BIOMARKERS

original reports abstract

Clinical and Molecular Characterization of *POLE* Mutations as Predictive Biomarkers of Response to Immune Checkpoint Inhibitors in Advanced Cancers

Benjamin Garmezy, MD[1]; Jinesh Gheeya, MD[2]; Heather Y. Lin, PhD[3]; Yuefan Huang, MS[4]; Taebeom Kim, PhD[4]; Xianli Jiang, PhD[4]; Kyaw Z. Thein, MD[5]; Patrick G. Pilié, MD[6]; Fadl Zeineddine, MD[7]; Wanlin Wang, MS[5]; Kenna R. Shaw, PhD[8]; Jordi Rodon, MD, PhD[5]; John Paul Shen, MD[7]; Ying Yuan, PhD[3]; Funda Meric-Bernstam, MD[5,8]; Ken Chen, PhD[4]; and Timothy A. Yap, MBBS, PhD, FRCP[5,8,9]

PURPOSE DNA polymerase epsilon is critical to DNA proofreading and replication. Mutations in *POLE* have been associated with hypermutated tumors and antitumor response to immune checkpoint inhibitor (ICI) therapy. We present a clinicopathologic analysis of patients with advanced cancers harboring *POLE* mutations, the pattern of co-occurring mutations, and their response to ICI therapy within the context of mutation pathogenicity.

METHODS We conducted a retrospective analysis of next-generation sequencing data at MD Anderson Cancer Center to identify patient tumors with *POLE* mutations and their co-occurring mutations. The pathogenicity of each mutation was annotated using InterVar and ClinVar. Differences in therapeutic response to ICI, survival, and co-occurring mutations were reported by *POLE* pathogenicity status.

RESULTS Four hundred fifty-eight patient tumors with *POLE* mutations were identified from 14,229 next-generation sequencing reports; 15.0% of *POLE* mutations were pathogenic, 15.9% benign, and 69.1% variant of unknown significance. Eighty-two patients received either programmed death 1 or programmed death ligand-1 inhibitors as monotherapy or in combination with cytotoxic T-cell lymphocyte-4 inhibitors. Patients with pathogenic *POLE* mutations had improved clinical benefit rate (82.4% *v* 30.0%; $P = .013$), median progression-free survival (15.1 *v* 2.2 months; $P < .001$), overall survival (29.5 *v* 6.8 months; $P < .001$), and longer treatment duration (median 15.5 *v* 2.5 months; $P < .001$) compared to those with benign variants. Progression-free survival and overall survival remained superior when adjusting for number of co-occurring mutations (≥ 10 *v* < 10) and/or microsatellite instability status (proficient mismatch repair *v* deficient mismatch repair). The number of comutations was not associated with response to ICI (clinical benefit *v* progressive disease: median 13 *v* 11 comutations; $P = .18$).

CONCLUSION Pathogenic *POLE* mutations were associated with clinical benefit to ICI therapy. Further studies are warranted to validate *POLE* mutation as a predictive biomarker of ICI therapy.

JCO Precis Oncol 6:e2100267. © 2022 by American Society of Clinical Oncology

ASSOCIATED CONTENT

Appendix

Data Sharing Statement

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

Accepted on December 28, 2021 and published at ascopubs.org/journal/po on February 2, 2022: DOI https://doi.org/10.1200/PO.21.00267

INTRODUCTION

DNA polymerase epsilon, encoded by the *POLE* gene, is a critical protein involved in DNA proofreading and replication.[1] POLE synthesizes the leading strand of DNA in the replication fork and has a 3′-5′ exonuclease domain that increases replication accuracy by approximately 100-fold through recognition and excision of mismatched base pairs.[2,3] Somatic and germline *POLE* proofreading defects, particularly mutations occurring in the exonuclease domain representing codons 268-471, are more often found in mismatch repair proficient tumors and associated with hypermutagenesis.[4-8]

Determining appropriate predictive biomarkers of response to optimize patient selection for immune checkpoint inhibitor (ICI) therapy remains a challenge.[9] The programmed death 1 (PD-1) inhibitor pembrolizumab is US Food and Drug Administration (FDA)-approved in multiple tumor-specific indications and also for histology-agnostic use in tumors that are microsatellite instability high (MSI-H), mismatch repair deficient (dMMR), and/or those with tumor mutation burden (TMB) ≥ 10 mutations/megabase.[10-13] Other ICI therapies have varied programmed death ligand-1 (PD-L1) or combined positive score cutoffs.[14] However, PD-L1 expression is often not predictive of ICI response.[15]

Wang et al[16] evaluated the prevalence of mutations in *POLE* and *POLD1*, another proofreading protein, in 47,721 patients with different cancer types via the

CONTEXT

Key Objective

Determining appropriate predictive biomarkers of response to optimize patient selection to immune checkpoint inhibitor (ICI) therapy remains a challenge. This retrospective clinicopathologic analysis of patients with POLE mutations examined the correlation between *POLE* pathogenicity and patient outcomes to ICI therapy. To our knowledge, this is the first and largest report of patient data in the context of *POLE* pathogenicity.

Knowledge Generated

Patients with pathogenic *POLE* mutations, compared to those with benign variants, had improved clinical benefit rate, median progression-free survival, median overall survival, and a longer duration on ICI treatment. Survival analyses remained superior when adjusting for number of co-occurring mutations within the tumor and/or microsatellite instability status.

Relevance

These findings support further study in patients with advanced solid tumors harboring *POLE* variants to further clarify the utility of *POLE* mutation location and pathogenicity as a predictive biomarker for ICI therapy.

cBioPortal database. They found variants in *POLE* and *POLD1* at mutational frequencies of 2.8% and 1.4%, respectively. Patients with one of these mutations had improved overall survival (OS; 34 *v* 18 months; $P = .004$) and were more likely to benefit from ICI therapy.[16] However, this study did not examine whether mutation location or pathogenicity had an effect on therapeutic response, a key consideration in the development of any clinical biomarker.

Here, we present a clinicopathologic analysis of patients with advanced cancers harboring *POLE* mutations at The University of Texas MD Anderson Cancer Center (MDACC). The primary aim of this study was to determine the correlation between *POLE* mutation pathogenicity and patient outcomes to ICI therapy. Secondary aims included determining the relationship of *POLE* mutations to patient prognosis, other ICI biomarkers, and co-occurring mutation patterns. To our knowledge, this is the first and largest report of patient data of this magnitude in the context of *POLE* pathogenicity.

METHODS

A retrospective electronic database search of Clinical Laboratory Improvement Amendments–certified next-generation sequencing data was conducted to identify MDACC patient tumors with *POLE* mutations and co-occurring mutations, as this previously has been shown to be a surrogate for TMB.[17,18] Clinical data through April 1, 2020, were collected from the electronic medical record. MDACC institutional review board approval was obtained before study initiation, and all data were collected and stored according to best practices, protecting patient confidentiality and data integrity.

The pathogenicity of each *POLE* mutation was annotated via InterVar[19] and ClinVar.[20] If one of these two sources indicated a variant of unknown significance (VUS), but the other provided a non-VUS annotation, then the non-VUS status was used. All mutations were then reviewed using peer-reviewed publications to further update pathogenicity[21-25] (Appendix 1). All *POLE* mutation annotations were checked independently by a second reviewer. Benign and likely benign as well as pathogenic and likely pathogenic were grouped together for analysis.

Descriptive statistics were used to summarize patient's characteristics. Chi-squared or Fisher exact tests were used to evaluate differences of category variables. The distributions of progression-free survival (PFS), OS, and time on first immunotherapy treatment were estimated using the Kaplan-Meier method.[26] Log-rank test[27] was performed to test the difference in survival between groups. The Cox proportional hazards model[28] were used for the multivariate analyses of survival, adjusting for MSI status and/or number of comutations (≥ 10 and ≥ 20).

See Appendix 1 for additional information.

RESULTS

Of 14,229 patients with solid tumors and available next-generation sequencing data, we identified 486 (3.4%) patients with a *POLE*-aberrant tumor. This percentage is comparable to that identified on The Cancer Genome Atlas database (4.0%, accessed on September 24, 2020). Of these 486 patients, 458 had comutation data and 453 had available clinical data in the electronic medical record.

POLE Mutation Pathogenicity

POLE mutations were annotated as the following (n = 453): pathogenic (n = 68, 15.0%), benign (n = 72, 15.9%), or variant of unknown significance (n = 313, 69.1%) mutations. Sixty-eight patients had a tumor with a mutation in the *POLE* exonuclease domain: 47.1% pathogenic, 8.8% benign, and 50% VUS (Fig 1).

Response to Immune Checkpoint Inhibition

Of the 453 patients with available clinical data, 172 had received treatment with either a PD-1 or PD-L1 (PD-1/L1) inhibitor. One hundred twenty-one patients were considered

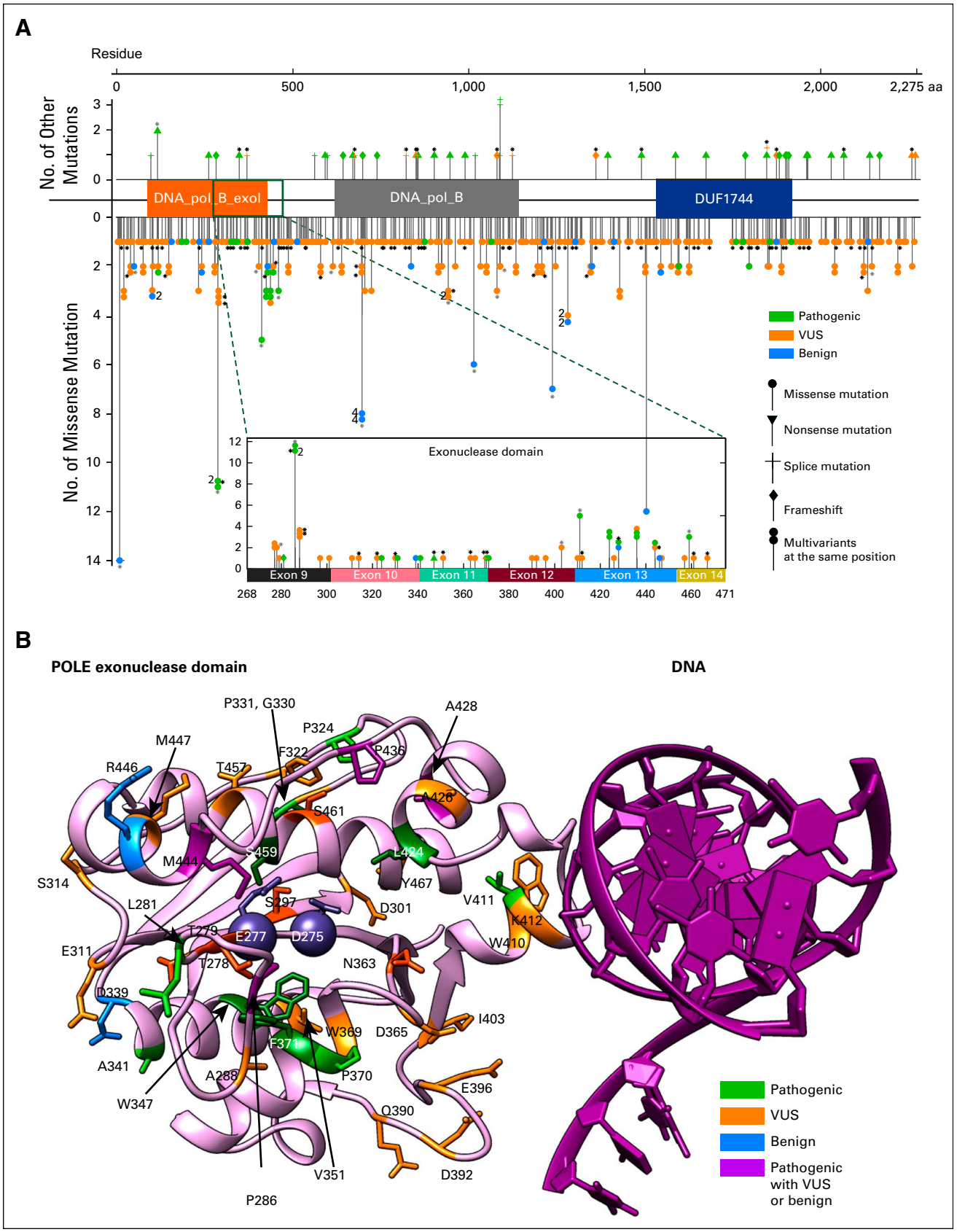

FIG 1. (continued on following page)

FIG 1. (Continued). (A) Distribution of *POLE* mutations within *POLE* whole-length sequence. Among 450 evaluable patients, one had a *POLE* amplification and 449 had a *POLE* mutation, contributing to 424 unique POLE variants, as plotted against the mutational sites. Each data point with certain symbol represents a unique variant. All 375 unique missense mutations are shown downward with solid circles. Thirteen frameshift mutations, 13 splice variants, 23 nonsense mutations are plotted upwards in respective symbols. Stacked symbols indicate different mutations have been found at the same position. For example, POLE_P286 has 11 missense mutations occurrences among 449 patients in total, with one pathogenic mutation (P286L) in two patients and another VUS mutation found in nine patients. Black asterisks next to some data points indicate that those variants are in patients containing multiple POLE mutations, while gray asterisks represent a mixture of patients with a single *POLE* mutation and multiple *POLE* mutations. Fifty unique variants (47 missense mutations) are found within the exonuclease domain (268-471). (B) POLE exonuclease domain mutations mapped to structure. The exonuclease domain of human POLE modeled by AlphaFold2[29] (pink) is aligned to *Saccharomyces cerevisiae* POLE-DNA complex[30] (DNA in purple) and then the POLE chain from *Saccharomyces cerevisiae* is removed. The two catalytic residues (D275 and E277) are shown as spheres. Mutations found in this domain are displayed in the structure with different colors indicating pathogenic status. Residues that are physically adjacent to the catalytic sites (all atom distance < 6 Å) are highlighted in darker colors, with VUS mutations S297, T278, T279, N363, and S461 in dark orange and pathogenic mutations M444, L424, S459, F371, W347, and P286 in dark green. These pathogenic mutations surround the two catalytic residues (D275 and E277)[31] and likely affect the catalytic pocket, whereas pathogenic mutations at V411 might affect DNA binding, although its location is distal to the catalysis center. All benign mutations occur at residues far from catalytic sites. Other residues W410, I403, D365, D396, and D392 may also contribute to DNA binding, although the functional annotation of those mutations remains unknown. Different mutations at A428 (A428T and A428S) lead to conflicting pathogenic status in available databases; no patient treated with anti–PD-1/L1-based therapy in this cohort had an A428 mutation. PD-1, programmed death 1; PD-L1, programmed death ligand-1; VUS, variant of unknown significance.

suitable for response analysis after excluding those with limited-stage disease, insufficient follow-up time for response evaluation (ie, had no restaging scans performed), and/or received their treatment as neoadjuvant, adjuvant, or maintenance therapy. Ninety-six of 121 (79.3%) had a tumor or molecular subtype with an FDA-approved indication for ICI therapy. Overall, 64 patients received PD-1/L1 inhibitors as monotherapy, 18 as combination therapy with a cytotoxic T-cell lymphocyte-4 (CTLA-4) inhibitor, and 39 in combination with either chemotherapy, a molecular targeted agent, or a vaccine (Table 1). See additional clinical information in Appendix Table A1.

Patients who achieved radiologic complete response (CR), partial response (PR), or stable disease (SD) were considered to have derived clinical benefit. Response data are shown in Table 2 and Appendix Table A1. Clinical benefit rate (CBR) of all 121 patients to PD-1/L1 inhibitor–based therapy was 55.4% (95% CI, 46.5 to 64.2). CBR was greater in patients with pathogenic *POLE* mutations when compared to patients with benign variants: 81.0% (pathogenic), 38.0% (benign); pathogenic versus benign, $P = .01$. We then grouped patients with benign or VUS mutations together as nonactionable variants, as this would be a meaningful distinction when selecting patients for therapy in clinic. CBR was also greater in patients with pathogenic mutations with tumors harboring pathogenic *POLE* mutations compared with nonactionable variants; 81.0% versus 50.0%, $P = .014$. The overall response rate (ORR, CR, and PR) was also higher in patients with pathogenic versus benign mutations (52.4% *v* 11.1%; $P = .008$) and trended toward significance with pathogenic versus nonactionable variants (52.4% *v* 31.0%; $P = .061$).

As 32.2% of patients received PD-1/L1 inhibitors in combination with another agent that could influence response, we next analyzed the 82 patients who received immunotherapy-only (IO-only) regimens, either PD-1/L1 inhibitor as monotherapy or dual therapy in combination with a CTLA-4 inhibitor. CBR was again higher in patients with pathogenic *POLE* mutations; pathogenic versus benign, 82.4% versus 30.0%, $P = .013$; 82% versus 53.8%, $P = .50$. There were no CR or PR in patients with benign mutations (ORR pathogenic *v* benign: 47.1% *v* 0%; $P = .019$).

Among patients who received IO-only therapy (n = 82), eight had pathogenic *POLE* mutations in the exonuclease domain (all had missense and two had additional nonsense mutations; Fig 1B and Appendix Table A1); response rate (RR) was 37.5% (3 of 8 patients). No patient had a benign variant in the exonuclease domain and one patient had a VUS in the exonuclease domain (missense, RR 0%). Nine patients had pathogenic mutations outside of the exonuclease domain: two had single missense mutations (RR 100%; 2 of 2 patients), three had single frameshift (RR 66.7%; 2 of 3 patients), one had splice (RR 100%), one had nonsense (RR 0%), and two had both frameshift and missense mutations (RR 0%). No responses were observed in the 10 patients with benign POLE mutations outside the exonuclease domain (missense, RR 0%; 0 of 10 patients). Fifty-four patients had VUSs outside the exonuclease domain (all missense, RR 31.5%; 17 of 54 patients).

Survival Analysis

Median PFS for the 121 patients that received therapy with an anti–PD-1/L1-based regimen was 5.4 (95% CI, 3.5 to 7.9) months and PFS at 12 months was 35% (95% CI, 26 to 44). Median PFS was greater in patients with pathogenic mutations compared with benign mutations: 15.1 versus 2.8 months; $P < .001$. Median PFS was 4.9 (95% CI, 3.1 to 11.4) months in patients with VUS (Fig 2A). Median PFS improved by > 10 months in patients with pathogenic mutations compared with nonactionable variants, although this did not reach statistical significance (15.1 *v* 4.2 months; $P = .075$).

TABLE 1. Baseline Characteristics of Patients With *POLE* Mutations in Patients Receiving Immunotherapy (N = 121)

Characteristic	Frequency Count	Percent of Total Frequency
Sex		
Female	40	67
Male	81	33
POLE pathogenic status		
Benign	10	8
Likely benign	8	7
Pathogenic	15	12
Likely pathogenic	6	5
VUS	82	68
Type of immunotherapy received		
Anti–PD-1/L1 monotherapy	64	53
Anti–PD-1/L1 plus anti–CTLA-4	18	15
Anti–PD-1/L1 plus other (non-ICI)	39	32
Primary histology		
NSCLC	27	22
Colorectal adenocarcinoma	21	17
Melanoma	19	16
Breast	8	7
Head and neck: squamous cell carcinoma	7	6
Urothelial carcinoma	6	5
Cholangiocarcinoma	5	4
Glioblastoma	4	3
Prostate adenocarcinoma	3	2
Sarcoma	3	2
Small-cell lung cancer	2	2
Uterine cancer	2	2
Pancreatic adenocarcinoma	2	2
Gastric adenocarcinoma	2	2
Neuroendocrine carcinoma	2	2
Other	8	6
Median age, years (range) at primary cancer diagnosis	63	14-90
Median age, years (range) at start of ICI therapy	64	16-90
Immunotherapy biomarkers		
Co-occurring mutation number		
All patients (N = 121)		
< 10	43	36
10-19	47	39
≥ 20	31	26
Pathogenic *POLE* (n = 21)		
< 10	5	24
≥ 10	18	76
< 20	13	62
≥ 20	8	38
Benign *POLE* (n = 18)		
< 10	2	11
≥ 10	16	89
< 20	13	72
≥ 20	5	28
PD-L1 status		
All patients (N = 121)		
Positive (≥ 1)	33	27
Negative	38	31
Unknown	50	41
Pathogenic *POLE* (n = 21)		
Positive	6	29
Negative	8	38
Unknown	7	33
Benign *POLE* (n = 18)		
Positive	0	0
Negative	6	33
Unknown	12	67
MSI status		
All patients (N = 121)		
pMMR	53	44
dMMR	16	13
Unknown	52	43
Pathogenic *POLE* (n = 21)		
pMMR	11	52
dMMR	2	10
Unknown	8	38
Benign *POLE* (n = 18)		
pMMR	11	61
dMMR	1	6
Unknown	6	33

Abbreviations: CTLA-4, cytotoxic T-cell lymphocyte-4; dMMR, deficient mismatch repair; ICI, immune checkpoint inhibitor; MSI, microsatellite instability; NSCLC, non–small-cell lung cancer; PD-1/L1, programmed death-1/programmed death ligand-1; pMMR, proficient mismatch repair.

Median OS from time of anti–PD-1/L1-based therapy for these patients was 29.5 (95% CI, 26.0 to not reached [NR]) and OS at 12 months was 77% (95% CI, 67 to 84); median follow-up was 15.6 months. Median OS was greater for patients with pathogenic mutations than benign variants: 29.5 versus 11.6 months; $P < .001$. Patients with VUS had median OS that was NR (Fig 3A).

TABLE 2. Best Response to Immune Checkpoint Inhibitor Therapy in Patients Receiving Immunotherapy

Response	Frequency Count	Percent of Total Frequency
All patients (N = 121)		
CR	8	7
PR	34	28
SD	25	21
PD	54	45
IO-only patients (n = 82)		
Pathogenic *POLE* (n = 17)		
CR	0	0
PR	8	48
SD	6	35
PD	3	18
Benign *POLE* (n = 10)		
CR	0	0
PR	0	0
SD	3	30
PD	7	70
VUS *POLE* (n = 55)		
CR	4	7
PR	17	31
SD	11	20
PD	23	42

Abbreviations: CR, complete response; IO, immunotherapy; PD, progressive disease; PR, partial response; SD, stable disease; VUS, variant of unknown significance.

Patients Who Received IO-Only Treatment Regimens (n = 82)

Among patients who received IO-only regimens, median PFS was 6.0 (95% CI, 4.1 to 13.3) months with a 12-month PFS of 41% (95% CI, 29 to 51). Median PFS was longer in patients with pathogenic compared with benign *POLE* mutations: 15.1 versus 2.2 months; $P < .001$. Patients with VUS had a median PFS of 6.2 (95% CI, 3.9 to 20.6) months (Fig 2B). With adjustment for comutation number (≥ 10 *v* < 10) and MSI/MMR status (microsatellite stable [MSS]/proficient mismatch repair *v* MSI-H/dMMR), patients with pathogenic mutations had a superior PFS than those with benign mutations (HR, 0.07; 95% CI, 0.02 to 0.31; $P < .001$), as did patients with VUS mutations compared with benign mutations (HR, 0.16; 95% CI, 0.05 to 0.53; $P = .003$). When adjusting for number of comutations (≥ 20 *v* < 20) and MSI status, patients with pathogenic mutations continued to have superior PFS than those with benign mutations (HR, 0.07; 95% CI, 0.02 to 0.31; $P < .001$), as did those with VUS mutations compared with benign mutations (HR, 0.12; 95% CI, 0.04 to 0.43; $P = .001$).

Among patients who received IO-only regimens, median OS from time of therapy start was 29.5 (95% CI, 26.0 to NR) months and OS at 12 months was 80% (95% CI, 68 to 87); median follow-up was 17.5 months. Median OS was greater in patients with pathogenic compared with benign *POLE* mutations: 29.5 months versus 6.8 months, $P < .001$. Median OS was NR in patients with VUS mutations (Fig 3B). Differences in median OS between patients with pathogenic and nonactionable variants were not statistically significant (29.5 months *v* NR; $P = .265$). Patients with pathogenic *POLE* mutations had greater median OS compared to patients with benign mutations when adjusting for number of comutations (≥ 10 *v* < 10: HR, 0.10 [95% CI, 0.03 to 0.40], $P = .001$; ≥ 20 *v* < 20: HR, 0.10 [95% CI, 0.03 to 0.41], $P = .001$) or MSI status (HR, 0.14; 95% CI, 0.03 to 0.81], $P = .028$).

Of note, patients with pathogenic compared with benign *POLE* mutations had longer median IO-only treatment duration of first IO-only therapy (15.5 *v* 2.5 months; $P < .001$). Patients with VUS had a median duration of 6.2 (95% CI, 3.6 to 14.2) months.

Total Population of Patients With POLE Mutations (N = 453)

Among the total population of patients with *POLE* mutations and available clinical information, median follow-up was 2.5 years and median OS was 6.8 (95% CI, 5.0 to 9.4) years. Median OS from time of diagnosis was greater for those with pathogenic *POLE* mutations compared to those with benign mutations (NR *v* 3.4 years; $P < .001$) and those with VUS (NR *v* 8.0 years; $P = .012$; Fig 3C). Patients with pathogenic mutations had a superior median OS compared to those with nonactionable variants (NR *v* 6.4 years; $P = .003$). Patients with pathogenic mutations continued to have superior OS with adjustment for TMB (≥ 10 *v* < 10) or MSI status (HR, 0.23; 95% CI, 0.07 to 0.75; $P = .014$).

Relationship of *POLE* Pathogenicity and Immunotherapy Biomarkers

Compared to patients with benign *POLE* mutations, those with pathogenic variants were significantly not more likely to have number of comutations ≥ 10 (76.2% *v* 88.9%; $P = .417$) or ≥ 20 (38.1% *v* 27.8%; $P = .734$), PD-L1–positive on pathology examination (42.9% *v* 0%; $P = .115$), or MSI-high status (13.3% *v* 8.3%; $P > .99$; Table 1).

Co-occurring Mutations

Figure 4A shows the landscape of comutations in all patients with *POLE* mutations (n = 450). The most common co-occurring mutations included *TP53* (52%), *ARID1A* (22%), *BRCA2* (21%), *KRAS* (21%), *NF1* (19%), *NOTCH3* (18%), *NOTCH1* (18%), *ATM* (18%), *PIK3CA* (17%), *SETD2* (17%), and *SMARCA4* (17%).

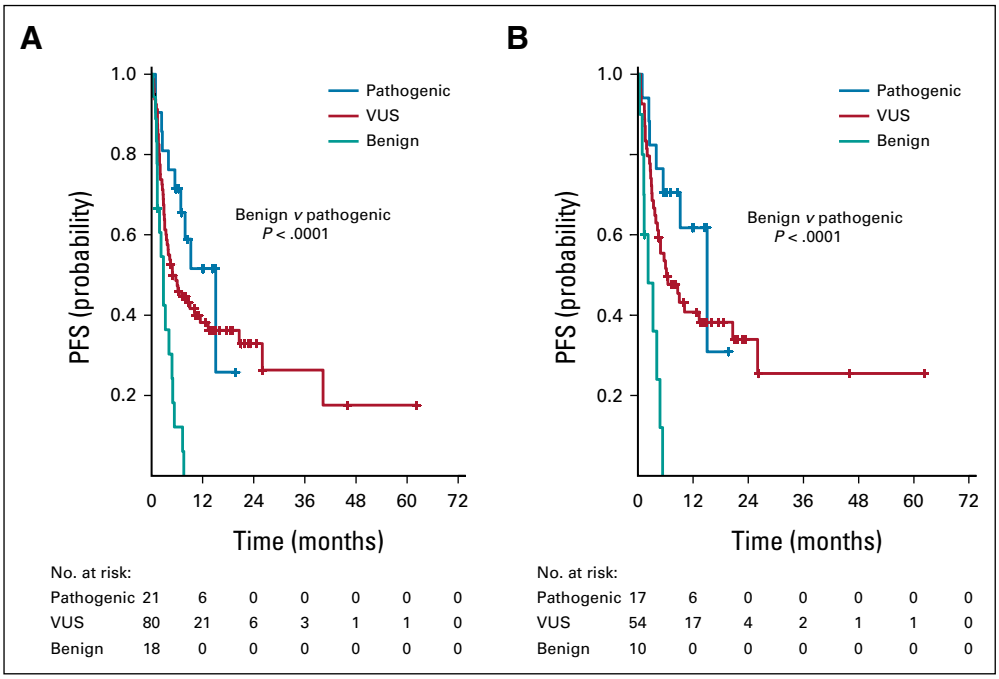

FIG 2. PFS for patients treated with an anti–PD-1/L1-based regimen. Among all 121 patients treated, two patients did not have data evaluable for survival analysis. Median PFS was as follows: pathogenic *POLE* mutation 15.1 months, VUS 4.9 months, and benign 2.8 months; pathogenic versus benign $P < .001$ (A). Among the 82 patients who received an immunotherapy-only regimen: pathogenic 15.1 months, VUS 6.2 months, benign 2.2 months; pathogenic versus benign $P < .001$. With adjustment for comutation number (≥ 10 *v* < 10) and MSI/MMR status (MSS/pMMR *v* MSI-H/dMMR), patients with pathogenic mutations had a superior PFS than those with benign mutations (HR, 0.07; 95% CI, 0.02-0.31; $P < .001$). When adjusting for number of comutations (≥ 20 *v* < 20) and MSI status, patients with pathogenic mutations continued to have superior PFS than those with benign mutations (HR, 0.07; 95% CI: 0.02-0.31; $P < .001$) (B). dMMR, deficient mismatch repair; MSI-H, microsatellite instability high; MSS, microsatellite stable; PD-1, programmed death 1; PD-L1, programmed death ligand-1; PFS, progression-free survival; VUS, variant of unknown significance.

Patients with pathogenic *POLE* mutations had more comutations in DNA damage response (DDR) pathway genes (pathogenic *v* benign *POLE* mutation): *ARID1A* (30.0% *v* 14.1%; $P = .032$), *ATM* (26.3% *v* 12.7%; $P = .059$), *ATR* (18.8% *v* 4.2%; $P = .012$), *ATRX* (20.0% *v* 7.0%; $P = .039$), *BRCA1* (20.0% *v* 9.9%; $P = .132$), *BRCA2* (26.3% *v* 14.1%; $P = .100$), *CDK12* (20.0% *v* 12.7%; $P = .323$), and *PALB2* (13.8% *v* 2.8%; $P = .036$; Figs 4B and 4C).

The number of mutations was not significantly associated with antitumor response to an IO-only regimen (clinical benefit *v* progressive disease [PD]: median 13 *v* 11; $P = .18$). However, there was a trend toward increased CBR in patients with ≥ 20 co-occurring mutations (odds ratio, 2.6; $P = .086$); there was no trend when a lower threshold of ≥ 10 co-occurring mutations was used (odds ratio, 1.3; $P = .65$). There was also no difference in OS in patients with low or high numbers of comutations. Overall, among the 29 patients with responses (CR or PR) to IO-only regimens, 10 (34%) had < 10 co-occurring mutations. Notably, the number of unique co-occurring DDR gene mutations was associated with clinical benefit to therapy (median 1 *v* 0; $P = .009$). Additionally, among patients with nonactionable *POLE* variants (n = 65), the number of comutations was not predictive of antitumor response (CR/PR *v* SD/PD, median 16 *v* 11; $P = .838$) or clinical benefit (CR/PR/SD *v* PD, 13 *v* 11; $P = .522$).

DISCUSSION

Current research studies have focused on the association between *POLE* mutations and tumors with a hypermutation phenotype,[5-7,21,32] providing the rationale for targeting patients with these tumors with immunotherapeutic agents. To the best of our knowledge, this study is the first to highlight the importance of the pathogenic status of *POLE* mutations. CBR in patients who received an IO-only regimen (PD-1/L1 inhibitor monotherapy or in combination with CTLA-4 inhibitor) was greater in patients with pathogenic *POLE* mutations compared to those with nonactionable variants (82.4% *v* 30.0%; $P = .013$) and there were no radiologic responses in patients with benign variants.

Mutations in the *POLE* exonuclease domain are associated with hypermutated cancers, antitumor responses to immunotherapy agents, and a trend toward improved

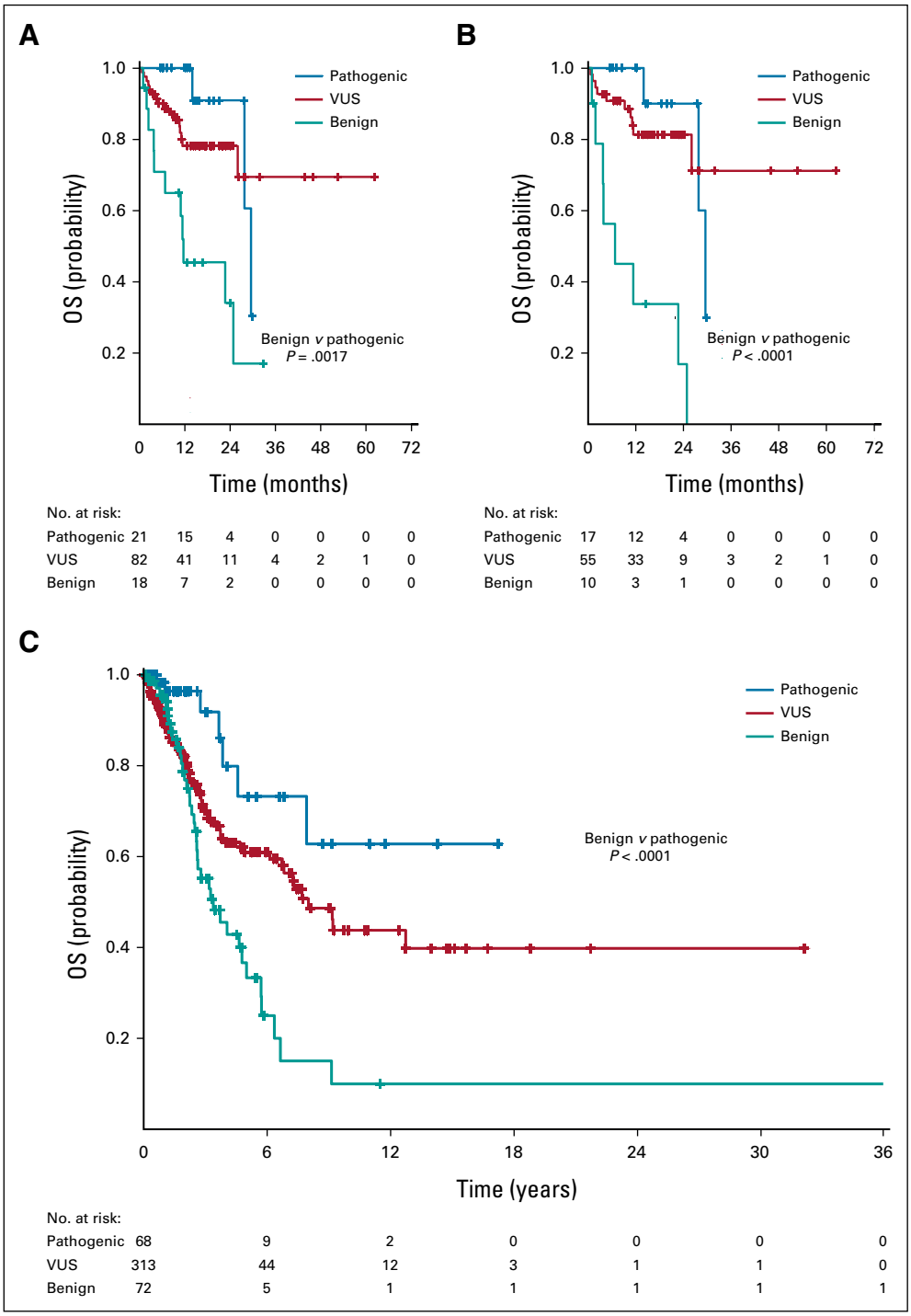

FIG 3. OS for patients treated with an anti–PD-1/L1-based regimen. Among all 121 patients treated, median OS was as follows: pathogenic *POLE* mutation 29.5 months, VUS NR, and benign 11.6 months; pathogenic versus benign $P < .001$ (A). Among the 82 patients who received an immunotherapy-only regimen: pathogenic 29.5 months, VUS NR, benign 6.8 months; pathogenic versus benign $P < .001$. Patients with pathogenic *POLE* mutations had greater median OS compared to patients with benign mutations when adjusting for number of comutations (≥ 10 *v* < 10: HR, 0.10 [95% CI, 0.03 to 0.40], $P = .001$; ≥ 20 *v* < 20: HR, 0.10 [95% CI, 0.03 to 0.41], $P = .001$) or MSI status (HR, 0.14; 95% CI, 0.03 to 0.81; $P = .028$) (B). (C) OS from time of diagnosis for all 453 patients with *POLE* mutations including patients who did not receive anti–PD-1/L1 therapy. Median OS was as follows: pathogenic *POLE* mutation NR, VUS 8.0 years, and benign 3.4 years; pathogenic versus benign $P < .001$. NR, not reached; OS, overall survival; PD-1, programmed death 1; PD-L1, programmed death ligand-1; VUS, variant of unknown significance.

prognosis.[3,4,7,33-37] Only 15 (12.4%) patients treated with a PD-1/L1 inhibitor had a mutation in the exonuclease domain. Comparative analysis relating *POLE* pathogenicity, location and type of mutation, and IO response was limited because of a small sample size. Although mutations in the exonuclease domain are often pathogenic, our study highlights that there are pathogenic alterations outside of this domain that may be successfully targeted by ICI therapy. Our cohort includes IO-only responses in patients with *POLE* missense, splice, and frameshift alterations outside of the exonuclease domain.

Patients with pathogenic mutations (n = 17) who received IO-only regimens had improved median PFS (15.1 *v* 2.2 months; $P < .001$), OS (29.5 *v* 6.8 months; $P < .001$), and duration on therapy (15.5 *v* 2.5 months; $P < .001$) than patients with benign mutations (n = 10). PFS differences remained significant when adjusting for MSI and number of comutations at either cutoffs of ≥ 10 or ≥ 20. Additionally,

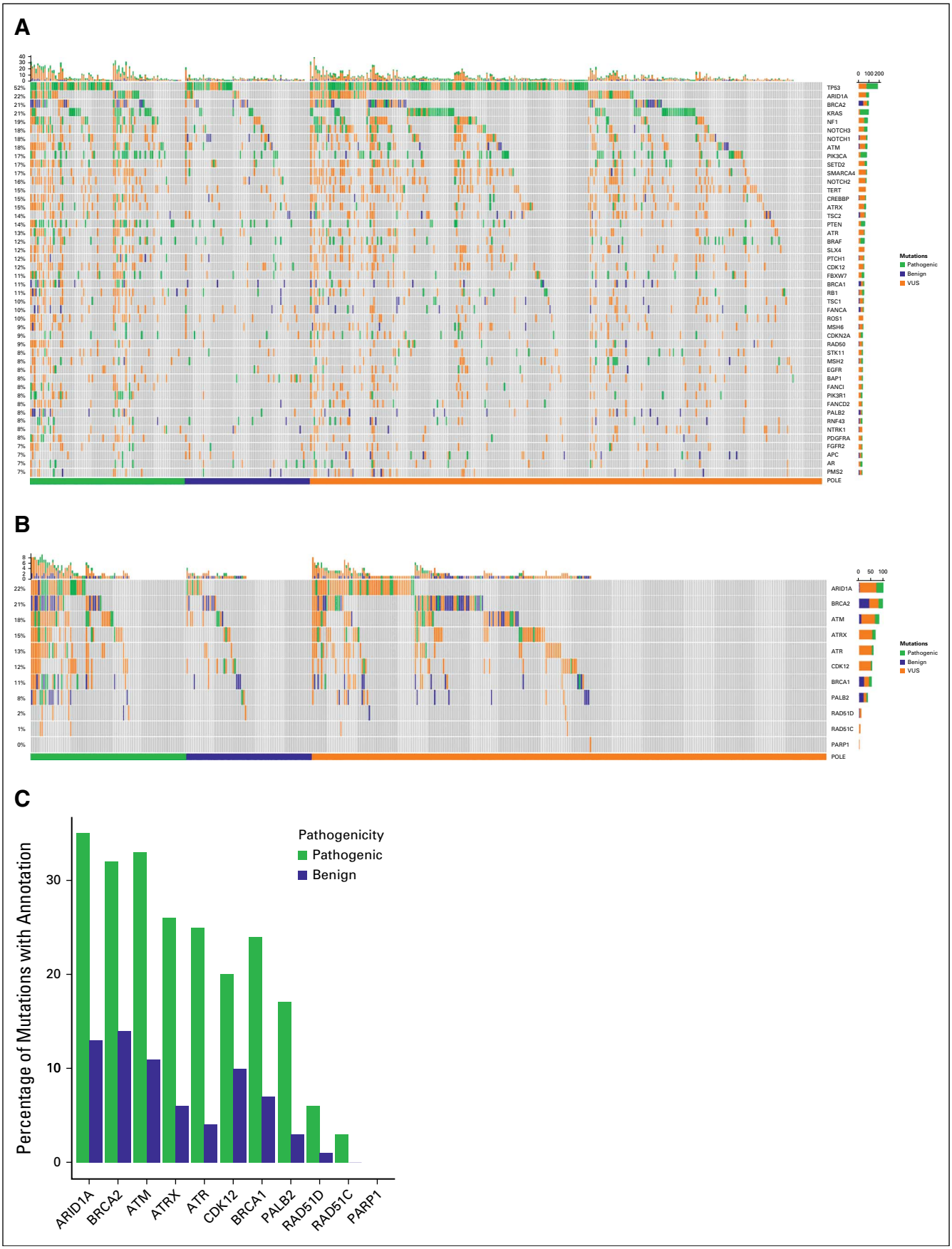

FIG 4. (continued on following page)

FIG 4. (Continued). (A) The landscape of co-occurring mutations in all patients with evaluable *POLE* mutations (n = 450). The most common co-occurring mutations included *TP53* (52%), *ARID1A* (22%), *BRCA2* (21%), *KRAS* (21%), *NF1* (19%), *NOTCH3* (18%), *NOTCH1* (18%), *ATM* (18%), *PIK3CA* (17%), *SETD2* (17%), and *SMARCA4* (17%). Mutations are classified as pathogenic (green), benign (blue), and VUS (orange). Patients with pathogenic *POLE* mutations had more comutations in DDR genes as shown in the (B) heat map and (C) bar graphs above. DDR, DNA damage response; VUS, variant of unknown significance.

including all patients with *POLE* mutations treated with IO-only regimens, 10 of 29 (34%) responses were found in tumors with < 10 co-occurring mutations. This provides preliminary evidence that these *POLE*-mutated tumors may be more immunogenic and/or responsive to ICI, irrespective of the number of comutations.

A major limitation of this study was that there was not a sufficient patient population to comprehensively evaluate the effect of *POLE* pathogenicity in historically immune-resistant tumors. Seventy-nine percent of patients included in our series had FDA-approved ICI indications based upon tumor or molecular subtype, limiting our ability to draw meaningful conclusions from the remaining immune-resistant patients. Nevertheless, responses observed across both immune-sensitive and immune-resistant patients indicate that *POLE* pathogenicity may indicate benefit to ICI therapy irrespective of traditional immunosensitivity of tumor histology or hypermutagenic status. These data highlight the need for further analysis in larger patient populations without FDA-approved ICI indications, including tumor types that have not previously been linked to *POLE* proofreading-defect tumorigenesis,[1,8] and prospective clinical testing. Another major limitation was that the majority (68%) of patients had a *POLE* VUS. In this series, 38% of patients with a VUS treated with an IO-only regimen had a response. Further work is necessary to better annotate these mutations to clarify potential immune sensitization differences within current VUSs within the context of intrinsic tumor immunosensitivity.

Data from an early phase trial of 16 patients with *POLE* mutations treated with nivolumab were recently presented.[38] At 84 days after treatment, ORR was 50% for patients with pathogenic mutations (n = 8) compared to 0% in patients with benign mutations (n = 5), consistent with our findings. A phase II trial is randomizing patients with *POLE*- or *POLD1*-mutated solid tumors to treatment with nivolumab plus ipilimumab versus nivolumab monotherapy (NCT03461952).[39] Another phase II trial is assessing treatment with the PD-L1 inhibitor durvalumab for patients with previously treated, metastatic, and MSI-high or *POLE*-mutated colorectal cancer (NCT03461952).[40] Additionally, the phase III POLEM study is randomly assigning patients with stage III MMR-deficient or *POLE* exonuclease domain mutant colon cancer to further adjuvant treatment with avelumab (*v* no intervention) after standard-of-care fluoropyrimidine-based chemotherapy (NCT03827044).[41] To improve the utility of *POLE* as a robust predictive biomarker of response to select patients for ICI therapy, on the basis of our data, these trials should also include assessments of the pathogenicity of *POLE* mutations, location within the gene, number of comutations, and associated tumor immune infiltration. Of note, a recent study found that nine of 11 tumor samples with *POLE* or *POLD1* mutations had high levels of tumor-infiltrating lymphocytes, although this must be placed into the context that these patients had an average TMB of 158 mutations/megabase.[37]

Among the total population, median OS was NR in patients with pathogenic mutations and was 3.4 years in patients with benign variants ($P < .001$). Previous data have shown that patients with either *POLE* or *POLD1* mutations have significantly longer OS compared with a wild-type population (2.8 *v* 1.5 years, respectively).[16] Our study is consistent with these data and adds to these findings by demonstrating the impact of *POLE* pathogenicity on survival outcomes.

To the best of our knowledge, this study is the first to provide a detailed description of the landscape of comutations in patients with *POLE*-mutated tumors. Interestingly, of patients who received an IO-only regimen, the number of comutations was not significantly associated with antitumor response. We also examined whether the number of comutations could predict for antitumor response in patients with *POLE* benign variants or VUS, but this analysis did not reveal significant results, indicating again that the number of mutations a tumor had was not the primary driver of response to ICI therapy. We also noted that tumors with pathogenic mutations were more likely to have comutations in DDR pathway genes and the number of unique co-occurring DDR mutations was associated with benefit to an IO-only regimen, but the sample size analyzed was small, limiting any formal conclusions to be made.

In summary, patients with pathogenic *POLE* mutations had improved antitumor responses and greater median PFS and OS with ICI therapy. These data should be interpreted within the context of a limited sample size and intrinsic tumor immune-sensitivity in most patients. Our findings nevertheless support further study in patients with advanced solid tumors harboring *POLE* pathogenic or VUS variants to further clarify the utility of *POLE* mutation location and pathogenicity as a predictive biomarker for ICI therapy.

AFFILIATIONS

[1]Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX
[2]The University of Texas Health Science Center at Houston, Houston, TX
[3]Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, TX
[4]Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX
[5]Department of Investigational Cancer Therapeutics (Phase I Clinical Trials Program), The University of Texas MD Anderson Cancer Center, Houston, TX
[6]Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX
[7]Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX
[8]Khalifa Institute for Personalized Cancer Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX
[9]The Institute for Applied Cancer Science, The University of Texas MD Anderson Cancer Center, Houston, TX

CORRESPONDING AUTHOR

Timothy A. Yap, MBBS, PhD, Investigational Cancer Therapeutics (Phase I Program), The University of Texas MD Anderson Cancer Center, 1400 Holcombe Blvd, Houston, TX 77030; e-mail: tyap@mdanderson.org.

PRIOR PRESENTATION

Some data included in this manuscript were presented as oral presentation at the 2020 Virtual ASCO Annual Meeting, May 29-31, 2020. Associated abstract: DOI: 10.1200/JCO.2020.38.15_suppl.3008 *Journal of Clinical Oncology* 38, 2020 (suppl 15; abstr 3008).

SUPPORT

Supported in part by The Cancer Prevention and Research Institute of Texas (award no. RP150535), the Sheikh Khalifa Bin Zayed Al Nahyan Institute for Personalized Cancer Therapy, National Center for Advancing Translational Sciences Grant No. UL1 TR000371 (Center for Clinical and Translational Sciences), and the MD Anderson Cancer Center Support Grant No. P30 CA016672. This work was supported by the National Cancer Institute (K22 CA234406 to J.P.S., P50CA221707 and P50CA127001 to Y.Y., and U01CA247760 to K.C.) and the Cancer Prevention & Research Institute of Texas (RP180248 to K.C. and RR180035 to J.P.S.; J.P.S. is a CPRIT Scholar in Cancer Research).

DATA SHARING STATEMENT

A data sharing statement provided by the authors is available with this article at DOI https://doi.org/10.1200/PO.21.00267.

AUTHOR CONTRIBUTIONS

Conception and design: Benjamin Garmezy, Ying Yuan, Timothy A. Yap
Financial support: Kenna R. Shaw, Ken Chen
Administrative support: Kenna R. Shaw, Funda Meric-Bernstam, Ken Chen, Timothy A. Yap
Provision of study materials or patients: Kyaw Z. Thein, Wanlin Wang, Kenna R. Shaw, Jordi Rodon, Funda Meric-Bernstam, Timothy A. Yap
Collection and assembly of data: Benjamin Garmezy, Jinesh Gheeya, Kyaw Z. Thein, Wanlin Wang, Kenna R. Shaw, Funda Meric-Bernstam, Timothy A. Yap
Data analysis and interpretation: Benjamin Garmezy, Jinesh Gheeya, Heather Y. Lin, Yuefan Huang, Taebeom Kim, Xianli Jiang, Kyaw Z. Thein, Patrick G. Pilié, Fadl Zeineddine, Jordi Rodon, John-Paul Shen, Ying Yuan, Funda Meric-Bernstam, Ken Chen, Timothy A. Yap
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

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

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

Benjamin Garmezy
Uncompensated Relationships: AVEO (Inst)

Xianli Jiang
Patents, Royalties, Other Intellectual Property: I am in the process of a patent application, which is nonrelevant to this study. The application is for United States Letters Patent, Serial No. 63/015,317

Patrick G. Pilié
Consulting or Advisory Role: Novartis
Patents, Royalties, Other Intellectual Property: Patent pending-biomarker

Kenna R. Shaw
Consulting or Advisory Role: Guidepoint Global
Research Funding: Guardant Health (Inst), Tempus (Inst), Philips Healthcare (Inst)

Jordi Rodon
Consulting or Advisory Role: Peptomyc, Kelun, Merck Sharp & Dohme, Spectrum Pharmaceuticals, Pfizer, Roche/Genentech, Ellipses Pharma, Ionctura, Novartis, Lilly, Orion, Servier, Molecular Partners, NovellusDx, Certara, Bayer, KisoJi Biotechnology
Research Funding: Novartis, Bayer, Spectrum Pharmaceuticals, Tocagen, Symphogen, BioAtla, Pfizer, Genmab, CytomX Therapeutics, Kelun, Takeda/Millennium, GlaxoSmithKline, Ipsen, Blueprint Medicines
Travel, Accommodations, Expenses: ESMO, Department of Defense, Louisiana State University, Huntsman Cancer Institute, Cancer Core Europe, Karolinska Cancer Institute, King Abdullah International Medical Research Center, Molecular Partners, Merck Sharp & Dohme, Kelun Pharmaceuticals/Klus Pharma, Bayer, WIN Consortium, Janssen
Other Relationship: European Journal of Cancer, Chinese University of Hong Kong, SOLTI, Elsevier, GlaxoSmithKline, Vall d'Hebron University Hospital Institute of Oncology, VHIO/Ministero De Empleo Y Seguridad Social

John-Paul Shen
Stock and Other Ownership Interests: Agios, Syndax
Consulting or Advisory Role: Engine Biosciences
Research Funding: Celsius Therapeutics

Ying Yuan
Honoraria: Ono Pharmaceutical
Consulting or Advisory Role: Boehringer Ingelheim, Amgen, AbbVie, Servier, Starpax Medical, Vertex, MicuRx Pharmaceuticals, BeyondSpring Pharmaceuticals, Bristol Myers Squibb/Celgene/Juno

Funda Meric-Bernstam
Employment: MD Anderson Cancer Center
Honoraria: Rutgers Cancer Institute of New Jersey
Consulting or Advisory Role: Samsung Bioepis, Xencor, Debiopharm Group, Silverback Therapeutics, IBM Watson Health, Roche, PACT Pharma, eFFECTOR Therapeutics, Kolon Life Sciences, Tyra Biosciences, Zymeworks, Puma Biotechnology, Zentalis, Alkermes, Infinity Pharmaceuticals, AbbVie, Black Diamond Therapeutics, Eisai, OnCusp Therapeutics, Lengo Therapeutics, Tallac Therapeutics, Karyopharm Therapeutics, Biovica

Speakers' Bureau: Chugai Pharma
Research Funding: Novartis (Inst), AstraZeneca (Inst), Taiho Pharmaceutical (Inst), Genentech (Inst), Calithera Biosciences (Inst), Debiopharm Group (Inst), Bayer (Inst), Aileron Therapeutics (Inst), PUMA Biotechnology (Inst), CytomX Therapeutics (Inst), Jounce Therapeutics (Inst), Zymeworks (Inst), Curis (Inst), Pfizer (Inst), eFFECTOR Therapeutics (Inst), AbbVie (Inst), Boehringer Ingelheim, Guardant Health (Inst), Daiichi Sankyo (Inst), GlaxoSmithKline (Inst), Seattle Genetics (Inst), Klus Pharma (Inst), Takeda (Inst)
Travel, Accommodations, Expenses: Beth Israel Deaconess Medical Center

Timothy A. Yap
Consulting or Advisory Role: Pfizer, EMD Serono, Clovis Oncology, Ignyta, AstraZeneca, Atrin Pharmaceuticals, Aduro Biotech, Merck, Almac Diagnstics, Bayer, Bristol Myers Squibb, Calithera Biosciences, Cybrexa Therapeutics, Janssen, Roche, Seattle Genetics, Axiom Biotechnologies, F-Star, Guidepoint Global, I-Mab, Repare Therapeutics, Rubius Therapeutics, Schrodinger, Varian Medical Systems, Zai Lab
Research Funding: AstraZeneca (Inst), Vertex (Inst), Pfizer (Inst), Bayer (Inst), Tesaro (Inst), Jounce Therapeutics (Inst), Seattle Genetics (Inst), Kyowa Hakko Kirin (Inst), Constellation Pharmaceuticals (Inst), Lilly (Inst), Artios (Inst), Clovis Oncology (Inst), Cyteir (Inst), EMD Serono (Inst), Forbius (Inst), F-Star (Inst), GlaxoSmithKline (Inst), Genentech (Inst), ImmuneSensor Therapeutics (Inst), Ipsen (Inst), Karyopharm Therapeutics (Inst), Merck (Inst), Novartis (Inst), Ribon Therapeutics (Inst), Regeneron (Inst), Repare Therapeutics (Inst), Sanofi (Inst), Scholar Rock (Inst)

No other potential conflicts of interest were reported.

REFERENCES

1. Rayner E, van Gool IC, Palles C, et al: A panoply of errors: Polymerase proofreading domain mutations in cancer. Nat Rev Cancer 16:71-81, 2016
2. Albertson TM, Ogawa M, Bugni JM, et al: DNA polymerase epsilon and delta proofreading suppress discrete mutator and cancer phenotypes in mice. Proc Natl Acad Sci USA 106:17101-17104, 2009
3. Church DN, Briggs SE, Palles C, et al: DNA polymerase ε and δ exonuclease domain mutations in endometrial cancer. Hum Mol Genet 22:2820-2828, 2013
4. Palles C, Cazier JB, Howarth KM, et al: Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. Nat Genet 45:136-144, 2013
5. Shlien A, Campbell BB, de Borja R, et al: Combined hereditary and somatic mutations of replication error repair genes result in rapid onset of ultra-hypermutated cancers. Nat Genet 47:257-262, 2015
6. Campbell BB, Light N, Fabrizio D, et al: Comprehensive analysis of hypermutation in human cancer. Cell 171:1042-1056.e10, 2017
7. Hatakeyama K, Ohshima K, Nagashima T, et al: Molecular profiling and sequential somatic mutation shift in hypermutator tumours harbouring POLE mutations. Sci Rep 8:8700, 2018
8. Park VS, Pursell ZF: POLE proofreading defects: Contributions to mutagenesis and cancer. DNA Repair (Amst) 76:50-59, 2019
9. Havel JJ, Chowell D, Chan TA: The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer 19:133-150, 2019
10. Rizvi NA, Hellmann MD, Snyder A, et al: Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 348:124-128, 2015
11. Goodman AM, Kato S, Bazhenova L, et al: Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther 16:2598-2608, 2017
12. Le DT, Durham JN, Smith KN, et al: Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 357:409-413, 2017
13. Le DT, Uram JN, Wang H, et al: PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 372:2509-2520, 2015
14. Guo L, Wei R, Lin Y, et al: Clinical and recent patents applications of PD-1/PD-L1 targeting immunotherapy in cancer treatment-current progress, strategy, and future perspective. Front Immunol 11:1508, 2020
15. Davis AA, Patel VG: The role of PD-L1 expression as a predictive biomarker: An analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. J Immunother Cancer 7:278, 2019
16. Wang F, Zhao Q, Wang Y-N, et al: Evaluation of POLE and POLD1 mutations as biomarkers for immunotherapy outcomes across multiple cancer types. JAMA Oncol 5:1504-1506, 2019
17. Roszik J, Haydu LE, Hess KR, et al: Novel algorithmic approach predicts tumor mutation load and correlates with immunotherapy clinical outcomes using a defined gene mutation set. BMC Med 14:168, 2016
18. Chalmers ZR, Connelly CF, Fabrizio D, et al: Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 9:34, 2017
19. Li Q, Wang K: InterVar: Clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. Am J Hum Genet 100:267-280, 2017
20. Landrum MJ, Lee JM, Benson M, et al: ClinVar: Improving access to variant interpretations and supporting evidence. Nucleic Acids Res 46:D1062-D1067, 2018
21. Ahn SM, Ansari AA, Kim J, et al: The somatic POLE P286R mutation defines a unique subclass of colorectal cancer featuring hypermutation, representing a potential genomic biomarker for immunotherapy. Oncotarget 7:68638-68649, 2016
22. Xing X, Kane DP, Bulock CR, et al: A recurrent cancer-associated substitution in DNA polymerase epsilon produces a hyperactive enzyme. Nat Commun 10:374, 2019
23. Guenther M, Veninga V, Kumbrink J, et al: POLE gene hotspot mutations in advanced pancreatic cancer. J Cancer Res Clin Oncol 144:2161-2166, 2018
24. Imboden S, Nastic D, Ghaderi M, et al: Phenotype of POLE-mutated endometrial cancer. PLoS One 14:e0214318, 2019
25. Davila JI, Chanana P, Sarangi V, et al: Frequent POLE-driven hypermutation in ovarian endometrioid cancer revealed by mutational signatures in RNA sequencing. BMC Med Genomics 14:165, 2021
26. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958
27. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163-170, 1966
28. Cox DR: Regression models and life-tables. J R Stat Soc Series B Methodol 34:187-202, 1972
29. Jumper J, Evans R, Pritzel A, et al: Highly accurate protein structure prediction with AlphaFold. Nature 596:583-589, 2021
30. Hogg M, Osterman P, Bylund GO, et al: Structural basis for processive DNA synthesis by yeast DNA polymerase varepsilon. Nat Struct Mol Biol 21:49-55, 2014
31. Henninger EE, Pursell ZF: DNA polymerase epsilon and its roles in genome stability. IUBMB Life 66:339-351, 2014
32. Yuza K, Nagahashi M, Watanabe S, et al: Hypermutation and microsatellite instability in gastrointestinal cancers. Oncotarget 8:112103-112115, 2017

33. Shinbrot E, Henninger EE, Weinhold N, et al: Exonuclease mutations in DNA polymerase epsilon reveal replication strand specific mutation patterns and human origins of replication. Genome Res 24:1740-1750, 2014
34. Fang H, Barbour JA, Poulos RC, et al: Mutational processes of distinct POLE exonuclease domain mutants drive an enrichment of a specific TP53 mutation in colorectal cancer. PLoS Genet 16:e1008572, 2020
35. Temko D, Van Gool IC, Rayner E, et al: Somatic POLE exonuclease domain mutations are early events in sporadic endometrial and colorectal carcinogenesis, determining driver mutational landscape, clonal neoantigen burden and immune response. J Pathol 245:283-296, 2018
36. Billingsley CC, Cohn DE, Mutch DG, et al: Prognostic significance of POLE exonuclease domain mutations in high-grade endometrioid endometrial cancer on survival and recurrence: A subanalysis. Int J Gynecol Cancer 26:933-938, 2016
37. Keshinro A, Vanderbilt C, Kim JK, et al: Tumor-infiltrating lymphocytes, tumor mutational burden, and genetic alterations in microsatellite unstable, microsatellite stable, or mutant POLE/POLD1 colon cancer. JCO Precis Oncol 5:817-826, 2021
38. Rousseau B: High activity of nivolumab in patients with pathogenic exonucleasic domain POLE (edPOLE) mutated Mismatch Repair proficient (MMRp) advanced tumours. Ann Oncol 31:S462-S504, 2020 (suppl 4; abstr 5260)
39. Rizvi N, Tang P, Bhardwaj N, et al: Nivolumab +/- ipilimumab in patients with hypermutated cancers detected in blood: NIMBLE. Cancer Immunol Res 7:B207, 2019 (abstr B207)
40. Durvalumab for MSI-H or POLE Mutated Metastatic Colorectal Cancer (NCT03435107), 2020, https://clinicaltrials.gov/ct2/show/NCT03435107
41. Lau D, Kalaitzaki E, Church DN, et al: Rationale and design of the POLEM trial: Avelumab plus fluoropyrimidine-based chemotherapy as adjuvant treatment for stage III mismatch repair deficient or POLE exonuclease domain mutant colon cancer: A phase III randomised study. ESMO Open 5:e000638, 2020
42. den Dunnen JT, Antonarakis SE: Mutation nomenclature extensions and suggestions to describe complex mutations: A discussion. Hum Mutat 15:7-12, 2000
43. Woolson RFC, William R: Statistical Methods for the Analysis of Biomedical Data (ed 2). New York, NY, John Wiley & Sons, 2002

APPENDIX 1. SUPPLEMENTAL MATERIAL

Methods

Clinical data were collected from the MD Anderson Cancer Center electronic medical record between June 18, 2019, and April 1, 2020, and included basic demographic parameters, tumor stage, pathology, and treatment response, as well as survival data. Next-generation sequencing data were collected on April 29, 2019, and again on January 14, 2020, from five panels: FoundationOne (Foundation Medicine, Cambridge, MA), FoundationOne CDx (Foundation Medicine, Cambridge, MA), STGA-DNA 2018 (MD Anderson Cancer Center, Houston, TX), Tempus xT (Tempus, Chicago, IL), and MI Profile (Caris Life Sciences, Irving, TX). The five panels included the following numbers of genes: FoundationOne (416), FoundationOne CDx (324), STGA-DNA (147), Tempus xT (648), and MI Profile (260). Next-generation sequencing data collected included gene symbols/names, protein changes, Human Genome Variation Society (HGVS) expressions for transcripts and corresponding proteins,[42] and complementary DNA changes. Initial annotation of *POLE* mutation pathogenicity was conducted via InterVar[19] and ClinVar.[20] Review of peer-reviewed published literature through August 15, 2021, further identified known pathogenic *POLE* hotspot mutations, including *P286R*, *V411L*, *V411R*, *V411M*, *S297F*, *A456P*, and *S459F*.[21-25] Patients with these mutations that had previously been labeled as having a variant of unknown significance were updated to pathogenic.

In the analyses of progression-free survival (PFS), overall survival (OS) and time on immunotherapy of the patients receiving immunotherapy (IO), PFS was defined as the time from treatment initiation to the time of progression or death, whichever occurred first; OS was defined as the time from treatment initiation to death; and time on immunotherapy was defined as the time from first IO initiation to the end of first IO treatment or last follow-up. For the analysis of OS with both the patients receiving IO and those who did not receive IO included, OS was defined as the time of diagnosis to death. For events that have not occurred by the time of data analysis, times were censored at the last contact at which the patient was known to be progression-free for PFS or the last time the patient was known to be alive for OS.

Additional statistical methods included the following: Wilcoxon rank-sum or Kruskal-Wallis tests were used to detect differences for continuous variables between groups.[43] A two-proportion Z-test was used to compare the percentage of patients with a DNA damage response comutation among patients with pathogenic versus benign *POLE* mutations. The Fisher's exact test was used to determine the association between response and comutation load. Odds ratio estimation, confidence intervals, and *P* value were calculated in R package Epitools.[26] The *P* value was adjusted for multiple hypotheses testing using the Bonferroni-Holm method.

TABLE A1. Additional Data for Patients Receiving Immunotherapy

Patient No.	Age at Diagnosis (years)	Sex	Cancer Type	*POLE* Pathogenic Status	Pole Mutation Alteration[a]	Pole Mutation cDNA Change	Mutation in Exonuclease Domain[b]	Variant Type	Number of Comutations in Patient Tumor	NGS Panel	Genes Tested in Panel[c]	MSI Status	Treatment[d]	Response
1	59	F	Uterine	Pathogenic	POLE_S459F		Y	Missense	15	FoundationOne	416	Stable	Dual IO	PD
2	24	M	Colorectal	Pathogenic	POLE_P286R	c.857C>G	Y	Missense	160	FoundationOne CDx	324	Stable	Dual IO	SD
3	71	M	Colorectal	Pathogenic	POLE_P286R POLE_R114* POLE_V2152M	c.857C>G c.340C>T c.6454G>A	Y	Missense Nonsense Missense	77	STGA-DNA 2018	147	Stable	Mono IO	SD
4	33	M	Colorectal	Pathogenic	POLE_E537D POLE_P286R Y2265*	c.1611G>T c.857C>G c.6795C>A	Y	Missense Missense Nonsense	83	STGA-DNA 2018	147	Stable	Mono IO	PR
5	54	M	NSCLC	Pathogenic	POLE_c.286-1G>T	c.286-1G>T	Y	Splice	9	STGA-DNA 2018	147	Stable	Combo	PR
6	41	M	Colorectal	Pathogenic	POLE_ R579HNonsense POLE_V411L	c.1736G>ANonsense c.1231G>T	Y	MissenseNonsense Missense	80	STGA-DNA 2018	147	Stable	Mono IO	SD
7	53	M	Colorectal	Pathogenic	POLE_S459F	c.1376C>T	Y	Missense	27	STGA-DNA 2018	147	Stable	Mono IO	PR
8	71	M	Melanoma	Likely pathogenic	POLE_ P286LNonsense POLE_V1972M	c.857C>TNonsense c.5914G>A	Y	Missense	67	STGA-DNA 2018	147	—	Dual IO	PR
9	72	M	HNSCC	Likely pathogenic	POLE_P436L	c.1307C>T	Y	Missense	40	STGA-DNA 2018	147	Stable	Mono IO	SD
10	45	F	Cholangiocarcinoma	Likely benign	POLE_R446Q	c.1337G>A	Y	Missense	15	FoundationOne	416	Stable	Combo	PD
11	42	F	Breast (HR+)	Likely benign	POLE_R446Q		Y	Missense	13	Tempus xT Assay	648	—	Combo	PD
12	80	M	Melanoma	VUS	POLE_P436F	c.1306_1307delinsTT	Y	Missense	33	STGA-DNA 2018	147	Stable	Mono IO	PD
13	51	F	Breast (TNBC)	VUS	POLE_I403M	c.1209C>G	Y	Missense	12	STGA-DNA 2018	147	—	Combo	CR
14	56	M	NSCLC	VUS	POLE_S297P	c.889T>C	Y	Missense	1	STGA-DNA 2018	147	—	Combo	PD
15	70	F	HNSCC	VUS	POLE_Q390H	c.1170G>C	Y	Missense	6	STGA-DNA 2018	147	—	Combo	PR
16	76	F	Merkel cell carcinoma	Pathogenic	POLE_W671*	c.2013G>A	N	Nonsense	14	STGA-DNA 2018	147	—	Mono IO	PD
17	71	F	Neuroendocrine carcinoma	Pathogenic	POLE_R260*	c.778C>T	N	Nonsense	16	STGA-DNA 2018	147	Stable	Combo	PD
18	69	F	NSCLC	Pathogenic	POLE_ c.1686+1G>T	c.1686+1G>T	N	Splice	6	STGA-DNA 2018	147	—	Mono IO	PR
19	59	M	Melanoma	Likely pathogenic	POLE_P324S	c.970C>T	N	Missense	8	STGA-DNA 2018	147	Stable	Dual IO	PR
20	50	F	Breast (HR+, HER2–)	Likely pathogenic	POLE_F699fs*11	c.2091_2092insC	N	Frameshift	16	FoundationOne CDx	324	—	Combo	PR
21	76	M	Melanoma	Likely pathogenic	POLE_P943S POLE_R1082fs*24	c.2827C>T c.3244del	N	Missense Frameshift	12	STGA-DNA 2018	147	—	Mono IO	SD
22	27	F	NSCLC	Likely pathogenic	POLE_V1887fs*36		N	Frameshift	42	FoundationOne	416	Stable	Mono IO	PR
23	49	M	Colorectal	Likely pathogenic	POLE_S643fs*149	c.1926del	N	Frameshift	19	STGA-DNA 2018	147	High	Mono IO	PD
24	81	M	NSCLC	Likely pathogenic	POLE_E740fs*52	c.2218del	N	Frameshift	7	STGA-DNA 2018	147	—	Mono IO	PR
25	65	M	Colorectal	Likely pathogenic	POLE_R1364fs*5 POLE_R1823C	c.4090del c.5467C>T	N	Frameshift Missense	16	STGA-DNA 2018	147	High	Dual IO	SD

(Continued on following page)

TABLE A1. Additional Data for Patients Receiving Immunotherapy (Continued)

Patient No.	Age at Diagnosis (years)	Sex	Cancer Type	*POLE* Pathogenic Status	Pole Mutation Alteration[a]	Pole Mutation cDNA Change	Mutation in Exonuclease Domain[b]	Variant Type	Number of Comutations in Patient Tumor	NGS Panel	Genes Tested in Panel[c]	MSI Status	Treatment[d]	Response
26	57	M	NSCLC	Likely pathogenic	POLE_A183S	c.547G>T	N	Missense	6	STGA-DNA 2018	147	Stable	Dual IO	PR
27	67	M	Cancer of unknown primary	Likely pathogenic	POLE_ c.1106+1G>T POLE_G1860W	c.1106+1G>T c.5578G>T	N	Splice Missense	16	STGA-DNA 2018	147	Stable	Combo	PR
28	69	M	NSCLC	Benign	POLE_P697A	c.2089C>G	N	Missense	14	FoundationOne	416	—	Mono IO	PD
29	73	M	NSCLC	Benign	POLE_R1508H	c.4523G>A	N	Missense	18	FoundationOne	416	—	Mono IO	PD
30	64	F	Breast (TNBC)	Benign	POLE_P99L	c.296C>T	N	Missense	31	FoundationOne	416	Stable	Mono IO	SD
31	71	M	Cholangiocarcinoma	Benign	POLE_G6R	c.16g>C	N	Missense	13	FoundationOne	416	—	Combo	PD
32	67	M	HNSCC	Benign	POLE_E2140K	c.6418G>A	N	Missense	6	STGA-DNA 2018	147	—	Mono IO	SD
33	72	M	NSCLC	Benign	POLE_G6R		N	Missense	23	FoundationOne	416	Stable	Combo	SD
34	58	F	Glioblastoma	Benign	POLE_R1508H	c.4523G>A	N	Missense	45	FoundationOne	416	Stable	Mono IO	PD
35	61	M	Pancreas	Benign	POLE_R1508H		N	Missense	45	FoundationOne	416	Stable	Combo	PR
36	47	F	Colorectal	Benign	POLE_G6R		N	Missense	19	FoundationOne	416	Stable	Combo	SD
37	43	F	Breast (HR+)	Benign	POLE_R1508H		N	Missense	17	FoundationOne CDx	324	Stable	Combo	PR
38	58	M	Prostate	Likely benign	POLE_F837S	c.2510T>C	N	Missense	17	FoundationOne	416	Stable	Mono IO	PD
39	51	M	NSCLC	Likely benign	POLE_S1353G	c.4057A>G	N	Missense	30	FoundationOne	416	—	Mono IO	PD
40	66	F	Uterine	Likely benign	POLE_P697R	c.2090C>G	N	Missense	8	STGA-DNA 2018	147	Stable	Dual IO	PD
41	34	M	RCC: clear cell	Likely benign	POLE_F837S		N	Missense	11	FoundationOne CDx	324	Stable	Dual IO	PD
42	68	M	Prostate adenocarcinoma	Likely benign	POLE_G1216S	c.3646G>A	N	Missense	10	STGA-DNA 2018	147	Stable	Combo	PD
43	43	M	Colorectal	Likely benign	POLE_A512T	c.1534G>A	N	Missense	17	STGA-DNA 2018	147	High	Mono IO	SD
44	78	M	NSCLC	VUS	POLE_R231C	c.691C>T	N	Missense	10	STGA-DNA 2018	147	Stable	Combo	PD
45	26	F	Glioblastoma	VUS	POLE_A1150T	c.3448G>A	N	Missense	30	STGA-DNA 2018	147	Stable	Combo	PD
46	69	M	Bladder	VUS	POLE_R93I	c.278G>T	N	Missense	15	STGA-DNA 2018	147	Stable	Mono IO	SD
47	49	F	Breast (HR+, HER2–)	VUS	POLE_R231C		N	Missense	20	FoundationOne	416	Stable	Combo	PD
48	32	F	Breast (TNBC)	VUS	POLE_G2266S		N	Missense	18	FoundationOne	416	Stable	Combo	PD
49	90	M	Urothelial: upper tract	VUS	POLE_V540I	c.1618G>A	N	Missense	34	STGA-DNA 2018	147	—	Mono IO	PR
50	53	M	NSCLC	VUS	POLE_R1136Q	c.3407G>A	N	Missense	21	FoundationOne	416	—	Mono IO	PR
51	49	F	Colon: mucinous adenocarcinoma	VUS	POLE_A1510V	c.4529_4530delinsTG	N	Missense	10	STGA-DNA 2018	147	High	Mono IO	SD
52	63	M	NSCLC	VUS	POLE_R37Q	c.110G>A	N	Missense	26	FoundationOne	416	—	Mono IO	PR
53	64	F	NSCLC	VUS	POLE_A724V	c.2171C>T	N	Missense	17	FoundationOne	416	—	Mono IO	PD
54	64	M	Melanoma	VUS	POLE_P557L	c.1670C>T	N	Missense	5	STGA-DNA 2018	147	—	Mono IO	PR
55	56	F	NSCLC	VUS	POLE_Q1293H	c.3879G>T	N	Missense	9	STGA-DNA 2018	147	—	Combo	PR
56	78	F	Bladder: urothelial	VUS	POLE_R1193T	c.3578G>C	N	Missense	8	STGA-DNA 2018	147	—	Mono IO	PR
57	58	F	Melanoma	VUS	POLE_R1556W	c.4666C>T	N	Missense	7	STGA-DNA 2018	147	—	Mono IO	CR
58	62	M	HNSCC	VUS	POLE_G2226K	c.6676_6677GG>AA	N	Missense	203	FoundationOne	416	Stable	Mono IO	PD

(Continued on following page)

TABLE A1. Additional Data for Patients Receiving Immunotherapy (Continued)

Patient No.	Age at Diagnosis (years)	Sex	Cancer Type	*POLE* Pathogenic Status	Pole Mutation Alteration[a]	Pole Mutation cDNA Change	Mutation in Exonuclease Domain[b]	Variant Type	Number of Comutations in Patient Tumor	NGS Panel	Genes Tested in Panel[c]	MSI Status	Treatment[d]	Response
59	64	M	Bladder: urothelial	VUS	POLE_K1276M POLE_P696L	c.3827A>T c.2087C>T	N	Missense Missense	6	STGA-DNA 2018	147	—	Mono IO	SD
60	72	M	Melanoma	VUS	POLE_P1210L	c.3629C>T	N	Missense	25	STGA-DNA 2018	147	—	Combo	CR
61	84	M	NSCLC	VUS	POLE_L938F	c.2812C>T	N	Missense	11	STGA-DNA 2018	147	—	Mono IO	CR
62	73	F	NSCLC	VUS	POLE_E2275*	c.6822_6823delinsTT	N	Nonsense	10	STGA-DNA 2018	147	—	Combo	CR
63	31	M	Colorectal	VUS	POLE_S1644L POLE_T2049A	c.4931C>T c.6145A>G	N	Missense Missense	18	STGA-DNA 2018	147	High	Mono IO	PR
64	54	M	Melanoma	VUS	POLE_R1324C	c.3969_3970CC>AT	N	Missense	23	FoundationOne CDx	324	Stable	Mono IO	PD
65	64	M	Liposarcoma	VUS	POLE_G702R	c.2104G>A	N	Missense	3	STGA-DNA 2018	147	—	Mono IO	PD
66	50	M	Gallbladder	VUS	c.5353A>G	chr12:133218258	N	Missense	16	POLE_T1785A	416	—	Dual IO	PR
67	66	M	NSCLC	VUS	POLE_R2225C	c.6673C>T	N	Missense	8	STGA-DNA 2018	147	—	Dual IO	PR
68	52	F	Glioblastoma	VUS	POLE_N518S	c.1553A>G	N	Missense	23	STGA-DNA 2018	147	—	Mono IO	PD
69	61	F	Breast (HR+, HER2–)	VUS	POLE_D1623Y	c.4867G>T	N	Missense	12	STGA-DNA 2018	147	—	Dual IO	PR
70	60	M	Prostate adenocarcinoma	VUS	POLE_T528M		N	Missense	24	MI Profile	260	Stable	Combo	PD
71	28	M	Neuroendocrine carcinoma	VUS	POLE_I635T	c.1904T>C	N	Missense	2	STGA-DNA 2018	147	—	Combo	PD
72	49	M	Chordoma	VUS	POLE_N1971I	c.5912A>T	N	Missense	2	STGA-DNA 2018	147	—	Mono IO	PD
73	75	F	Melanoma	VUS	POLE_G963S	c.2887G>A	N	Missense	6	STGA-DNA 2018	147	—	Mono IO	PD
74	79	M	Urothelial: small cell	VUS	POLE_Y100C	c.299A>G	N	Missense	18	STGA-DNA 2018	147	Stable	Mono IO	SD
75	13	M	Glioblastoma	VUS	POLE_R1308W POLE_V755M	c.3922C>T c.2263G>A	N	Missense Missense	19	STGA-DNA 2018	147	High	Combo	PD
76	66	M	Colorectal	VUS	POLE_R1570Q	c.4709G>A	N	Missense	10	STGA-DNA 2018	147	High	Combo	PR
77	58	M	HNSCC	VUS	POLE_E1035K	c.3103G>A	N	Missense	4	STGA-DNA 2018	147	—	Mono IO	PD
78	63	M	Colorectal	VUS	POLE_R1630Q		N	Missense	15	FoundationOne	416	Stable	Mono IO	PD
79	79	M	Urothelial	VUS	POLE_A1101L	c.3301_3302delinsTT	N	Missense	11	STGA-DNA 2018	147	Stable	Mono IO	CR
80	57	M	Colon adenocarcinoma	VUS	POLE_T1462A	c.4384A>G	N	Missense	11	STGA-DNA 2018	147	High	Mono IO	PD
81	78	M	Appendix	VUS	POLE_T1052M	c.3155C>T	N	Missense	13	STGA-DNA 2018	147	High	Mono IO	SD
82	54	F	Colorectal	VUS	POLE_R1284W	c.3850C>T	N	Missense	16	STGA-DNA 2018	147	High	Combo	CR
83	41	M	Colorectal	VUS	POLE_R150Q	c.449G>A	N	Missense	28	STGA-DNA 2018	147	High	Mono IO	PR
84	62	F	NSCLC	VUS	POLE_H532P	c.1595A>C	N	Missense	6	STGA-DNA 2018	147	Stable	Mono IO	PD
85	72	F	Pancreas	VUS	POLE_A1323V		N	Missense	18	FoundationOne	416	—	Dual IO	PD
86	50	M	Colorectal	VUS	POLE_K1058Q	c.3172A>C	N	Missense	8	STGA-DNA 2018	147	Stable	Combo	SD
87	36	F	Melanoma	VUS	POLE_R1364C POLE_S27F	c.4090C>T c.80C>T	N	Missense Missense	13	STGA-DNA 2018	147	—	Mono IO	PD
88	66	M	Melanoma	VUS	POLE_L1235F	c.3703C>T	N	Missense	23	STGA-DNA 2018	147	—	Dual IO	PR
89	54	M	Melanoma	VUS	POLE_S2093F	c.6278C>T	N	Missense	18	STGA-DNA 2018	147	—	Mono IO	PR
90	57	F	Melanoma	VUS	POLE_M1406K	c.4217T>A	N	Missense	3	STGA-DNA 2018	147	—	Dual IO	SD
91	70	M	NSCLC	VUS	POLE_D1165N	c.3493G>A	N	Missense	5	STGA-DNA 2018	147	—	Dual IO	PD

(Continued on following page)

TABLE A1. Additional Data for Patients Receiving Immunotherapy (Continued)

Patient No.	Age at Diagnosis (years)	Sex	Cancer Type	*POLE* Pathogenic Status	Pole Mutation Alteration[a]	Pole Mutation cDNA Change	Mutation in Exonuclease Domain[b]	Variant Type	Number of Comutations in Patient Tumor	NGS Panel	Genes Tested in Panel[c]	MSI Status	Treatment[d]	Response
92	78	M	Mixed hepatocellular/ cholangiocarcinoma	VUS	POLE_H511D	c.1531C>G	N	Missense	7	STGA-DNA 2018	147	Stable	Combo	PD
93	77	M	Small bowel	VUS	POLE_I1633T	c.4898T>C	N	Missense	7	STGA-DNA 2018	147	Stable	Combo	PD
94	63	M	NSCLC	VUS	POLE_R1858C	c.5572C>T	N	Missense	8	STGA-DNA 2018	147	Stable	Mono IO	PD
95	63	M	Cholangiocarcinoma	VUS	POLE_I484V	c.1450A>G	N	Missense	9	STGA-DNA 2018	147	High	Mono IO	PD
96	62	M	NSCLC	VUS	POLE_K1182N	c.3546G>C	N	Missense	8	STGA-DNA 2018	147	Stable	Mono IO	PD
97	69	M	HNSCC	VUS	POLE_E1377M	c.4129_4130delinsAT	N	Missense	16	STGA-DNA 2018	147	—	Combo	PD
98	70	M	Colorectal	VUS	POLE_R685Q	c.2054G>A	N	Missense	6	STGA-DNA 2018	147	High	Mono IO	SD
99	66	M	Bladder: urothelial (+ angiosarcoma)	VUS	POLE_E18K	c.52G>A	N	Missense	22	STGA-DNA 2018	147	—	Combo	SD
100	82	M	Colorectal	VUS	POLE_R1579H	c.4736G>A	N	Missense	6	STGA-DNA 2018	147	High	Mono IO	PD
101	36	F	NSCLC	VUS	POLE_E1949Q	c.5845G>C	N	Missense	7	STGA-DNA 2018	147	Stable	Combo	SD
102	50	M	HNSCC	VUS	POLE_R639C	c.1915C>T	N	Missense	6	STGA-DNA 2018	147	—	Combo	PR
103	62	M	Esophageal adenocarcinoma	VUS	POLE_R1630W	c.4888C>T	N	Missense	5	STGA-DNA 2018	147	High	Mono IO	CR
104	47	M	Gastric	VUS	POLE_G1262E	c.3785G>A	N	Missense	6	STGA-DNA 2018	147	Stable	Combo	PD
105	73	M	Melanoma	VUS	POLE_E1085K	c.3253G>A	N	Missense	10	STGA-DNA 2018	147	Stable	Mono IO	PR
106	67	M	NSCLC	VUS	POLE_H182N	c.544C>A	N	Missense	13	STGA-DNA 2018	147	Stable	Combo	PD
107	47	F	NSCLC	VUS	POLE_V1158M	c.3472G>A	N	Missense	4	STGA-DNA 2018	147	—	Mono IO	PD
108	40	F	Colorectal	VUS	POLE_K666N	c.1998G>T	N	Missense	6	STGA-DNA 2018	147	Stable	Combo	PD
109	73	F	Skin: SCC	VUS	POLE_S917F	c.2750C>T	N	Missense	6	STGA-DNA 2018	147	—	Mono IO	PD
110	53	M	Melanoma	VUS	POLE_S1118F	c.3353C>T	N	Missense	42	STGA-DNA 2018	147	Stable	Dual IO	SD
111	39	M	Melanoma	VUS	POLE_A2142T	c.6424G>A	N	Missense	8	STGA-DNA 2018	147	—	Mono IO	PD
112	79	M	Melanoma	VUS	POLE_E1898K POLE_L1119F	c.5692G>A c.3354_3355delinsTT	N	Missense Missense	23	STGA-DNA 2018	147	Stable	Mono IO	PR
113	68	M	Melanoma	VUS	POLE_Y2151N	c.6451T>A	N	Missense	30	STGA-DNA 2018	147	—	Dual IO	PR
114	57	F	Colorectal	VUS	POLE_L1622P POLE_R759C	c.4865T>C c.2275C>T	N	Missense Missense	22	STGA-DNA 2018	147	High	Dual IO	PR
115	27	M	NSCLC	VUS	POLE_A2056V	c.6167C>T	N	Missense	14	STGA-DNA 2018	147	—	Mono IO	PD
116	79	F	NSCLC	VUS	POLE_W826C	c.2478G>C	N	Missense	8	STGA-DNA 2018	147	—	Mono IO	SD
117	55	M	Gastric adenocarcinoma	VUS	POLE_A1007P	c.3019G>C	N	Missense	2	STGA-DNA 2018	147	Stable	Mono IO	SD
118	45	F	Cervix: adenocarcinoma	VUS	POLE_K101E POLE_K101E		N	Missense Missense	11	FoundationOne FoundationOne CDx	324	Stable	Combo	PD

(Continued on following page)

TABLE A1. Additional Data for Patients Receiving Immunotherapy (Continued)

Patient No.	Age at Diagnosis (years)	Sex	Cancer Type	*POLE* Pathogenic Status	Pole Mutation Alteration[a]	Pole Mutation cDNA Change	Mutation in Exonuclease Domain[b]	Variant Type	Number of Comutations in Patient Tumor	NGS Panel	Genes Tested in Panel[c]	MSI Status	Treatment[d]	Response
119	72	F	NSCLC	VUS	POLE_P893L	c.2678C>T	N	Missense	16	STGA-DNA 2018	147	Stable	Mono IO	SD
120	86	M	NSCLC	VUS	POLE_S1893I	c.5678G>T	N	Missense	8	STGA-DNA 2018	147	—	Mono IO	PR
121	64	M	Melanoma	VUS	POLE_P1311R	c.3932C>G	N	Missense	5	STGA-DNA 2018	147	—	Combo	PD

Abbreviations: cDNA, complementary DNA; CR, complete response; CTLA-4, cytotoxic T-cell lymphocyte-4; CUP, cancer of unknown primary; F, female; HER2, human epidermal growth factor receptor 2; HNSCC, head and neck squamous cell carcinoma; HR, hormone receptor; IO, immunotherapy; M, male; MSI, microsatellite instability; N, no; NGS, next-generation sequencing; NSCLC, non–small-cell lung cancer; PD-1, programmed death 1; PD-L1, programmed death ligand-1; PR, partial response, SD, stable disease; RCC, renal cell carcinoma; SCC, squamous cell carcinoma; STGA, Solid Tumor Genetic Analysis; TNBC, triple-negative breast cancer; VUS, variant of unknown significance; Y, yes.

[a]Mutations with an * after the position are nonsense mutations; mutations with fs are frameshift mutations.

[b]Mutations in the exonuclease domain are within codons 268-471.

[c]Number of genes included in next-generation sequencing panel.

[d]Treatment categories included mono IO (single-agent immunotherapy treatment with anti–PD-1/L1 agent); dual IO (immunotherapy combination with anti–PD-1/L1 plus anti–CTLA-4); and combo (anti–PD-1/L1 therapy in combination with chemotherapy or another non-IO agent).