

## Two Months of Therapy: A Case of Pathologic Complete Response to Chemo-Immunotherapy in a Patient with Metastatic Colorectal Cancer

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### Introduction:

Colorectal cancer (CRC) is the fourth most common cancer in the United States (US), and the second leading cause of cancer related deaths. In the past decade, targeted therapies have increased the median overall survival (mOS) for metastatic CRC from 12 months to 30 months (1). Recent data emerging from The Cancer Genome Atlas have identified subtypes of colon cancer associated with common oncogenic pathways (2). One area of particular interest involves a subset of CRC with mismatch repair deficiency associated with a hypermutated phenotype. This phenotype of CRC potentially results from thousands of mutations linked to inherited mismatch repair mechanisms (e.g. Lynch Syndrome) or sporadic cancers associated with somatic mutations or epigenetic phenomenon including gene promoter methylation leading to silencing of gene expression. Knowledge of the mutations that are responsible for CRC biology provides opportunity for developing therapeutic targets.

Instability of microsatellites, both truncated and expanded, are observed in up to 20% of early stage CRCs and up to 5% of advanced stage CRC (3). High microsatellite instability (MSI), is often associated with a high mutation burden, in the range of thousands per mega base. MSI cancers increase recognition by the cytotoxic T lymphocytes, and carry a better prognosis compared to stable microsatellite tumors (4,5). Most CRC cases arising from MMR are secondary to somatic mutations, and the minority arise from germline mutations such as Lynch Syndrome (6). The four genes associated with Lynch Syndrome are MLH1, MSH2, MSH6 and PMS2. Sporadic deficiencies in MMR result from MLH1 methylation that produces complete loss of the MMR pathway (6). Immune surveillance plays a role in CRC regulation, and tumor dysregulation of immune checkpoints may contribute to immune resistance mechanisms (7). Therapeutic manipulation to promote CRC antitumor immunity has been studied. Two immune checkpoint inhibitors, CTLA4 and PD-1 antibodies, increase immune response in CRC tumors with high MSI (8). While the majority of patients with advanced CRC do not benefit from single agent immune checkpoint inhibitors, phase II studies suggest a disease control rate of 70% in patients treated with single agent nivolumab or pembrolizumab (9,10). Pembrolizumab is now approved for patients with mismatch repair deficient (dMMR) tumors, irrespective of the site of disease. Immunotherapy for CRC could involve 5-fluorouracil (5FU) to enhance anti-tumor immunity via elimination of myeloid derived tumor suppressive cells and increased IFN- $\gamma$  production by tumor specific CD8<sup>+</sup> cells. Preclinical studies have established the role of chemotherapy as immune modulating agents that could be synergistic with immune checkpoint inhibitors in CRC. In mouse models, oxaliplatin has been reported to induce immunogenic cell death leading

to release of neoantigens that are engulfed by dendritic cells and presented to tumor-specific CD8+ T cells (11, 12).

Based on the previously reported data, a clinical trial was designed to evaluate the safety and efficacy of mFOLFOX6 (5FU, leucovorin and oxaliplatin) in combination with pembrolizumab in patients with advanced, unresectable CRC irrespective of their MMR status. Here we report a complete pathologic response in a patient with dMMR CRC who received mFOLFOX6 with pembrolizumab after only 2 months of therapy as observed at time of surgical resection.

#### Discussion/Case:

A 28 year-old woman presented with diarrhea and hematochezia for several months. Colonoscopy demonstrated evidence of a large sigmoid colon mass that was biopsied as poorly differentiated adenocarcinoma of the colon. A three generation pedigree with emphasis on cancers and tumors was obtained during genetic counselling and revealed her paternal grandfather died from colorectal cancer at age 32. Immunohistochemical stains with working controls were performed on the specimen. Cancer cells are strongly and diffusely positive for keratin cocktail and CDX2 with negative staining for CK7, CK20, Synaptophysin, chromogranin and PAX-8. Immunohistochemical stains for mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2 were performed with evidence of loss of expression of MLH1 and PMS2 and normal expression of MSH2 and MSH6. Patient had genetic counseling to discuss confirmation of Lynch syndrome and recommended her father be tested for MLH1 mutation given paternal history of early CRC.

Further molecular studies showed *BRAF V600E* wild type and *KRAS G12D* mutation. Her tumor was evaluated by FoundationOne™ with evidence of *BRCA2*, *CDK12*, *PIK3CA*, *PTEN*, *APC*, *ARID1A*, *ARID2*, *CIC*, *CREBBP*, *FAT1*, *MLH1*, *MSH2*, *SPTA1* and *TP53* mutations, along with a high tumor mutation burden of 48.71 muts/Mb. Despite the retained expression of MSH2 and loss of PMS2 on IHC, the findings from FoundationOne™ next generation sequencing identified a mutation in *MSH2*. The patient had radiographic evaluation via positron emission tomography (PET) scan demonstrating evidence of large central hepatic mass with extrahepatic extensive lymphadenopathy in the porta hepatis and para-aortic region (Image 1).

She was enrolled on clinical trial NCT02375672 and treated with mFOLFOX6 every 2 weeks and pembrolizumab (200 mgIV Q 3 weeks). After 2 months of therapy, she experienced an excellent clinical response and partial immune RECIST and RECIST response (Image 2). Operative resection of the tumor required rectosigmoidectomy, partial cystectomy, left partial hepatectomy, and portocaval lymph node

dissection. Final pathology (Image 3) contained only acellular mucin and complete necrosis with associated necrotizing granuloma, clusters of foamy macrophages and microcalcifications present in pericolic region consistent with pathologic complete response. No viable tumor was observed in any of the resected samples. Immunohistochemical stains were performed on the resected specimen and showed that the lymphoid infiltrate surrounding the necrosis is composed mainly of T cells (positive for CD3, CD4 and CD8) with small amount of CD20 positive B cells (Image 4).

#### Discussion:

While mismatch repair deficiency for long was associated with improved survival for patients with early stage CRC (4,5), the association with advanced disease suggested of worse outcome. However, this phenotype is typically associated with increase tumor infiltrating lymphocytes that is associated with improved survival in a subset of patients with advanced CRC (9,10,13,14). It was not until recently where the correlation between mutation burden, tumor infiltrating lymphocytes and the response to immune checkpoint point has been established for patients with CRC. In fact, the FDA has granted in 5/2017 approval for the use of pembrolizumab based on the presence of mismatch repair deficiency irrespective of the tissue of origin. Since the use of pembrolizumab is limited to < 5% of all patients with advanced incurable CRC, efforts are underway to overcome innate resistance to immunotherapy; one approach by combining chemotherapy that can lead to immunogenic cell death and other agents that can enhance tumor lymphocyte trafficking (15, 16).

Both chemotherapy and radiation provoke adaptive immune responses in CRC promoting tumor cell death, and current research is examining the benefits of combining current treatments with immunotherapy. 5-FU and oxaplatin are thought to aid immune mediated death, and based on this thought two trials (NCT02375672, NCT02268825) are studying the combination of FOLFOX and pembrolizumab in GI and colon cancers (17). This patient as well as others are still being followed on the NCT02375672, and the preliminary results of the study have been reported at ASCO (18). In a small study of 14 chemorefractory patients, a combination of atezolizumab, anti-PD-L1 monoclonal antibody, and bevacizumab, VEGF blockade, without chemotherapy demonstrated disease response (7%) or stability (64%) (NCT01633970) (17). Additional CRC studies of chemo-immunotherapy combinations include a regimen of bevacizumab and FOLFOX leading to increased inflammatory helper T-cells (19). Lastly a combined chemo-immunotherapy trial with FOLFOX, bevcizumab and atezolizumab in treatment naive patients has shown 87% of the patients demonstrating response or stable disease (20). Multiple other studies are underway examining various chemo-immunotherapy combinations in advanced CRC cases (21).

While responses to single agent PD-1 inhibitors have been reported, and a complete pathologic response has been documented after extended course of pembrolizumab (13), our report describes a

case of dramatic response to a combination of chemotherapy and pembrolizumab with complete tumor necrosis and pathologic response within only 2 months of therapy. While dMMR can explain the benefit from pembrolizumab, number of molecular abnormalities could have contributed to massive antigen presentation associated with chemotherapy. This in part due to the activity associated with mFOLFOX6 in first line setting (response rate of ~50%) and the presence of abnormalities in DNA homologous recombination repair genes (*BRCA2*, *ARID1A*, and *ARID2*) that may confer sensitivity to platinum based therapy.

This case raises number of questions regarding: 1- the optimal imaging modality to assess for treatment response, 2- the role of chemotherapy to enhance responses to immune checkpoint inhibitors, especially to enhance activity in dMMR or to overcome innate resistance in proficient MMR, and finally, 3- the ideal duration of therapy. Several studies have demonstrated prolonged and sustained responses in a subset of patients with melanoma and other solid tumors who discontinued therapy due to variable reasons including initial progression that was followed by delayed response. Future studies are needed to answer these important questions especially in the light of cost associated with this novel class of drugs, and risk of immune mediated toxicity.

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Image 1: PET/CT scan demonstrating large hepatic mass and large sigmoid colon mass extrahepatic extensive lymphadenopathy in the porta hepatis



Image 2: CT scan after 2 months of therapy showing radiographic response



Image 3: A. Partial hepatectomy showing complete necrosis and necrotizing granuloma with no viable tumor. B. Rectosigmoid Colon resection showing acellular mucin and complete necrosis, necrotizing granuloma, clusters of foamy macrophages.

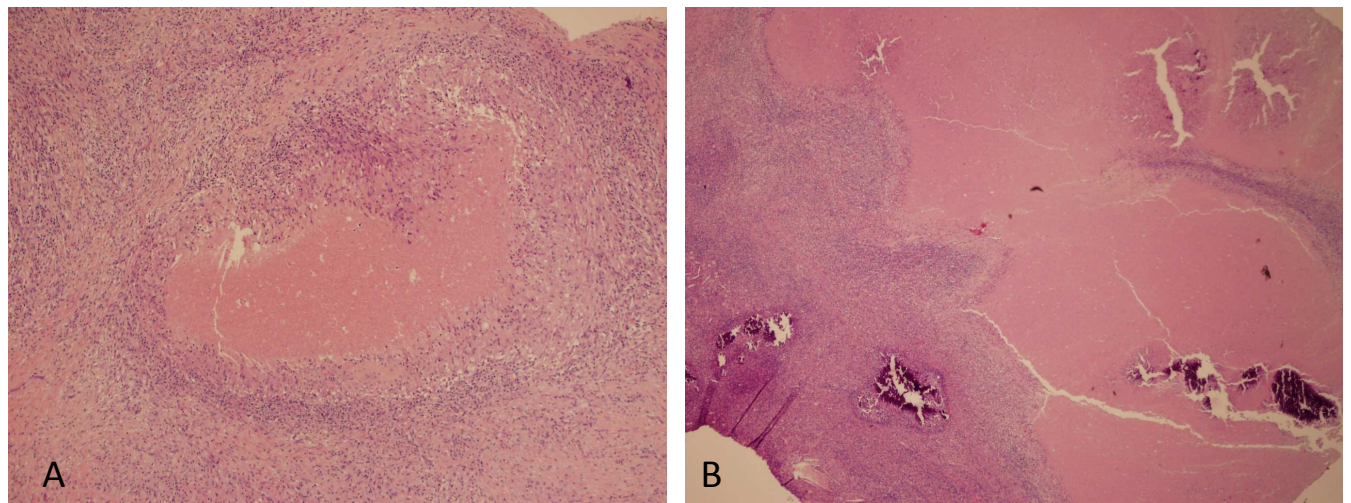


Image 4: Immunohistochemical stains show that the lymphoid infiltrate surrounding the necrosis is composed mainly of T cells. Positive for CD3 (A), CD4 (B) and CD8 (C) with small amount of CD20 positive B cells (D).

