paraffin-embedded). Preliminary evidence suggested that PurIST subtypes may have prognostic value, with classical patients showing higher response rates and survival benefits from FFX than basal patients.[20,21]

In this prospectively designed retrospective study, we report the results of a clinical validation study with prespecified end points and acceptance criteria of the PurIST classifier as a laboratory-developed test using a real-world data set of patients with advanced PDAC. The study was designed to validate the use of PurIST subtypes as a prognostic marker for patients with PDAC receiving FFX as first-line (1L) as well as to establish PurIST as a predictive biomarker to identify patients more likely to respond to FFX versus GnP.

PATIENTS AND METHODS

Patients

A deidentified, all-comers cohort was drawn from the Tempus clinical-genomic database and used for all subsequent analyses. Patient records were eligible for inclusion in the study if they were advanced-stage PDAC, defined as stages III and IV unresectable tumors, with biopsies from either primary or metastasized tumors, had no previous surgery, were treatment-naïve at the time of biopsy, had no delays in biopsy collection or NGS testing, and were treated with either FFX or GnP as 1L systemic therapy with at least one cycle. All data were deidentified in accordance with the Health Insurance Portability and Accountability Act. Line of therapy was determined using clinician notes and clinical abstraction methods. Dates of diagnosis and sequencing spanned years 2017-2023. Clinical data abstraction was performed for records with pancreatic cancer diagnosis, stage, metastatic status, age, sex, race, smoking status,

cytotoxic treatment, and performance status (PS) stage (Data Supplement—Clinical Data Abstraction). Sequencing was completed between 2017 and 2023 (Data Supplement—DNA and RNA sequencing). The study and analytic approach were prospectively designed and planned in writing, as per the study's statistical analysis plan.

NGS-Based DNA and RNA Sequencing

The Tempus testing platform consists of a targeted DNA sequencing assay (xT), which includes 648 genes, and an exome capture RNA sequencing assay (xR).[22,23] The xR assay panel uses Integrated DNA Technologies xGen Exome Research Panel v2 backbone, which consists of >415,000 individually synthesized probes and spans a 34-Mb target region (19,433 genes) of the human genome. Additional Tempus-specific custom spike-in probes are included to enhance target region detection (eg, fusion and viral probes). The target depth of sequencing is 25 million paired-end reads. The xR assay is used clinically for reporting gene fusions, alternative gene splicing, and gene expression algorithms.[24,25]

The PurIST Subtyping Model

PurIST is a top scoring pairs (*k*-TSPs) gene signature model that assesses rank-based expression levels of 16 genes sorted into eight gene pairs from a single sample to estimate the likelihood of that sample being basal.[20] The normalized expression values of the 16 PurIST genes were calculated on the basis of the standard Tempus xR method of normalization based on transcript length, Guanine-Cytosine content, and library size as previously described.[25]

Adaptation and Validation of the PurIST Model on the Tempus RNA-seq Platform

To assess the fidelity and analytical validity of the PurIST model using the Tempus RNA-seq platform, the following experiments were performed:

1. Methodological validation: Outputs of the PurIST pipeline described in Rashid et al[20] were compared with outputs obtained from the whole-transcriptome xR assay at Tempus (Data Supplement, Fig S1 and Table S3).
2. Orthogonal gene expression quantification validation: Gene quantification of PurIST genes using the Tempus xR assay was compared with digital droplet polymerase chain reaction gene quantification (Data Supplement, Fig S2, Tables S4 and S5).
3. Analytical validation of PurIST on the Tempus platform, assessing limit of detection, intraassay precision, interassay precision, and intersequencer concordance (Data Supplement, Table S6).

All experiments met prespecified performance metrics. See the Data Supplement (Methods) for a complete description of experiments and results.

Confidence Scoring and Implementation of an Indeterminate Subtype Class

The PurIST model outputs a continuous score, enabling the identification of low-confidence or indeterminate results when the expressions of gene pairs used in the algorithm provide results that are within statistical margins of error of the definitions for classical and basal subtypes. We quantified this uncertainty using a confidence score calculated on the basis of the gene expression values of all 16 genes and implemented a threshold of confidence below which samples are classified as indeterminate. See the Data Supplement (Methods) for details.

Study Design

This is a prospectively designed, retrospective study (ie, an observational review that looks back in time to collect patient information while also prospectively following patients until a prespecified data extraction date) to validate the performance of the PurIST algorithm in PDAC stage III unresectable or stage IV patients. A prespecified statistical plan was developed before the start of the study to measure the association of PurIST subtypes with clinical outcomes in a real-world clinicomolecular data set (Data Supplement, Methods). Eligible patients were classified using the PurIST model into one of the following subtypes: basal, classical, or indeterminate (representing a low-confidence classification). Patients who received FFX as 1L with either a classical or basal subtype call were analyzed for the primary end point. Because of the lack of equipoise in the choice between FFX and GnP in patients with higher ECOG PS, the comparison of FFX to GnP was prespecified in the subset of classical patients with an ECOG PS of 0 or 1.

Study End Points

The primary study end point was the overall survival (OS) comparison between basal and classical subtypes in patients treated with FFX as 1L. The secondary end point was the OS comparison of classical subtype patients receiving 1L GnP versus 1L FFX. OS was defined as the time from the index date (1L initiation date for FFX or GnP) to the date of death from any cause. Any patient not known to have died at the time of analysis was censored on the last recorded date on which the patient was known to be alive.

Statistical Analysis

Primary End Point Analysis

A Cox proportional-hazard (Cox PH) model was fit to the data for the OS comparison between basal and classical subtypes in patients receiving 1L FFX. Risk set adjustment was performed to account for patients who entered the study upon sequencing versus at treatment initiation. The primary end point was evaluated at a one-sided significance level of .05.

Secondary End Point Analysis

A multivariable Cox PH model was used to compare OS between classical subtype patients receiving 1L GnP versus 1L FFX, with prespecified adjustment for age and ECOG (0 and 1). As in the primary end point, the secondary end point was evaluated at a one-sided significance level of .05. Multiplicity control was achieved by using a gatekeeping strategy, that is, the secondary end point was evaluated upon primary end point success.

Patient Clinical and Pathologic Characteristics

Differences in mean/counts among groups were calculated using two-sample *t* tests for numeric variables and Pearson's chi-squared test for categorical variables. Statistical significance was determined at $P < .05$.

RESULTS

Patient Characteristics

Of the 931 patients included in the study population, 65.3% (n = 608) were classified as classical, 27.4% (n = 255) as basal, and 7.3% (n = 68) as an indeterminate subtype (Fig 1). The ratio of classical to basal patients was consistent with previous studies.[19,20] In a comparison of clinical traits, basal patients were more likely than classical to be male (62% *v* 51%, respectively; $P = .003$) and more likely to have a liver metastasis (64% *v* 53%; $P = .005$). Patients who were younger and had lower ECOG scores were more likely to receive FFX than GnP ($P < .001$ and $P < .001$, respectively; Data Supplement, Table S1).

Primary End Point Analysis: Comparison of Classical Versus Basal Subtypes in 1L FFX-Treated Patients

The primary analyses in our study evaluated PurIST subtypes as a prognostic biomarker in 1L FFX-treated patients (n = 536). Within this treatment group, 70.5% (n = 378) of patients were classified as classical and 29.5% (n = 158) as basal (Table 1), consistent with expected proportions (Data Supplement, Table S2). Basal subtype patients had significantly shorter OS than classical subtype patients (hazard ratio [HR], 1.86 [95% CI, 1.49 to 2.33]; $P < .001$), with median OS values of 7.0 months (95% CI, 6.0 to 8.2) in 1L FFX basal subtype patients and 11.8 months (95% CI, 10.3 to 12.9) in 1L FFX classical subtype patients (Fig 2A). Table 1 shows that

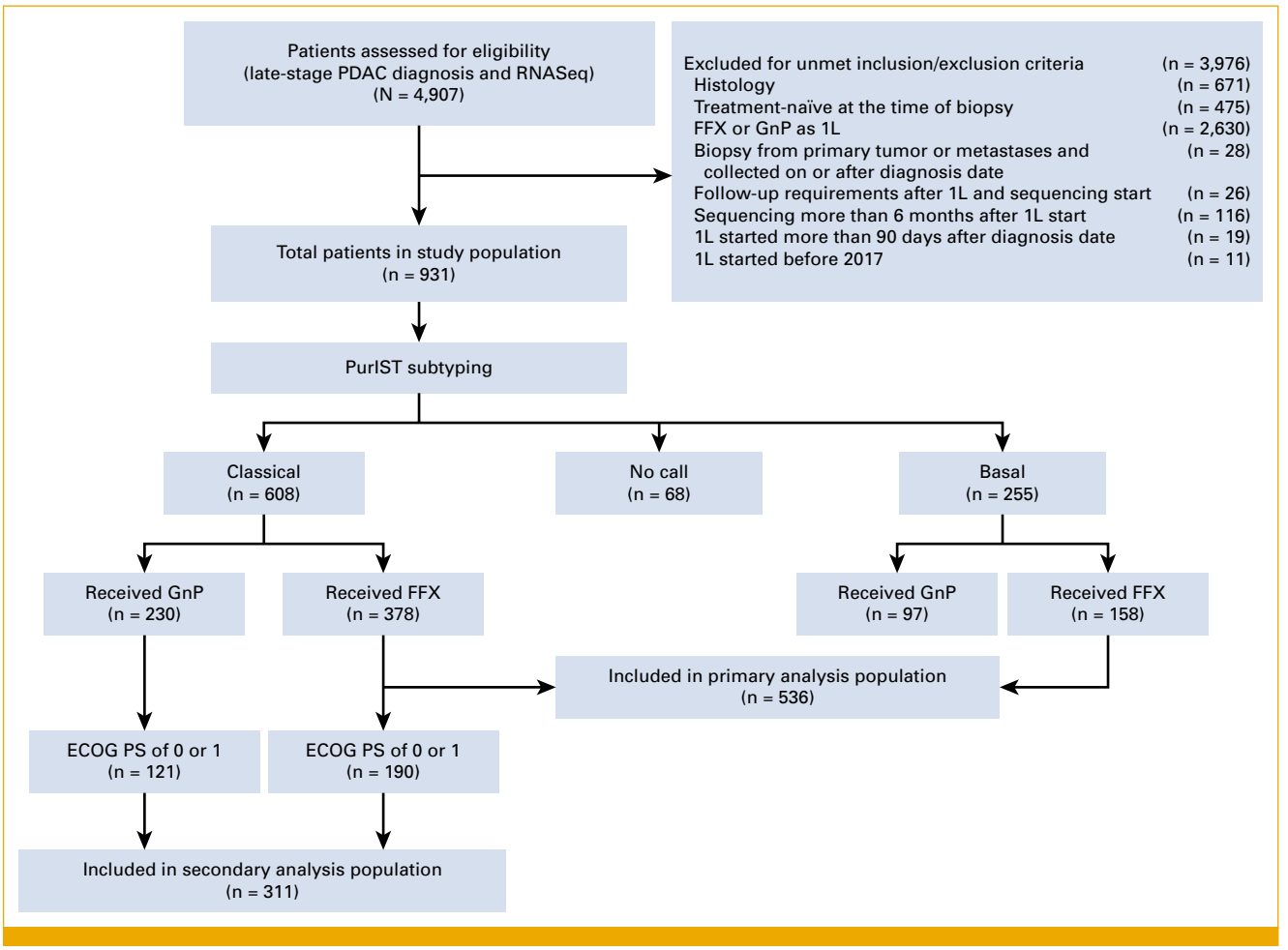

FIG 1. CONSORT diagram. 1L, first-line; ECOG PS, Eastern Cooperative Oncology Group performance status; FFX, FOLFIRINOX; GnP, gemcitabine + nab-paclitaxel; PDAC, pancreatic ductal adenocarcinoma; PurIST, purity independent subtyping of tumors.

TABLE 1. Patient Clinical and Pathologic Characteristics in the Primary Analysis Population (FFX-treated patients)

Characteristic	Overall, n = 536	Basal, n = 158	Classical, n = 378	*P**
Age, years				.4
Mean (SD)	63.3 (9.2)	63.8 (9.3)	63.0 (9.2)	
Median	64	64	63	
IQR	(57.0-70.0)	(58.0-70.0)	(57.0-70.0)	
Min/max	36.0/88.0	36.0/88.0	36.0/86.0	
Sex, No. (%)				.002
Female	232 (43)	52 (33)	180 (48)	
Male	304 (57)	106 (67)	198 (52)	
Race, No. (%)				.3
White	292 (56)	90 (57)	202 (53)	
Black	29 (6)	5 (3)	24 (6)	
Other	52 (9)	19 (12)	33 (9)	
Other race	32 (6)	11 (7)	21 (6)	
American Indian or Alaska Native	5 (1)	1 (1)	4 (1)	
Asian	14 (3)	6 (4)	8 (2)	
Native Hawaiian or Other Pacific Islander	1 (0)	1 (1)	0 (0)	
Missing	163 (29)	44 (28)	119 (31)	
CCI				.4
Mean (SD)	0.8 (1.3)	0.8 (1.5)	0.7 (1.2)	
Median	0	0	0	
IQR	(0.0-1.0)	(0.0-1.0)	(0.0-1.0)	
Min/max	0.0/10.0	0.0/10.0	0.0/6.0	
ECOG, No. (%)				.3
0	144 (27)	49 (31)	95 (25)	
1	141 (26)	46 (29)	95 (25)	
2	22 (4)	5 (3)	17 (4)	
3	2 (<1)	0 (0)	2 (1)	
Missing	227 (42)	58 (37)	169 (45)	
Stage, No. (%)				.6
III	7 (1)	1 (1)	6 (2)	
IV	529 (99)	157 (99)	372 (98)	
Biopsy tissue site, No. (%)				.2
Primary tumor	153 (29)	39 (25)	114 (30)	
Metastases	380 (71)	117 (74)	263 (70)	
Unknown	3 (<1)	2 (1)	1 (<1)	
HRR mutation, No. (%)	54 (10)	19 (12)	35 (9)	.4
BRCA mutation, No. (%)	21 (4)	7 (4)	14 (4)	.9
Liver metastases present, No. (%)	307 (57)	103 (65)	204 (54)	.022
Smoking status, No. (%)				.4
History of smoking	236 (44)	64 (41)	172 (46)	
No history of smoking	255 (48)	82 (52)	173 (46)	
Unknown	45 (8)	12 (8)	33 (9)	

NOTE. HRR pathway: presence of a somatic mutation in a homologous recombination repair pathway–associated gene (*BRCA1, BRCA2, PALB2, ATM, ATR, ATRX, BAP1, BARD1, BRIP1, CHEK1, CHEK2, RAD50, RAD51, RAD51B, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG,* and *FANCL*). *BRCA* mutation: presence of a somatic mutation in *BRCA1* or *BRCA2*. **P*-values were calculated using two sample t-test for numeric variables and Pearson's Chi-squared test for categorical variables.

Abbreviations: CCI, Charlson comorbidity index; HRR, homologous recombination repair; ECOG, Eastern Cooperative Oncology Group; FFX, FOLFIRINOX; min/max, minimum/maximum.

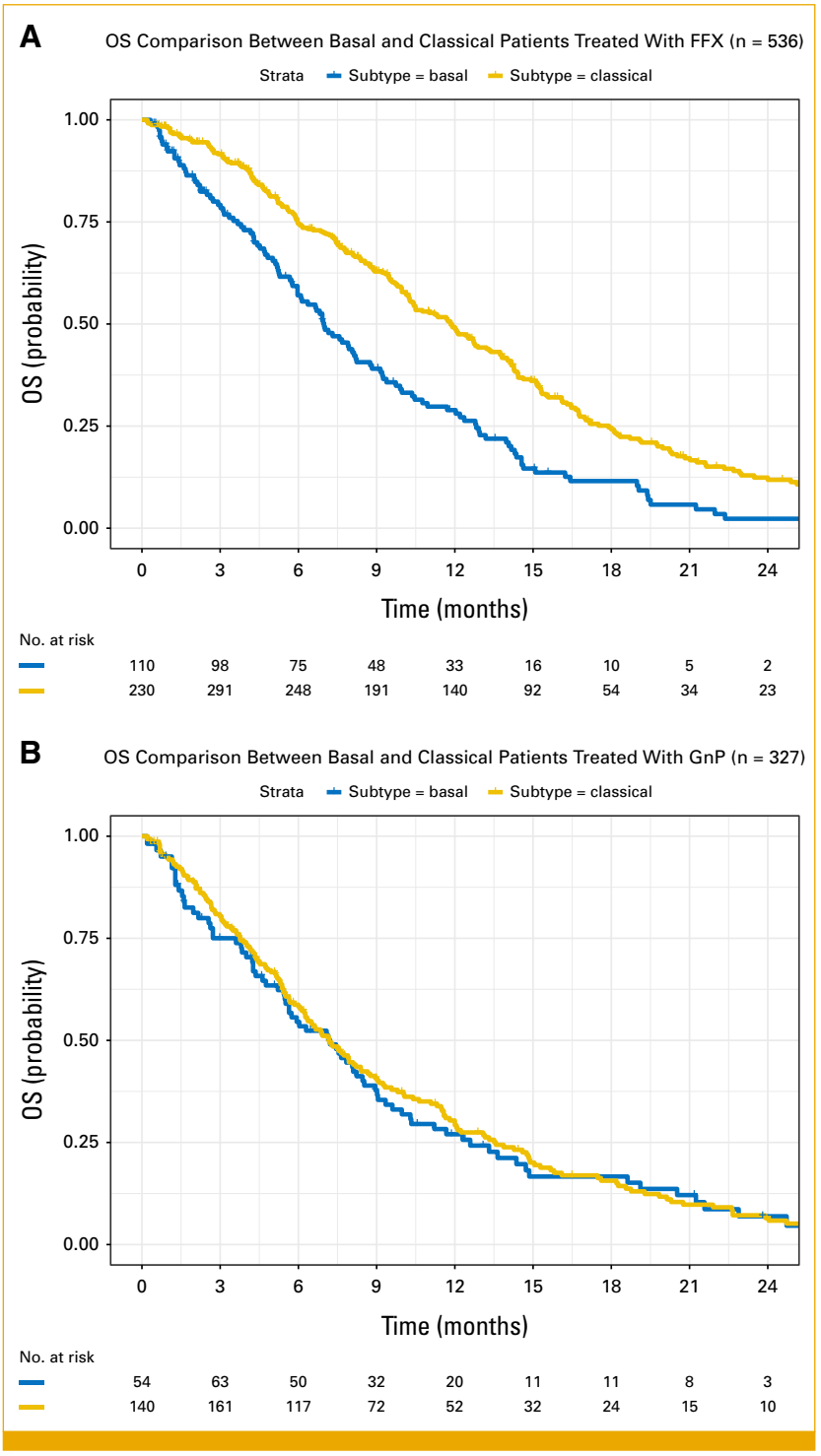

FIG 2. (A) Primary end point—OS comparison between basal and classical subtypes in patients treated with FFX. For patients treated with FFX 1L (n = 536), basal subtype patients have a higher risk of death versus classical subtype patients (HR, 1.86; $P = < .001$). Similarly, basal patients were less likely than classical in the 1L FFX treated group to survive to 12 months (28.9% *v* 48.9%; $P < .001$). (B) Supportive analysis—OS comparison between basal and classical subtypes in patients treated with GnP (n = 327). 1L, first-line; FFX, FOLFIRINOX; GnP, gemcitabine + nab-paclitaxel; HR, hazard ratio; OS, overall survival.

sex and the presence of liver metastasis are not balanced between the subtypes. A sensitivity analysis was performed by repeating the primary analysis after adjusting for these two variables, and the results remained significant (Data Supplement, Fig S3). In a post hoc analysis, we assessed whether there was any difference in OS between basal and classical subtype patients in the GnP-treated cohort and observed no significant difference (7.21 *v* 7.18 months, respectively, HR, 1.02; *P* = NS; Fig 2B).

Secondary End Point Analysis: Comparison of 1L FFX Versus 1L GnP in Classical Subtype Patients

Of the 608 patients labeled with classical subtype tumors, 51.1% (n = 311) had a good PS with an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1 and were included in the secondary end point analysis. Among these patients, 61% (n = 190) received FFX as 1L and 39% (n = 121) received GnP as 1L (Table 2). In this cohort, patients

TABLE 2. Patient Clinical and Pathologic Characteristics in the Secondary End Point Population (classical patients with ECOG PS of 0 or 1)

Characteristic	Total, n = 311	FFX, n = 190	GnP, n = 121	*P*
Age, years				<.001
Mean (SD)	66.9 (9.7)	63.0 (8.6)	73.0 (8.0)	
Median	67.0	63.0	74.0	
IQR	(60.0-74.0)	(57.0-69.0)	(69.0-78.0)	
Min/max	38.0/88.0	38.0/84.0	46.0/88.0	
Sex, No. (%)				>.9
Female	149 (48)	90 (47)	59 (49)	
Male	162 (52)	100 (53)	62 (51)	
Race, No. (%)				.4
White	177 (57)	104 (55)	73 (60)	
Black	20 (6)	11 (6)	9 (7)	
Other	26 (8)	15 (8)	11 (9)	
Other race	18 (6)	9 (5)	9 (7)	
American Indian or Alaska Native	4 (1)	3 (2)	1 (1)	
Asian	4 (1)	3 (2)	1 (1)	
Missing	88 (28)	60 (32)	28 (23)	
CCI				<.001
Mean (SD)	1.0 (1.5)	0.7 (1.2)	1.4 (1.8)	
Median	0	0	1	
IQR	(0.0-1.0)	(0.0-1.0)	(0.0-2.0)	
Min/max	0.0/10.0	0.0/5.0	0.0/10.0	
ECOG, No. (%)				<.001
0	130 (42)	95 (50)	35 (29)	
1	181 (58)	95 (50)	86 (71)	
Stage, No. (%)				>.9
III	3 (1)	2 (1)	1 (1)	
IV	308 (99)	188 (99)	120 (99)	
Biopsy tissue site, No. (%)				.8
Primary tumor	97 (31)	58 (31)	39 (32)	
Metastases	214 (69)	132 (69)	82 (68)	
HRR mutation, No. (%)	32 (10)	22 (12)	10 (8)	.5
BRCA mutation, No. (%)	12 (4)	9 (5)	3 (2)	.5
Liver metastases present, No. (%)	164 (53)	102 (54)	62 (51)	.8
Smoking status, No. (%)				.3
History of smoking	147 (47)	84 (44)	63 (52)	
No history of smoking	133 (43)	88 (46)	45 (37)	
Unknown	31 (10)	18 (9)	13 (11)	

Abbreviations: CCI, Charlson comorbidity index; HRR, homologous recombination repair; ECOG PS, Eastern Cooperative Oncology Group performance status; FFX, FOLFIRINOX; GnP, gemcitabine + nab-paclitaxel; min/max, minimum/maximum.

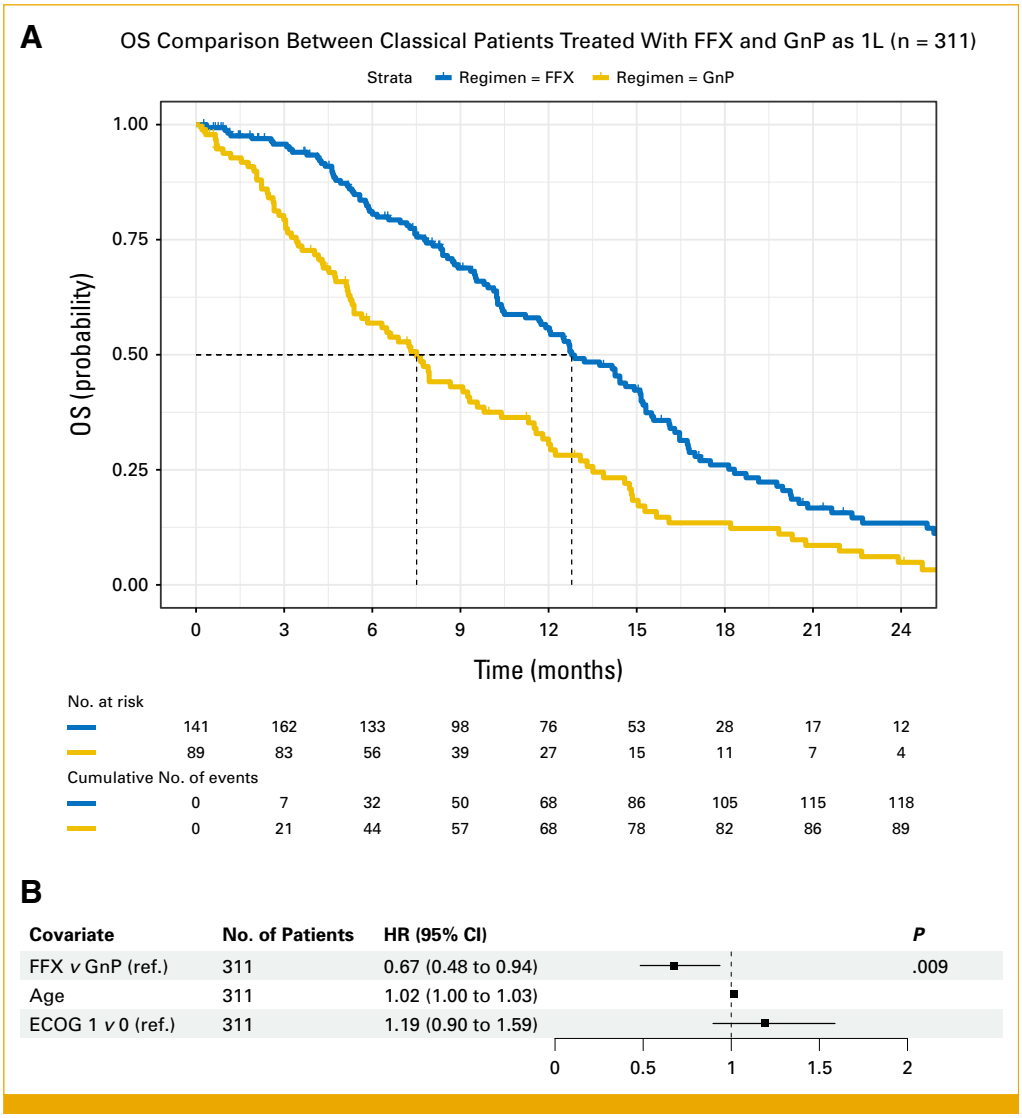

FIG 3. Secondary end point analysis—OS comparison between classical patients receiving 1L GnP versus classical subtype receiving 1L FFX. (A) Kaplan-Meier curves, not adjusted for age or ECOG. (B) Results of a multivariate Cox PH model adjusting for age and ECOG. (C) Kaplan-Meier curves, not adjusted for age or ECOG, for all regimen-subtype combinations, patients with ECOG values of 0 or 1 only. (D) Interaction test between 1L regimen (GnP *v* FFX) and PurIST subtype (basal *v* classical), for patients with ECOG values of 0 or 1 only. *P* value is from two-sided test. 1L, first-line; Cox PH, Cox proportional-hazard; ECOG, Eastern Cooperative Oncology Group; FFX, FOLFIRINOX; GnP, gemcitabine + nab-paclitaxel; HR, hazard ratio; OS, overall survival; PurIST, purity independent subtyping of tumors; ref., reference; TX, treatment. (continued on following page)

who were younger and had lower ECOG PS scores (0 *v* 1) were more likely2 to receive FFX than GnP (*P* < .001; Table 2). Patients treated with FFX had significantly longer OS than GnP (HR, 0.55 [95% CI, 0.42 to 0.71]; *P* < .001), with a median OS of 12.89 months (95% CI, 11.7 to 15.1) for FFX treated patients and a median OS of 7.64 months (95% CI, 5.6 to 9.6) for GnP (Fig 3A).

In the secondary end point analysis, a multivariable Cox PH model adjusted for age and ECOG PS showed a 33% relative risk (RR) reduction in death in classical subtype patients treated with FFX versus GnP (HR, 0.67 [95% CI, 0.48 to 0.94]; *P* < .01; Fig 3B), with neither age nor ECOG PS contributing significantly to OS in the model. In a supportive sensitivity analysis that included patients with higher and missing ECOG PS, this differential response to treatment was maintained with a 26% RR reduction in death in classical subtype patients treated with FFX versus GnP, after adjusting for age and ECOG (Data Supplement, Fig S4).

In a Kaplan-Meier analysis of basal subtype patients, the median OS for patients receiving FFX was 6.92 months (95% CI, 5.97 to 9.74) versus 7.84 months (95% CI, 5.64 to 11.67) for GnP (Fig 3C). This difference was not significant, in contrast to the significant treatment association observed in classical subtype patients (Fig 3A), suggesting that

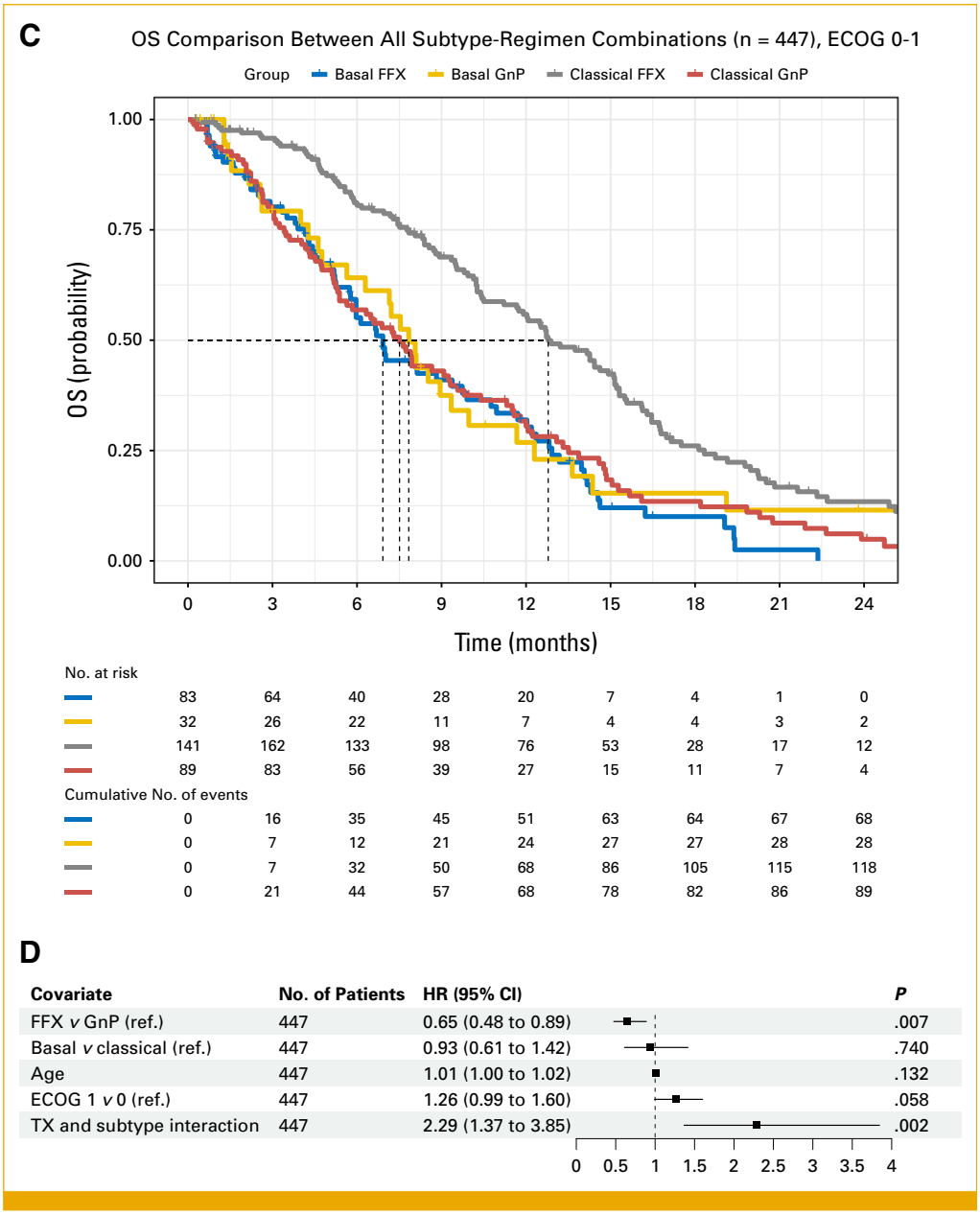

FIG 3. (Continued).

treatment outcomes are subtype-dependent. This hypothesis was formally tested in a supportive analysis, where an interaction effect between the treatment regimen and PurIST subtype was evaluated in a multivariable model controlling for age and ECOG PS. This interaction was highly significant (HR, 2.29 [95% CI, 1.37 to 3.85]; $P < .002$), strengthening the evidence of PurIST subtypes as a predictive biomarker of treatment outcomes (Fig 3D).

DISCUSSION

We performed an analytical and clinical validation of the PurIST molecular subtyping algorithm on a prospectively designed retrospective study using a real-world cohort of patients with advanced PDAC treated with 1L FFX or GnP. Using a prespecified statistical analysis plan, PurIST was validated as a robust prognostic classifier in FFX-treated patients, with basal subtype patients having significantly worse outcomes than classical subtype patients, while in GnP-treated patients, no prognostic association was observed. In a secondary analysis restricted to classical subtype patients with good PS, FFX-treated patients had significantly longer OS times than those treated with GnP while controlling for ECOG status and age. Finally, in a post hoc analysis, a significant interaction effect was observed between subtype and treatment regimen in a multivariable Cox

regression model, strengthening the evidence of PurIST subtypes as a predictive biomarker of treatment response.

In the current treatment paradigm of advanced-stage unresectable pancreatic cancer, FFX and GnP are both used in the 1L setting. Randomized controlled trials, for example, JCOG1611-GENERATE, and NAPOLI-3,[6,8] and retrospective studies[9-14] have shown variability in 1L regimen efficacy, and have motivated molecular biomarker studies, for example, COMPASS[26] and GEMPREDICT,[27] to optimize and personalize 1L treatment. A PurIST-driven biomarker approach was implemented in the PASS-01 trial[7] demonstrating the feasibility of multiomic testing and disease subtyping for informing clinical management in the advanced setting. PurIST subtyping using the Tempus RNA-seq platform is a clinically available assay that can fulfill this unmet clinical need for biomarker-informed clinical management in the 1L advanced-stage setting. Interestingly, in a recent Real World Data paper by Singh et al[29] evaluating basal and classical subtypes in late-stage PDAC using RNA-seq, classical patients were shown to have significantly longer OS for both FFX and GnP in the 1L setting. This differs from our findings that classical and basal patients on GnP had similar OS leading to a significant interaction between subtype and treatment. There is limited information describing the clinical characteristics of the cohort in the study by Singh et al[29] such as ECOG status, so it is difficult to speculate what could be driving the difference in findings between the two studies. Of note, the models in the study by Singh et al[29] and in this study do at least differ in the handling of borderline results: Singh et al[29] excluded weak basal and classical patients, while Tempus uses an indeterminacy prediction discussed in the Methods section to exclude borderline patients.

Limitations of this study reflect the real-world, retrospective nature of the validation cohort. Although the analyses controlled for confounding variables such as age and ECOG status, as a nonrandomized study, additional biases may be unaccounted for. The generalizability of findings, however, is strengthened by clinical patterns observed in the study cohort that are consistent with previous observations. Specifically, we observed a younger median age for patients receiving FFX-based treatments, and males had significantly worse OS, both across and within subtypes and treatment groups.[30,31] Moreover, patients with liver metastases had worse OS in the FFX and GnP treatment groups and were more likely to be basal subtype as previously described.[32-36]

As PurIST subtypes reflect intrinsic differences in tumor biology, their utility as a treatment biomarker is likely to generalize to other FFX-like regimens with similar mechanisms of action, such as NALIRIFOX, although formal biomarker subtype comparisons will be needed to confirm. Although this study focused on unresectable PDAC patients, PurIST subtyping may also have value in the perioperative setting for (neo)adjuvant treatment decision making. With current innovations in blood and image-based cancer screening methods, pancreatic cancer diagnosis may shift significantly to earlier-stage disease, which could make perioperative biomarkers a significant clinical unmet need.[37] Currently, the PANCREAS trial is evaluating the use of the PurIST algorithm to prospectively guide neoadjuvant treatment to FFX or GnP on the basis of classical or basal classification, respectively (ClinicalTrials.gov identifier: NCT04683315).

The results of this study support the use of PurIST molecular subtyping as a diagnostic tool that can be used in conjunction with clinical features for informing treatment decisions in the 1L, advanced-disease setting. Future prospective studies are recommended to demonstrate the clinical utility of PurIST as a predictive biomarker with improvements to patient outcomes in both the advanced and (neo)adjuvant settings.

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