Hormone Receptor–Positive Breast Cancer Sensitive to Pembrolizumab: Evidence of the Pathogenicity of the \textit{MLH1} Variant 1835del3

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\textbf{ABSTRACT}

A woman with estrogen/progesterone receptor–positive, \textit{ERBB2}-negative metastatic breast cancer developed progressive disease despite treatment with multiple hormonal and chemotherapeutic modalities. She carried a germline variant of \textit{MLH1} (1835del3), also known as c.1835_1837del and v612del, the pathogenicity of which has not been conclusively determined. \textit{MLH1} staining was not seen on immunohistochemical staining of her tumor tissue. The patient experienced a >5-year dramatic response to 4 doses of pembrolizumab. Family studies revealed multiple other relatives with the \textit{MLH1} 1835del3 variant, as well as multiple relatives with colon cancer. The one relative with colon cancer who underwent genetic testing demonstrated the same variant. Laboratory studies revealed that the patient’s tumor showed loss of heterozygosity (LOH) in the \textit{MLH1} region, high levels of microsatellite instability, and a high tumor mutational burden. LOH in the \textit{MLH1} region, along with the remarkable clinical response to pembrolizumab treatment and the presence of the same \textit{MLH1} variant in affected relatives, supports the hypothesis that the \textit{MLH1} variant is pathogenic. Given the patient’s family history, this likely represents an uncommon presentation of Lynch syndrome. Physicians should be alert to evaluate patients for targetable genetic variants even in unlikely clinical situations such as the one described here.

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\textbf{Case Report}

This patient presented in 2008 with right-sided breast cancer, with 70% estrogen receptor (ER) expression and 2% to 5% progesterone receptor (PR) expression, and \textit{ERBB2}-negative breast cancer, and provides data to suggest that the dysfunction of the c.1835 1837del variant of \textit{MLH1}, whose pathogenicity has been uncertain, was the driver of the disease, rendering it susceptible to PD-1 blockade. This study received Providence St. Joseph Institutional Review Board approval (study 2021000589).

\begin{itemize}
  \item Breast cancer has, until recently, not been considered amenable to treatment with immunotherapy. However, recent studies have demonstrated the utility of PD-1 blockade for the treatment of triple-negative breast cancer,\(^{1,2}\) as well as the effectiveness of immunotherapy in patients with high tumor mutational burden (TMB) and/or high microsatellite instability (MSI-H), regardless of the tissue of origin.\(^{3–10}\) Although cancers in patients with Lynch syndrome have been shown to represent a specific group of patients with MSI who may respond to such treatment, there has been controversy as to whether breast cancer should be included among the cancers that are associated with this syndrome, and which Lynch syndrome genes might be associated with breast cancer.\(^{11–29}\)
  
  This report documents a remarkable response to a short course of pembrolizumab in a woman (self-reported female sex) with hormone receptor–positive, \textit{ERBB2}-negative breast cancer, and provides data to suggest that the dysfunction of the c.1835 1837del variant of \textit{MLH1}, whose pathogenicity has been uncertain, was the driver of the disease, rendering it susceptible to PD-1 blockade.
  
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One year later her disease progressed again, and she received everolimus, fulvestrant, exemestane, and leuprolide. In 2014, she developed biopsy-proven liver metastases, with ER and PR expression 99%, HER2/neu-negative, and Ki-67 index of 5% (Figure 1). PD-L1 test results on this specimen were completely negative (University of Washington Medical Center [UWMC] OncoPlex), as was a study for MSI. The patient received capecitabine, to be followed subsequently by ixabepilone, eribulin, and cyclophosphamide/methotrexate/5-fluorouracil (CMF) without response.

In February 2017 the patient experienced disease progression in her liver and multiple areas in the abdomen, pelvis, rectum, bladder, and cervical lymph nodes, with ureteral obstruction requiring stenting. The rectal lesion was ER-positive, whereas the bladder lesion was reported as ER 95%, PR 15% (Figure 2). Tissue from a cervical node showed ER and PR 80%, ERBB2-negative. Samples of this node were analyzed at the New York Genome Project as well as at Foundation Medicine (Table 1). The patient experienced a 3-month partial response to CPT-11, but no response thereafter to vinorelbine, docetaxel, or doxorubicin.

In October 2017 the patient experienced a bowel obstruction from tumor progression in the rectum and pelvis, necessitating an ileostomy. Because of the finding of MSI-H and a high TMB in tumor tissue along with the presence of an MLH1 variant that might be pathogenic, she received 4 doses of pembrolizumab postoperatively, resulting in a spectacular complete remission of her disease. The pembrolizumab was limited to 4 doses because the patient developed significant hepatitis, which resolved with discontinuation of treatment and a short course of glucocorticoids. The patient was found to have an asymptomatic brain metastasis in July 2020 and a single ileum bone metastasis in January 2021, both of which resolved with stereotactic radiation. As of April 2023, the patient remains free of detectable disease and has received no systemic therapy for breast cancer since January 2018. Colonoscopy in October 2021 revealed one tubular adenoma.

Tissue from the liver, rectum, bladder, and cervical node all were thought to be consistent with metastatic breast cancer by pathologic evaluation. All specimens were hormone receptor-positive and ERBB2-negative. Additionally, all were positive for mammaglobin and GATA3. The bladder and cervical node specimens were also tested for gross cystic disease fluid protein-15 and both were positive (Figures 1 and 2).

The patient’s 2 sons, her brother (who has had colon cancer), her brother’s daughter, and a paternal cousin were all found to carry the same variant of MLH1 as the patient (Figure 3). The patient’s father, though not genetically tested, had colon cancer twice and stomach cancer at ages 49, 58, and 79 years, respectively.

**Laboratory Studies**

Because her family history raised the possibility of Lynch syndrome (Figure 3), a Myriad Colaris study was performed on peripheral blood, demonstrating a variant of...
unknown significance (VUS) MLH1 (1835del3), but no variants in MSH2 or MSH6 (Table 1). EPCAM and PMS2 were not included in the Colaris panel at that time. Myriad BRC1 germline sequencing revealed no abnormal variants in 2008, and BRCA germline BART (BRACAnalysis Large Rearrangement Test) testing for deletions and duplications was negative in 2010. In 2014, Myriad’s MyRisk 25-gene hereditary cancer panel, which included EPCAM and PMS2, again demonstrated the same MLH1 variant, which they considered a VUS, but no other variants were detected.

Tissue from the 2014 liver biopsy confirmed the MLH1 1835del3 variant. Two PIK3CA mutations were discovered as well (Table 1). MSI testing was negative (UW-OncoPlex cancer gene panel) and this laboratory classified the MLH1 variant as likely pathogenic. They also noted loss of heterozygosity (LOH) at the MLH1 locus. Studies of the cervical

![Figure 2. Rectal metastasis: (A) hematoxylin-eosin (original magnification ×20); (B) estrogen receptor (original magnification ×40); (C) mammaglobin (original magnification ×40); and (D) GATA3 (original magnification ×40).](image)

<table>
<thead>
<tr>
<th>Table 1. Somatic Mutations Found in &gt;1 Genomic Evaluation</th>
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<tr>
<td><strong>Tissue</strong></td>
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<td><strong>Collection year</strong></td>
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<td><strong>Genomic laboratory</strong></td>
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<tr>
<td>MLH1 V612del3</td>
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<tr>
<td>PIK3CA H1047R</td>
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<tr>
<td>PIK3CA K111E</td>
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<td>ESR1 Y537s</td>
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<td>TSC2 G654</td>
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<td>ARIDIA N1510fs*22</td>
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nodes (removed in 2017) by both the New York Genome Project and Foundation Medicine confirmed both PIK3Ca variants and noted an ESR1 mutation. The New York Genome Project reported 94 single nucleotide variants and 18 copy number alterations, including the MLH1 1835del3 variant, which they considered likely pathogenic. They reported LOH in the 3p24.3-p13 region, which includes MLH1. Foundation Medicine reported 10 abnormalities, including MLH1 1835del3, which they reported as a VUS. They reported MSI-H and a TMB of 47 mutations per megabase (mut/Mb. Manual review of the FoundationOne CDx copy number data also supported LOH at the MLH1 locus (B. Decker, oral and email communication, March 28, 2023).

Tissue from the patient’s original breast removal was assessed for mismatch repair protein expression and was negative for MLH1/PMS2 by IHC with intact MSH2 and MSH6. Tissue from a bladder metastasis surgically obtained in 2017 was negative for MLH1 promoter hypermethylation (ARUP Laboratories).

In summary, genomic studies were performed on peripheral blood at 1 laboratory and on tumor tissue at 3 laboratories during the course of this patient’s disease (Table 1). All 4 found the MLH1 1835del3 variant, but a definitive classification of this variant as pathogenic was not uniform, with 2 laboratories calling it likely pathogenic and 2 a VUS. There was considerable overlap among additional variants found in the tissues from metastatic sites submitted for genomic evaluation (Table 1). Genomic sequencing was not performed on the primary breast cancer tissue.

Two laboratories tested for MSI. One of the laboratories that reported the MLH1 1835del3 variant did not find MSI-H expression, whereas the other did. The New York Genome Project did not report a specific TMB but did report many VUS. Both PIK3Ca mutations were found in tumor tissue in all 3 laboratories, and the ESR1 mutation was found in the two most recently obtained samples, potentially a result of technical differences among laboratories, tumor heterogeneity, or the evolution of the tumor over time. Hypermethylation of MLH1 was excluded (ARUP Laboratories). LOH in the region of the MLH1 variant was noted by the New York Genome Project.

Given the uncertainty of the significance of the MLH1 1835del3 variant as the cause of MSI-H and high TMB in this patient’s tumor, we examined MLH1 protein expression in both the patient’s tissue and peripheral blood mononuclear cells (PBMCs), as well as PBMCs from her sons, compared with patients with breast cancer who did not have an MLH1 mutation. We hypothesized that MLH1 protein produced from the MLH1 1835del3 variant allele may be unstable, resulting in reduced expression of MLH1 in tissue and/or PBMCs.
Pathogenicity of MLH1 Variant 1835del3

Utilizing immunoaffinity enrichment multiple reaction monitoring (immuno-MRM) mass spectrometry, we quantified the peptide IAAGEVIQRPAIK as a stoichiometric surrogate for the expression level of MLH1 protein from PBMCs and biopsies of lymph nodes containing metastatic disease. In noncycling PBMCs, the patient (and her sons) showed relatively low expression of MLH1 protein compared with a small number of additional patients with breast cancer (Figure 4), suggesting that the variant allele may result in lower expression of MLH1 protein, although a larger number of patients would need to be included in the study to establish a normal reference range. Interestingly, we found that MLH1 protein expression in the patient’s metastasis-containing lymph node was quite low compared with additional patients with breast cancer (Figure 4), possibly due to either LOH in the patient’s metastatic tumor or tissue heterogeneity among the biopsy samples. A larger sample size would be required to fully establish a normal reference range.

Discussion

This case highlights the difficulties in assessing the clinical impact of VUS, as well as determining whether breast cancer is a Lynch syndrome–associated condition. Notably, this patient’s breast cancer phenotype is not one usually associated with sensitivity to immunotherapy. The ER and PR positivity and the mammaglobin and GATA3 positivity in all samples as well as the frequent overlap of variants other than MLH1 in the metastatic tissues are consistent with the conclusion that all of the tumor deposits from this patient were metastatic breast cancer and that they evolved from a common breast cancer ancestor, rather than colon cancer.

This patient exhibited MSI-H and high TMB. Mismatch repair deficiency (dMMR) is quite uncommon in breast cancer. It can also be caused uncommonly by apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminase. In addition, sporadic cases can be caused by hypermethylation of MLH1 promoter, though hypermethylation was excluded in this case.

High TMB in breast cancer is most common in ERBB2-amplified disease, followed by triple-negative tumors, and least commonly in hormone receptor–positive tumors. PD-L1 positivity is somewhat uncommon in breast cancer, being reported in <10% of ER-positive cases and at somewhat higher frequency in triple-negative disease. The correlation of PD-L1 positivity with TMB appears to vary among tumors types. In breast cancer, ≤20% of tumors with high TMB are positive for PD-L1.

As of December 2022, the MLH1 1835del3 variant is classified as likely pathogenic by ClinVar (although one laboratory classifies it as a VUS) and UW-OncoPlex, pathogenic by VarSome and the New York Genome Project, and a VUS by Myriad and Foundation Medicine. One patient with this variant has been reported with a family history meeting Amsterdam I criteria. The MLH1 1835del3 variant is an in-frame TTG deletion resulting in deletion of a single valine residue while otherwise preserving the sequence’s reading frame. Establishing pathogenicity in nontruncating variants such as this can be challenging, particularly because nontruncating variants have the potential to impact normal MMR function while still exhibiting residual protein expression in tumor. Functional studies by Raevaara et al demonstrated that the MLH1 1835del3 variant was associated with decreased protein expression, though near normal

Figure 4. Immunoaffinity enrichment multiple reaction monitoring (immuno-MRM) mass spectrometry quantification of the expression level of MLH1 protein from PBMCs and from biopsies of lymph nodes containing metastatic disease. MLH1 protein expression is plotted as the peak area ratio (endogenous:standard)/μg. For PBMCs, samples with appreciable blood contamination (determined by intense hemoglobin bands in SDS-PAGE of protein lysates) or protein degradation were disqualified. Lymph node biopsies were required to have tumor area >20% for analysis. Box plots show the median (horizontal bar), interquartiles (box), and 5th to 95th percentiles (vertical bar). Samples of note, including the breast cancer case study in this report and PBMC samples from her 2 sons, are indicated on the plot.

Abbreviation: PMBC, peripheral blood mononuclear cell.
in vitro MMR activity. This study predicted the variant to be pathogenic. The variant has also been observed in families meeting clinical criteria for Lynch syndrome, although segregation data are lacking. This variant is not observed in population databases (eg, The Genome Aggregation Database [gnomAD]).

Although pathogenic variants of MLH1 have one of the highest reported risks of colorectal cancer among the dMMR deficiencies, there has been considerable debate as to whether breast cancer should be included in the Lynch syndrome family of tumors. Given the strong family colon cancer history in this case (including multiple family members with the same variant, one of whom has colon cancer) and the MLH1 deficiency (in the absence of hypermethylation), we hypothesize that the MLH1 deficiency caused by the MLH1 1835del3 variant combined with LOH led to MSI-H and a high TMB and would qualify this as an example of Lynch syndrome. Although we cannot discount the observation of improved responses to PD-1 blockers in general in patients who develop significant adverse events to their use, the remarkable response to a very short course of pembrolizumab would also be consistent with Lynch syndrome, given that other researchers have reported better response rates in patients with colon cancer with dMMR than in those who do not harbor these changes.

Conclusions

We believe this patient with breast cancer demonstrated an uncommon example of Lynch syndrome caused by a variant in MLH1 not previously conclusively proven to be pathogenic. Patients with phenotypes not often associated with variants amenable to treatment, as in this case of hormone receptor–positive, ERBB2-negative breast cancer, can sometimes demonstrate unexpected targetable mutations. The FDA approval of immunotherapy for TMB-high and MSI-H cancers regardless of tissue of origin should broaden the ability of clinicians to obtain genomic data on virtually all patients with metastatic cancer. Furthermore, it is important to periodically reevaluate VUS because their significance may become clearer over time.

Acknowledgments

We would like to honor our coauthor and long-time colleague Uliana Voytovich, who recently passed away while in Ukraine performing humanitarian work in the service of those affected by the war.
Pathogenicity of MLH1 Variant 1835del3


