Molecular and Radiological Features of Microsatellite Stable Colorectal Cancer Cases With Dramatic Responses to Immunotherapy

BRIDGET P. KEENAN¹, KATHERINE VAN LOON¹, ANURADHA D. KHILNANI²,
NICHOLAS FIDELMAN³, SPENCER C. BEHR³, CHLOE E. ATREYA¹ and DAVID Y. OH¹

¹Division of Hematology/Oncology, Department of Medicine, University of California, San Francisco, CA, U.S.A.;

²Merck & Co., Inc., Kenilworth, NJ, U.S.A.;

³Department of Radiology and Biomedical Imaging, University of California, San Francisco, CA, U.S.A.

Abstract. *Background/Aim:* The majority of colorectal cancer (CRC) cases, which are microsatellite stable (MSS) and do not harbor mismatch repair deficiency/microsatellite instability, are resistant to immunotherapy. Identification of patients with exceptional responses in MSS CRC and predictive biomarkers is an unmet need that needs to be addressed. *Case Report:* We report three cases of MSS CRC with durable clinical benefit from immunotherapy with anti-PD-1 checkpoint inhibitors. Two cases bear a *POLE* P286R mutation, which has been associated with lack of immunotherapy response in MSS CRC. Two cases bear alterations in Ataxia-Telangiectasia Mutated (*ATM*) which may contribute to observed responses, including interaction with a co-administered intratumoral stimulator of interferon genes (*STING*) pathway agonist in one patient. *Conclusion:* Novel DNA damage repair alterations, including mutations in *ATM*, can provide insight into additional mechanisms by which genomic alterations can sensitize MSS CRC to diverse immunotherapies.

Colorectal cancer (CRC) ranks in the top five cancer types in terms of incidence and mortality worldwide (1). In the minority of CRC tumors with microsatellite instability or mismatch repair deficiency (MSI/MMRd), response rates to checkpoint inhibitors (CPI) blocking PD-1 or CTLA-4 range from 30-55%, leading to their approval, whereas microsatellite stable/mismatch repair proficient colorectal cancer (MSS/pMMR CRC) has a near-zero

response rate (2-4). However, there are indications that CPI can benefit MSS CRC patients; a phase II trial of anti-PD-L1 plus anti-CTLA-4 demonstrated an overall survival benefit for MSS CRC compared to best supportive care, despite few objective responses (5).

Identifying exceptional MSS CRC responders to CPI is critical, but our knowledge of biomarkers that predict these responses is incomplete. Although pembrolizumab was recently approved for tumor mutational burden (TMB)-high [≥ 10 mutations/megabase (MB)] advanced cancers agnostic of tumor type, based on the KEYNOTE-158 study, this study did not include CRC patients (6), and response rates for TMB-high MSS CRC patients (defined as ≥ 9 mutations/MB) are known to be low (11%) (7).

Mutations in genes involved in DNA replication (such as *POLE* or *POLD1*) that create a hyper-mutated (but MSS) phenotype have been associated with durable response to CPI, including in CRC, but not all *POLE* mutations predict CRC response (8-11). In particular, the common proofreading P286R mutation did not demonstrate CPI response in MSS CRC and was associated with low CD8⁺ tumor infiltration, indicating that this mutation did not sensitize immune responses to these tumors (11). Other alterations in DNA damage repair (DDR) pathway genes have also been posited to predict immunotherapy responses in retrospectively analyzed cohorts (12). We present here three cases with perturbations involving DNA replication and/or DDR pathways, two with high TMB associated with *POLE* P286R, but also two with separate mutations in *ATM* which may interact with a co-administered *STING* pathway agonist.

Case Report

Case 1. A 28-year-old female presented with diarrhea, fatigue, and anemia. A diagnostic colonoscopy showed a near-obstructing mass in the transverse colon, with biopsies confirming poorly

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Correspondence to: David Y. Oh, MD, Ph.D., University of California, 513 Parnassus Avenue, HSE301, Box 0519, San Francisco, CA 94143, USA. Tel: +1 4155149301, e-mail: David.oh@ucsf.edu

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Figure 1. (A) Axial post contrast CT image through the liver showing the peripherally enhancing 4.8 cm right hepatic lobe metastasis with internal calcifications (white arrow). (B) CT performed after right hemicolectomy showing increased size of the right hepatic lobe metastasis (white arrow) to 5.5 cm (36 HU). (C) First follow-up CT after induction of immunotherapy demonstrating continued increase size of right hepatic lobe metastasis now measuring 6.4 cm (white arrow, 33 HU). Additionally, there is a new subtle 0.6 cm lesion just anterior to this (white arrowhead). (D) Second follow-up CT after initiation of immunotherapy demonstrating increased size of the right hepatic lobe metastasis measuring 7.7 cm, but increasing central necrosis (26 HU). A smaller lesion (white arrowhead) is again seen, but more well-defined due to the interval necrosis.

differentiated pMMR adenocarcinoma. Imaging revealed synchronous liver masses (largest 4.8 cm, Figure 1A). She received 4 cycles of neoadjuvant FOLFOX, with a subsequent CT scan revealing progression of liver metastases (Figure 1B). Resection of the colon mass revealed scant residual tumor cells in the primary site and 34 negative lymph nodes. Germline testing was negative; however, UCSF500 tumor molecular profiling demonstrated POLE (P286R) mutation, MSS, and high TMB (198.8 mutations/MB; Table I).

She was treated post-operatively with nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks (for 4 doses) followed by nivolumab every 2 weeks. After 4 cycles of combined CPI, re-imaging showed continued growth in the largest liver metastases, with peripheral coarse calcification, enhancing internal rim, and hypoattenuating outer rim (Figure 1C-D). She subsequently underwent partial hepatectomy; pathology from the surgical specimen demonstrated mucin with inflammatory cells and no detectable tumor.

Table I. Summary of microsatellite status, tumor mutational burden, and somatic mutations.

Gene or molecular feature	Case 1	Case 2	Case 3
Microsatellite status	MSS	MSS	MSS
Tumor mutational burden	198.8 mutations/MB (high)	168 mutations/MB (high)	9 mutations/MB (intermediate)
APC	R1273*, S1503*, R2204*	E1577*, L68*, S1222*, R2237*	P692fs*26, T1556fs*3
POLE	P286R	P286R	-
ATM	-	ATM R250* – subclonal	S2408L
TP53	-	F270S	G245S, P190L
Others		ALK Y984C, KDR D39Y, MAP2K1 D67N, PTEN D107Y, E7*, BCL6 R594Q, CBFB E152K – subclonal, CDH1 R598Q, CREBBP G1411E – subclonal, CYLD E626*, EPHA7 R69Q, R895*, ESR1 R256Q, FANCG E492*, INPP4B R250Q, MAP2K4 E107*, E202*, E203*, MLL2 R5448*, MSH6 E368*, PIK3R1 E520*, R461*, R649Q, PRKCI R480C, RB1 E137*, R255*, SETD2 E1922*, SLIT2 D1076N, R205*, SPTA1 E211*, TOP1 R89Q	KRAS G13D, NF1 C2453*, PIK3CA E545G, ACVR1B R420*, DDR1 R514C, FAM123B I332fs*45, FANCC Truncation exon 8

MSS: Microsatellite stable; MB: megabase; *stop codon.

Case 2. A 37-year-old male developed abdominal pain with 20-pound weight loss. A colonoscopy revealed a mass in the ascending colon, and biopsies confirmed a mucinous poorly differentiated adenocarcinoma. Right hemicolectomy revealed a stage IIIC tumor (pT4aN2b) with a positive visceral peritoneal margin and 24 out of 24 positive lymph nodes. After 8 cycles of adjuvant capecitabine and oxaliplatin, imaging revealed metachronous liver metastases, prompting the initiation of FOLFIRI and bevacizumab. Following progression in liver metastases and a subsequent referral to our institution, Foundation One testing revealed a POLE (P286R) mutation, an ATM mutation (predicted loss of function), and an MSS, hyper-mutated phenotype (168 mutations/MB; Table I). Germline testing did not reveal pathogenic mutations. After treatment with 4 cycles of pembrolizumab, imaging showed stability in the liver metastases with some growth in retroperitoneal and gastrohepatic lymph nodes, although with decreased attenuation, compared to pre-treatment scans (Figure 2A-B). Over the course of the following two years, the patient completed 49 cycles of pembrolizumab (two years) with subsequent tumor shrinkage (Figure 2C). Imaging three months after discontinuation of therapy showed continued decrease in liver metastases and lymph nodes (Figure 2D). During pembrolizumab, CEA decreased from baseline of 13.4 to 2.2 µg/l (reference, normal <5.1 µg/l).

Case 3. A 53-year-old woman presented with a 5 cm partially obstructing cecal mass and metastatic liver lesions. Biopsy of the cecal mass demonstrated poorly differentiated adenocarcinoma. Foundation One tumor molecular profiling revealed a potentially pathogenic ATM mutation, TMB 9 mutations/MB, and MSS (Table I). She initiated 5-fluorouracil and oxaliplatin (FOLFOX) with a mixed

response, then 5-fluorouracil and irinotecan (FOLFIRI) with bevacizumab with stable disease. She underwent right hepatectomy and right colon resection with negative margins, but residual disease was confirmed. Following surgery, she developed an anastomotic leak and post-operative chemotherapy was deferred. She subsequently underwent microwave ablation of two hepatic lesions. Prior to treatment with immunotherapy, scans showed enlarging liver metastases and new sub-centimeter lung nodules (Figure 3A). She was enrolled in a phase I clinical trial of intra-tumoral MK-1454, a STING agonist, which was injected into a single segment 3 liver metastasis at a dose of 540 µg weekly for 6 weeks then every 3 weeks, with 200 mg i.v. pembrolizumab every three weeks. After three cycles, CT scans showed decrease in pulmonary nodules and increased size of liver lesions (Figure 3B), although there was marked central necrosis and decreased attenuation. Study treatment was discontinued. Her CEA had risen while on study (CEA pre-treatment: 29.6, peak on-treatment: 72, normal: <5.1 µg/l). CT scans obtained 3 and 5.5 months after the last on-study treatment showed regression in liver metastases with further decreased attenuation and no new lesions (Figure 3C and 3D); CEA and liver enzymes had also returned to normal limits. At 9 months post-treatment without any further cancer-directed therapy, she had continued regression, no abnormal uptake in liver or other regions on PET/CT, and no tumor-associated mutations detectable on Guardant 360 blood test.

Disclaimers. Human investigations were performed after approval by local Ethics Committee. All patient data presented within is de-identified and written consent was obtained from patients to use their images.

Figure 2. Axial CT images from two different levels, one through the region of upper abdomen (top) and another in the mid-abdomen (bottom) at four different time points. (A) Baseline CT images prior to initiation of immunotherapy shows the treated liver metastases (*) and an enlarged right pericaval lymph node (arrowhead) measuring up to 1.9 cm (37 HU). (B) CT images four months following immunotherapy showing new gastrohepatic lymphadenopathy (white arrow, 27 HU) as well as slight increase in right pericaval lymph node now measuring 2 cm in short axis (arrowhead, 32 HU). Liver metastases (*) were not significantly changed. (C) Six-month follow-up CT showing slight decrease of both the retroperitoneal (arrowhead, 36 HU) and gastrohepatic lymph nodes (arrow, 27 HU). (D) Follow-up CT three years after immunotherapy demonstrating decreased size of the retroperitoneal (26 HU) and gastrohepatic lymph nodes (11 HU). Liver metastases (not shown) also decreased in size.

Figure 3. Top image is an axial CT image through the segment 3 lesion that had percutaneous injection. Bottom image is a separate CT image showing examples of other liver metastases. (A) Baseline CT image showing the 3.3 cm hepatic segment 3 metastasis [* , 49 Hounsfield units (HU)] as well as multiple adjacent hepatic metastasis (arrows). (B) CT images 2 months following initiation of the therapy showing increase in size of the hepatic metastases (white arrows) with injected lesion now measuring up to 5.9 cm with central necrosis (*, 43 HU) as well as new liver metastasis (white arrowhead). (C) Follow-up CT 3 months after cessation of therapy (4.5 months after initiation of therapy) demonstrating decreased hepatic metastases with segment 3 metastasis (*) now measuring 3.1cm (37 HU). Other liver metastases have also decreased in size. (D) Images 5.5 months after cessation of therapy (7 months after initiation of treatment) showing continued decrease in size as well as development of internal calcifications.

Discussion

Given the inherent resistance of most MSS CRC to CPI, small subsets of responders can provide valuable insight into mechanisms of response. Tumors with DDR pathway alterations may have a more immunogenic phenotype due to an increased number of mutations and neo-antigens (13). In breast, ovarian, and pancreatic tumors, increased tumor-infiltrating lymphocytes and immunogenic gene signatures within tumors have been associated with DDR mutations (13). However, it has also been demonstrated in BRCA1/2-mutated breast cancer that tumors had less immune cell infiltration and evidence of T cell cytolytic activity, despite higher TMB and the presence of neoantigens (14). This underscores the importance of looking beyond the quantity

of antigens generated by specific DDR pathway alterations to the quality of putative antigens that are produced, which can be crucial to immunotherapy response (15). This is particularly relevant in MSI/MMRd tumors, in which insertion-deletion (indel) mutations contribute to responsiveness to immunotherapy (16, 17), but also in MSS/pMMRd CRC as in this report, where high TMB alone does not confer response (7). Hence MSS CRC patients with non-MMR DDR alterations and exceptional CPI response can reveal other potential predictive biomarkers, but specific mutations must be carefully weighed against existing data (in the context of that tumor type) that they can associate with anti-tumor immunity and CPI response.

Two of our reported cases (Cases 1 and 2) bear the POLE P286R mutation, with a hypermutated phenotype (TMB >100

mutations/MB) indicating that this is a functional mutation, in agreement with other reports (18). While cases of POLE-mutated MSS CRC that sustained durable clinical benefit from immunotherapy have been reported (8, 11, 19), evidence for the association of specific POLE alterations and CPI response are scant. However, in available data, the POLE P286R mutation found in our patients was associated with CPI non-response and low CD8⁺ T cell infiltration in 2 other MSS CRC patients, directly indicating that neither this POLE alteration nor the associated high TMB were immunogenic (11). In an analysis associating pathogenic POLE mutations with immunotherapy response, there were many co-occurring mutations in DDR genes (10). While we cannot exclude a possible contribution of high TMB and/or POLE P286R alterations to responses in our patients, definitive evidence for their predictive value awaits prospective trials of MSS CRC patients with these biomarkers, and underscores the possibility that other DDR alterations may be responsible for the responses we observed.

An alternate explanation may involve alterations in the ATM pathway. Two cases presented herein (Cases 2 and 3) had somatic ATM mutations, a protein that orchestrates the repair of DNA double-stranded breaks (20). In particular, Case 3 had neither high TMB nor POLE alterations, pointing to a possible role for ATM. In addition to receiving pembrolizumab, this patient was also given intratumoral injections with MK-1454, a cyclic dinucleotide that activates the STING pathway and has shown efficacy in several tumor types (21, 22). Intriguingly, in both mice and patient samples, ATM deficiency leads to enhanced type I interferon signaling in response to unrepaired DNA damage, in a manner dependent on STING pathway activation (23, 24). Hence, this particular case highlights that in the absence of MSI, high TMB, or POLE alterations, ATM loss could synergize with immunotherapy targeting the STING activation pathway in conjunction with CPI. Perturbations in ATM may also synergize with other DDR mutations to promote response (25), as in Case 3's co-occurring mutations in TP53 and FANCC (26). It is also noteworthy that the MK-1454 was injected into liver metastases, as the presence of liver metastases has been associated with decreased response to CPI (27). Delivering immunotherapy directly to liver metastases is one approach to address this possible barrier to efficacy.

An additional feature of these cases was initial growth of tumors followed by shrinkage or stabilization of tumor size (pseudoprogression) (28, 29). In clinical trials of intratumoral immunotherapy, progression before response including development of new lesions has been noted in patients who ultimately responded (30, 31). In this series, pseudoprogression was accompanied by decreased attenuation and progressive necrosis. Changes in CT attenuation correlating with tumor necrosis may be a feature of both targeted therapies and immunotherapy (32, 33).

In conclusion, we present three MSS/pMMR CRC cases with sustained dramatic responses to CPI. While two cases harbored known functional alterations in POLE (P286R), with associated hypermutated phenotype, these alterations are not necessarily predictive of response in MSS CRC based on published reports. We identified potentially pathogenic mutations in other DDR pathway genes, notably ATM, which may be responsible for exceptional CPI responses in MSS CRC, including synergy with a STING pathway agonist. Exploration of additional mechanisms that confer response to immunotherapy in CRC is crucial to turning challenges into opportunities.

Conflicts of Interest

Dr. Anuradha Khilnani is a paid employee of Merck. Dr. Nicholas Fidelman, Dr. Chloe Atreya, and Dr. David Oh receive research funding from Merck (Merck & Co., Inc., Kenilworth, NJ, USA). The rest of the authors declare no potential conflicts of interest. Dr. Katherine Van Loon: research funding: Celgene Cancer Care Links; paid consultant: Amgen. Dr. Nicholas Fidelman: research funding: Sirtex Medical, Boston Scientific. Dr. Spencer Behr: advisory board member: Advanced Accelerator Applications (AAA); paid consultant: Cancer Targeting Technologies (CTT). Dr. Chloe Atreya: research funding (institution): Bristol-Meyers Squibb, Novartis, Guardant Health, Kura Oncology; advisory board member: Pionyr Immunotherapeutics, Array Biopharma. Dr. David Oh: research funding: Roche/Genentech, PACT Pharma; paid consultant: Maze Therapeutics. Research support: Dr. Oh is funded by the National Institute of Allergy and Infectious Diseases (K08AI139375).

Authors' Contributions

B.P.K., C.A., and D.Y.O. conceptualized the manuscript. S.C.B. provided and annotated images. B.P.K., K.V.L., A.D.K., N.F., S.C.B., C.A., and D.Y.O. wrote and edited the manuscript.

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