

# Complete and Prolonged Response to Immune Checkpoint Blockade in *POLE*-Mutated Colorectal Cancer

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## INTRODUCTION

Genomic sequencing of colorectal cancers reveals that 16% have a very high tumor mutation burden (TMB).<sup>1</sup> Three quarters of such tumors display microsatellite instability (MSI) associated with silencing or somatic mutation of mismatch repair (MMR) genes. One quarter are microsatellite stable (MSS) with somatic mutation of the replicative DNA polymerase gene *POLE*. Among advanced colorectal cancers, the prevalence of *POLE* mutations remains unknown, but only 3.5% are defective in MMR.<sup>2</sup> *POLE* proofreading and MMR act in concert to correct replication errors. Because MMR-deficient tumors often respond to immune checkpoint therapy,<sup>3-5</sup> *POLE*-mutated tumors might also respond. This report presents a patient who had metastatic colorectal cancer with an ultra-high TMB, intact MMR, and a pathogenic p.Val411Leu *POLE* mutation, and who experienced a complete and sustained response to the programmed death 1 (PD-1) checkpoint inhibitor pembrolizumab.

## CASE REPORT

A 44-year-old man presented with a near obstructing 15-cm rectal mass. Family history was negative for colorectal cancer. He underwent partial sigmoid colon resection and colostomy. Pathology revealed moderately differentiated adenocarcinoma with two involved lymph nodes, classified as stage IIIC disease (pT4bN1b according to the American Joint Committee on Cancer staging system, eighth edition). He received neoadjuvant radiation with capecitabine followed by definitive surgery with low anterior resection, intraoperative radiotherapy, radical cystectomy, and construction of an ileal conduit. Pathology showed abundant mucin without residual tumor cells. After adjuvant chemotherapy with fluorouracil and oxaliplatin, computerized tomography (CT) scan showed no evidence of disease.

Three years later, biopsy of an enlarging left supraclavicular lymph node revealed a *KRAS*-mutated MSS adenocarcinoma with abundant extracellular mucin and a lack of programmed death ligand 1 (PD-L1)

tumor cell expression by both E1L3N and SP263 antibody clones. The tumor-infiltrating lymphocytes (TILs) could not be assessed, because this was a lymph node metastasis and the primary tumor was unavailable.

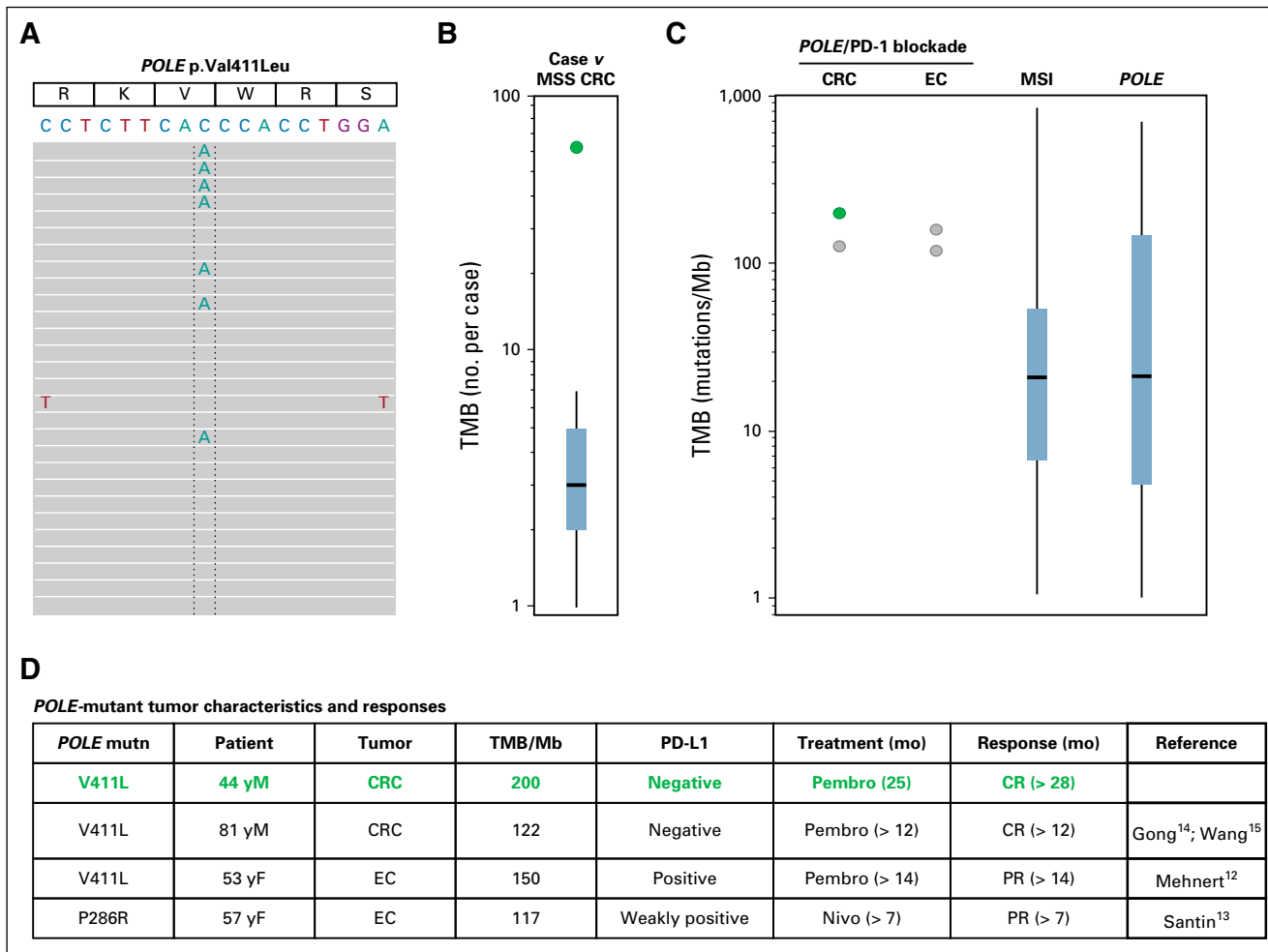
The patient received fluorouracil and irinotecan plus bevacizumab. Treatment was complicated by a small bowel fistula, which required discontinuation of bevacizumab, partial small bowel resection, takedown of an enterorectal fistula, and placement of a permanent rectal tube.

After a 3-month recovery from surgical complications, imaging showed new pulmonary metastases. The combination of fluorouracil and irinotecan was restarted. Progressive disease led to retreatment with fluorouracil and oxaliplatin, which was aborted after a severe oxaliplatin hypersensitivity reaction. Enrollment in a clinical trial assigned the patient to the regorafenib control arm, but treatment was aborted after 5 days for gross hemoptysis and a decrease in hemoglobin from 10.1 to 7.0 g/dL. With extensive pulmonary and nodal metastases and a large pelvic tumor, he enrolled in hospice. The hemoptysis resolved, and performance status improved, so the patient was treated with trifluridine and tipiracil. Five months later, he suffered toxicities of pancytopenia and fatigue and developed progressive disease. The enlarging left supraclavicular nodal mass led to Horner syndrome with ptosis and near syncope, which required palliative radiation.

The left supraclavicular lymph node specimen obtained 3 years after surgery displayed approximately 20% tumor purity by histology. Genomic profiling with the Stanford Solid Tumor Actionable Mutation Panel, a hybrid capture-based next-generation sequencing assay, revealed an ultra-high TMB relative to colorectal carcinomas analyzed on the same panel (Fig 1B). Additional testing demonstrated intact expression of MMR proteins as well as MSS by polymerase chain reaction. This prompted a search for the cause of the striking TMB. An updated version of the Stanford Solid Tumor Actionable Mutation Panel that included exon 9 and 13 of the *POLE* gene identified a pathogenic

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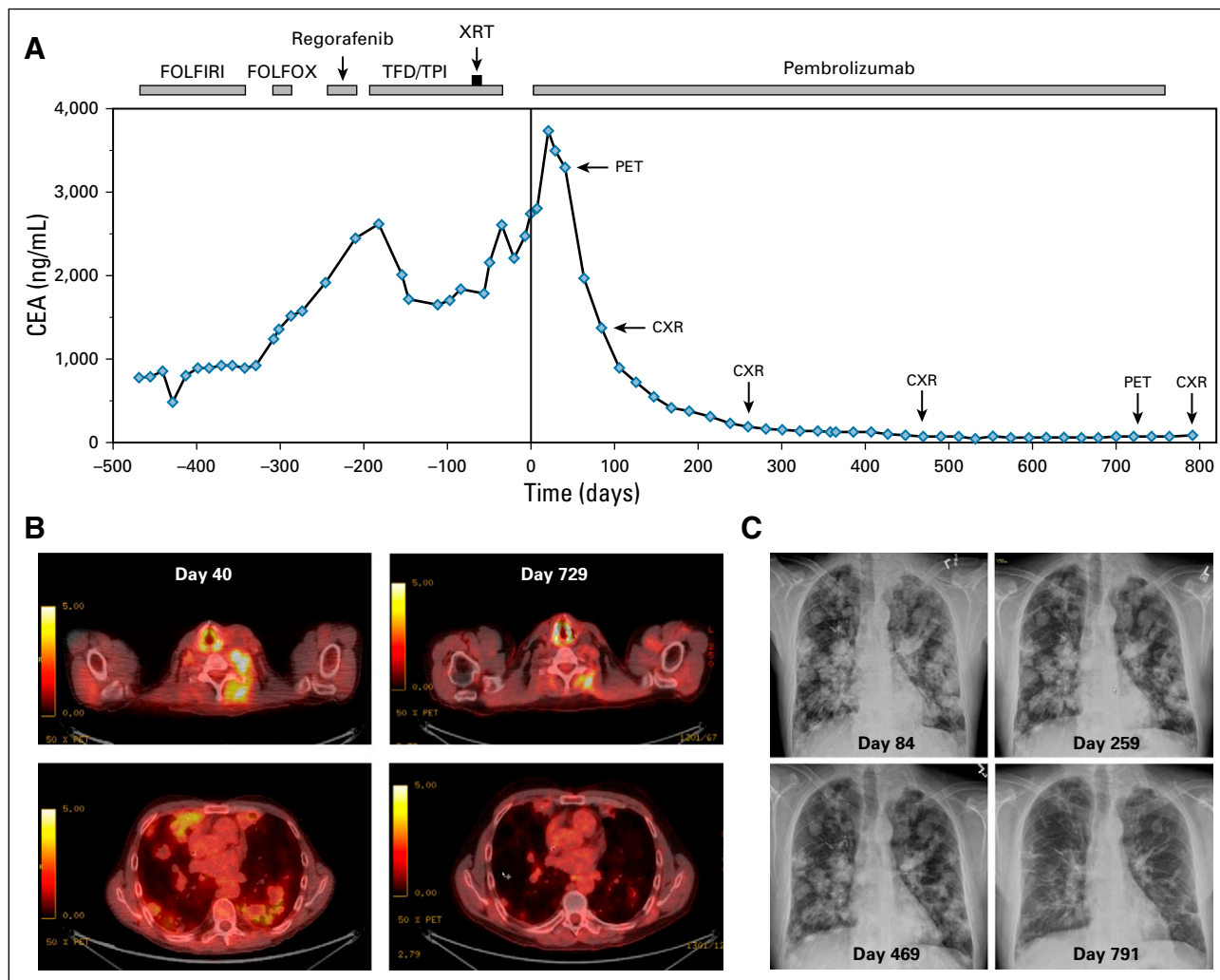


**FIG 1.** Characterization of patient's tumor. (A) *POLE* mutation. The exon 13 mutation c.1232G>T leads to amino acid substitution p.Val411Leu. The mutation is depicted as C>A in this representative subset of the data, because *POLE* is encoded on the minus strand. We detected a total of 495 C>A transversions of 1,982 reads, which provided a variant allele fraction of 25%. (B) Tumor mutation burden (TMB). The green dot indicates the number of nonsynonymous mutations in the patient's tumor detected by the targeted 196-gene Stanford Solid Tumor Actionable Mutation Panel. The boxplot shows the median, interquartile range, and standard deviation for the TMB in 82 microsatellite-stable (MSS) colorectal cancers (CRCs) detected by the Stanford Solid Tumor Actionable Mutation Panel at the time of patient testing. (C) Estimated TMB of patient's tumor versus other tumors. The green dot indicates the patient's tumor. The gray dots indicate TMB for those *POLE*-mutated tumors reported to have responded to programmed death 1 (PD-1) blockade: two CRCs and two endometrial cancers (ECs). The box plots show the TMB for a survey of 859 tumors with likely driver mutations in mismatch repair genes that would produce microsatellite instability (MSI) and 102 tumors with known or likely functional *POLE* mutations (adapted from Chalmers et al<sup>16</sup>). (D) *POLE*-mutant tumors that responded to PD-1 blockade. The green text indicates the patient's tumor. The table shows the *POLE* mutation (mutn); patient age and sex (male [M] or female [F]); tumor type; TMB per megabase of sequenced DNA; programmed death ligand 1 (PD-L1) staining of tumor cells; PD-1 blockade with pembrolizumab (pembro) or nivolumab (nivo) with treatment duration in months (mo); and complete response (CR) or partial response (PR) with response duration in months.

mutation in the *POLE* proofreading exonuclease domain (p.Val411Leu; c.1231G>T) with a variant allele fraction of 25% (Fig 1A), consistent with estimated tumor purity. On the basis of the ultra-high TMB, estimated at 200 mutations/Mb (Fig 1C), our molecular tumor board recommended immunotherapy with an immune checkpoint inhibitor.

Pembrolizumab was obtained for compassionate use. Treatment led to a transient increase in the carcinoembryonic antigen (CEA) from 2,742 ng/mL to a peak of 3,727 ng/mL followed by a decline to a plateau of 57 to

83 ng/mL which was maintained through 25 months of treatment and an additional 3 months of follow-up (Fig 2A). This was associated with resolution of pain and normalization of performance status from Eastern Cooperative Oncology Group status of 2 to 0. Much of the residual CEA level may have been attributable to inflammation from the persistent enterorectal fistula. Serial positron emission tomography/CT scans showed gradual and sustained decrease in tumor size with complete resolution of metabolic activity by day 729 (Fig 2B). The chest x-ray showed slow,



**FIG 2.** Tumor response to treatment. (A) Carcinoembryonic antigen (CEA). Bars above the graph indicate periods of treatment with fluorouracil and irinotecan (FOLFIRI), fluorouracil and oxaliplatin (FOLFOX), regorafenib, trifluridine (TFD) and tipiracil (TPI), radiation therapy (XRT) to the left supraclavicular mass, and the programmed death 1 (PD-1) inhibitor pembrolizumab. Time is shown as days relative to the initiation of pembrolizumab on day 0. (B) Positron emission tomography (PET)/computed tomography scan. Images show tumor burden on days 40 and 729 of pembrolizumab treatment. Metabolic activity in the neck and lung showed near-complete resolution. The day 729 left cervical mass was interpreted as lacking hypermetabolic activity on the basis of a comparison with the peritracheal signal from the blood pool. (C) Chest x-ray (CXR). Images show tumor burden on days 84, 259, 469, and 791. Note the delayed disappearance of masses in contrast to the rapid decline in CEA.

but ultimately complete, disappearance of the pulmonary masses (Fig 2C). The delayed response was consistent with slow clearance of mucin after tumor cell death.

The patient experienced a brief episode of localized herpes zoster and later a brief episode of asymptomatic grade 1 transaminitis. Each episode was addressed by withholding one cycle of pembrolizumab, and each quickly resolved without sequelae.

**DISCUSSION**

In recent years, immune checkpoint blockade has emerged as a safe and effective treatment of many solid tumors. In particular, tumors with high level of MSI and mismatch repair deficiency have shown dramatic responses to treatment with immune checkpoint inhibitors.<sup>4,5</sup> Similarly, accumulating

evidence suggests that TMB alone, independent of MMR status, correlates with response to immune checkpoint blockade for some tumor types.<sup>3,6-9</sup> Hence, MSS tumors with an ultra-mutated phenotype as a result of mutations in *POLE* or *POLD1*<sup>10,11</sup> represent an intriguing subset of tumors that may also respond to immune checkpoint inhibitors.

The case presented here adds to the few reports of *POLE*-mutated tumors that responded to PD-1 checkpoint blockade. Previous reports include two endometrial cancers<sup>12,13</sup> and a single colorectal cancer described at 4 and 12 months of follow-up.<sup>14,15</sup> The four responsive tumors, including the tumor in this report, displayed mutations in the exonuclease domain of *POLE* (Fig 1D) and ultra-high mutation burdens (greater than 100 mutations/Mb, ie, more than 5-fold greater than the median TMB

reported for MSI and *POLE*-mutated tumors<sup>17</sup> [Fig 1C]). By contrast, the responsive tumors were inconsistent in PD-L1 expression (Fig 1D).

Unlike patients in the other cases, the patient in this report began treatment with the greatest extent of disease and enjoyed the longest sustained response (which continued beyond 28 months). The response was complete by chest x-ray and positron emission tomography/CT scan (Figs 2A and 2B). The dramatic decline in CEA led to residual CEA levels, which may reflect residual fistulas and tissue damage from previous tumor invasion. Finally, the patient's clinical course illustrates the potential pitfalls in early evaluation of tumor response. Radiologic density of tumors failed to change significantly for 8 months before finally disappearing at 28 months.

The dramatic response in this patient shows the potential benefit of evaluating MSS tumors for *POLE* and possibly *POLD1* mutations. However, it is important to realize that some *POLE* or *POLD1* mutations, particularly previously uncharacterized mutations, may prove to be passenger alterations with no effect on TMB.<sup>10</sup> A prospective analysis of 80,853 patients with advanced solid tumors revealed known genomic alterations in *POLE* in only 259 patients (0.3%), with a median TMB of 31 mutations/Mb.<sup>17</sup> The most common mutation was p.R446Q (n = 77), which is uncharacterized, associated with low TMB (less than five

mutations/Mb), and predominantly germline. The two next-most-common mutations, p.P286R (n = 41) and p.V411L (n = 29), are both functional, associated with high TMB (greater than 20 mutations/Mb), predominantly somatic, and enriched in colorectal cancer and endometrial carcinoma. These were the mutations present in the four *POLE*-mutant tumors that have responded to PD-1 checkpoint blockade in this and other published reports (Fig 1D).

However, not all *POLE*-mutated tumors respond to checkpoint blockade. Two colorectal cancers with the p.P286R mutation showed progressive and stable disease after 1 and more than 10 months of follow-up.<sup>15</sup> Both cases showed low levels of CD8<sup>+</sup> TILs. In fact, in a cohort of five MSI tumors and three *POLE*-mutated tumors, high levels of CD8<sup>+</sup> TILs occurred in all four patients who experienced response and none of the four patients who did not experience response ( $P = .0007$ ).<sup>15</sup>

It is still unknown if responses to PD-1 checkpoint blockade will occur for tumors with *POLD1* mutations or *POLE* mutations outside the exonuclease domain or if responses will occur in *POLE*-mutant tumors with a less extreme TMB (ie, 10 to 100 mutations/Mb). These unanswered questions, along with the dramatic and prolonged response reported here, strongly emphasize the importance of ongoing clinical trials to evaluate responses to immune checkpoint inhibitors in tumors that harbor *POLE* mutations.<sup>18-20</sup>

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## EQUAL CONTRIBUTION

R.S. and D.F.S. contributed equally to this work as primary authors. G.C. and C.J.S. contributed equally to this work as corresponding authors.

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**Collection and assembly of data:** All authors

**Provision of study material or patients:** Robyn Silberman, Gilbert Chu

**Data analysis and interpretation:** All authors

**Manuscript writing:** All authors

**Final approval of manuscript:** All authors

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### Robyn Silberman

**Stock and Other Ownership Interests:** Loxo

**Honoraria:** Lexicon

**Consulting or Advisory Role:** Lexicon

### David F. Steiner

**Employment:** Google

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**Employment:** Genentech, Roche

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### Gilbert Chu

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