RESEARCH

Hormone receptor expression of colorectal cancer diagnosed during the peri-partum period

Jordyn Silverstein1, Wesley Kidder1,2, Susan Fisher3, Thomas A Hope1,4, Samantha Maisel1, Dianna Ng1,5, Jessica Van Ziffle5, Chloe E Atreya1,2 and Katherine Van Loon1,2

1Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, California, USA
2Division of Hematology and Oncology, Department of Medicine, University of California, San Francisco, California, USA
3Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, California, USA
4Department of Radiology and Biomedical Imaging, University of California, San Francisco, California, USA
5Department of Pathology, University of California, San Francisco, California, USA

Correspondence should be addressed to K Van Loon: katherine.vanloon@ucsf.edu

Abstract

Background: Colorectal carcinoma (CRC) during the peri-partum period is challenging to diagnose due to the overlapping symptoms of CRC and pregnancy. This is the first case series to investigate clinicopathologic, hormonal and molecular features of CRC diagnosed during the peri-partum period. We hypothesized that advanced presentations of CRC could possibly be mitigated by pregnancy-related hormonal factors.

Methods: We conducted a retrospective review of five women diagnosed with CRC during the peri-partum period and studied the clinical and molecular features of their cancer.

Results: All patients presented with stage IV CRC at diagnosis; three had primary tumors in the rectum and two had primary tumors in the sigmoid colon. The liver was the most common metastatic site (three of five women). Immunohistochemistry stains were negative for estrogen receptors alpha (ERα) and beta (ERβ), and one tumor demonstrated low-level positivity for PR (1%). Formalin-fixed and paraffin-embedded (FFPE) biopsies from each case were tested with next-generation sequencing and found that all tumors were mismatch repair (MMR) proficient, and three harbored a KRAS mutation. Germline testing showed no predisposition to CRC; however, several somatic variants of undetermined significance (VUS) were identified.

Discussion: CRC in the peri-partum period poses significant risk factors for presentations with advanced disease due to diagnostic challenges. While our study provides no evidence that pathogenesis of CRC during pregnancy is driven by elevated estrogen and/or progesterone levels during pregnancy, additional putative etiologic factors, including placental growth factors, the immunsuppressive state of pregnancy and other physiologic processes during pregnancy, warrant future study.

Case reports

Patient 1: A 34-year-old female presented with bloody stools, both before and during pregnancy, which were attributed to hemorrhoids. Following her vaginal delivery, she continued to experience bright red blood per rectum, which she treated with diet modifications. At 10 months post-partum, fullness in the right upper quadrant was noted on examination, and an abdominal ultrasound detected hepatomegaly with bilobar hepatic masses. A CT scan of the chest/abdomen/pelvis confirmed numerous hypodense metastases within the liver, with associated compression of the right portal vein, distal sigmoid colon thickening with adenopathy (Fig. 1). A colonoscopy identified a partially circumferential mass in the sigmoid colon, which was biopsied and pathology demonstrated invasive adenocarcinoma.

Key Words
- colorectal cancer
- pregnancy
- estrogen
- progesterone
- molecular features
Patient 2: A 30-year-old female experienced a self-limited episode of diarrhea at 25 weeks gestational age for which she was diagnosed with acute gastroenteritis. At 37 weeks, a cesarean section was performed due to arrest of descent during labor. At 2 weeks post-partum, she began experiencing copious diarrhea that was attributed to post-operative bowel changes. Treatment for \textit{Clostridium difficile} with metronidazole and then oral vancomycin had little impact on her symptoms. Diarrhea persisted with more than ten loose stools per day and electrolyte derangements. A flexible sigmoidoscopy was performed after patient reported the passage of air and stool per vagina, which revealed an area of ulceration and nodularity in the upper rectum. Biopsies confirmed invasive adenocarcinoma. At the time of her diagnosis, she had lost 30 pounds from her pre-pregnancy weight, was incontinent of feces and had developed a rectovaginal fistula. Magnetic resonance imaging of the pelvis at diagnosis revealed a bulky infiltrative high rectal mass extending into the right pelvic sidewall, with direct invasion into the fundus of the uterus and fistulization into the endometrial canal (Fig. 2). A staging PET/CT demonstrated no evidence of metastatic disease; however, peritoneal carcinomatosis was discovered during a laparoscopic diversion procedure.

Background

In the United States, colorectal cancer (CRC) is the third most common cause of cancer-related death (1). Over the past two decades, CRC incidence has declined by 3–4% each year (2). Consistent with CRC screening patterns (3), this decrease in CRC incidence is primarily among patients above the age of 50 years and greatest in patients above the age of 70 years (4). Meanwhile, the incidence of CRC in the United States is increasing among young and middle-aged adults, especially in obese women (5). Among adults between the ages of 20–39, colon cancer rates have increased by 1 to 2.4% annually since the mid-1980s; among adults between ages 40 and 54, rates have increased by 0.5–1.3% annually since the mid-1990s (6, 7).

CRC in pregnant women is rare, with estimated incidence rates between 0.002 and 0.2% of pregnancies (8). An increase in the incidence of CRC during pregnancy has been reported over time but has been assumed to be attributable, at least in part, to trends toward increasing maternal age (9). Prior studies have not consistently identified any association between reproductive history and CRC risk, although some studies have reported an inverse correlation between increased parity and CRC risk (10, 11, 12). Higher parity was however shown in one study to increase risk of CRC in the proximal colon (13). Additionally, hormone replacement therapy has been associated with reduced risk for CRC (14).

There have been few studies of patients with metastatic CRC diagnosed during the peri-partum period (15, 16, 17). A 1993 study examined 41 cases of pregnant women with metastatic CRC, reporting that the majority of cases (64%) originated in the rectum (9). A systematic review of 119 case reports of CRC diagnosed during pregnancy, compared mode of presentation, outcomes of the mother and fetus and treatment decisions (18). None of these...
prior studies have evaluated relevant factors of disease biology or molecular features of the tumors.

Following our observation of five women who presented to our institution with advanced CRC diagnosed during the post-partum period, we hypothesized that advanced presentations of CRC diagnosed in the peripartum period could possibly be driven by pregnancy-related hormonal factors. While a few prior studies have sought to evaluate the expression of estrogen receptors (ERs) and progesterone receptors (PRs) in colorectal tumor cells, results have been mixed. Earlier studies reported that 20–90% of CRC tumors express ER and PR (19, 20, 21); however, more recent data using the currently accepted methodology of immunohistochemistry (IHC) with ER and PR antibodies demonstrated no ER or PR expression in a sample of 156 CRC tumors (22, 23). None of these studies included patients with CRC diagnosed during the post-partum period. Herein, we review the clinical presentations of five cases of CRC diagnosed in the post-partum period, with correlative evaluations of molecular features.

Methods

Patient selection

We performed a retrospective review of five cases of advanced CRC diagnosed in post-partum women cared for at the UCSF Helen Diller Comprehensive Cancer Center during 2016–2017. Convenience sampling was employed, and patients were identified by physician recall. Each patient provided informed consent to participate in a research protocol for the molecular characterization of gastrointestinal malignancies, approved by the UCSF Committee on Human Research (#13-12574).

Pathologic interpretation

Formalin-fixed and paraffin-embedded (FFPE) specimens from either the primary or a metastatic site obtained at the time in closest proximity to pregnancy were obtained. Histology and specimen adequacy were confirmed by a pathologist (D N).

Immunohistochemistry

Immunohistochemistry for estrogen receptor alpha (ERα) and PR were performed using a validated protocol already in use for routine clinical practice in a licensed and accredited anatomic pathology laboratory. Estrogen receptor beta (ERβ) was optimized for dilutions between 1:100 and 1:250, using a tissue microarray with ten cores of tonsil tissue and two cores of placentation tissue, as well as one case each of normal testis, normal adrenal gland and tonsil with reactive hyperplasia. For ERα and PR, a previously tested ER-positive and PR-positive breast cancer was used as a positive control. For ERβ IHC, a tonsil with reactive lymphoid hyperplasia was used as a positive control.

The slides were deparaffinized in Clear-Rite™ 3 (xylene substitute) and hydrated in graded alcohols, deionized water. The staining was performed at room temperature manually. For ERα, ERβ and PR, endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min (H1009; Sigma-Aldrich). The slides were rinsed in tap water for 2 min, and then deionized water for ten dips. For ERα and PR, heat-induced epitope retrieval (HIER) was performed using a Pascal (Dako) with Tris–EDTA pH 9.0 (BP152-500; Fisher Scientific) at 97°C for 60 min, and for ERβ HIER was performed using a pressure cooker (Fagor, Scottsdale, AZ, USA) with citrate pH 6.0 (C7129-500G; Sigma-Aldrich) at 121°C for 10 min, left in the pressure cooker which is removed from the heat source to begin cooling for 10 min and then the container of buffer is removed from the pressure cooker and immersed in a cool water bath for 10 min. The slides were then rinsed in tap water for 1 min and held in distilled water. For ERα, ERβ and PR IHC, the slides were rinsed and immersed in phosphate buffered saline with 1% Tween 20 (P1379-100mL; Sigma-Aldrich) for 10 min. ERα and ERβ IHC slides were incubated for 30 min with primary antibody at 1:100 dilution, and PR IHC slides for 30 min at 1:50 dilution. The slides were rinsed twice with phosphate buffered saline with 1% Tween 20 for 3 min each time. The slides were then incubated with the labeled polymer (Dako Envision+ Rabbit secondary, K400311-2, lot number: 10124979; Dako Envision+ Mouse secondary, K400111-2, Secondary Lot #: 10125910) for 30 min. The slides were rinsed with phosphate buffered saline with 1% Tween 20 for 3 min, twice. The ERα and PR slides were incubated with diamino benzidine (DAB) (K346811-2; Dako) for 3 min, while the ERβ was incubated in DAB for 10 min. The slides were treated with a copper sulfate solution for DAB enhancement for 1 min, and counterstained with Mayer's hematoxylin (HXMHHGAL; American Mastertech) for 3 min. The slides were then washed in 0.08% ammonium hydroxide for four minutes (10600; Electron Microscopy Sciences) to blue the hematoxylin. The slides were rinsed with tap water for at least one minute between each step.
Details of the antibodies used are summarized in Supplemental Table A (see section on supplementary data given at the end of this article). ERα, ERβ, and PR status were reported as binary variables (positive or negative). For ERα, ERβ and PR, a sample was reported as positive when ≥1% of tumor cells showed any nuclear staining and negative when <1% of invasive cells showed any nuclear staining. Cancer tissue was compared to the normal tissue on the same slide with the same thresholds.

**Next-generation sequencing**

All tumors underwent next-generation sequencing (NGS) using the UCSF500 Cancer Gene Panel, which analyzes the exons of 479 genes, and select introns of 47 genes. Genomic DNA was extracted from both tumor and normal tissue using the DNA FFPE TissueKit (Qiagen), and quantified by Qubit fluorometry (Thermo Fisher Scientific). A DNA mass of 250 ng was further processed by KAPA HyperPrep Kits for Illuma – Kapa Biosystems. Target enrichment was performed by hybrid-capture (Roche Nimblegen), and sequencing on an Illumina HiSeq 2500 in rapid run mode (2 × 101 bp read length). An average read depth of 500× was obtained for tumor sequencing, and an average read depth of 300× was obtained for normal sequencing. Sequence reads are de-duplicated to allow for accurate allele frequency determination and copy number calling. Filtering of common germline polymorphisms present dbSNP, as well as technology-specific sequencing artifacts was performed before data analysis. Sanger sequencing was performed using standard reagents and methods; primer sequences for NRAS, KRAS, BRAF and EGFR are available upon request.

PCR products were purified using the Exo/SAP method, and sequencing reactions are performed using Big Dye v3.1 (Applied Biosystems). The sequencing products were separated by capillary electrophoresis on an Applied Biosystems 3500XL Genetic Analyzer. Sequence traces were analyzed using Mutation Surveyor (Softgenetics). Methylation-specific PCR for MLH1 promoter methylation was performed following DNA bisulfite conversion with the EZ DNA Methylation-Lightning™ kit (Zymo) and the EpTect HRM PCR Kit (Qiagen) (24). PCR-based microsatellite instability (MSI) was assessed by a commercial assay (Promega) and separated by capillary electrophoresis on an Applied Biosystems 3500XL Genetic Analyzer.

**Results**

**Patient characteristics**

The clinicopathologic characteristics of the five patients are summarized in Table 1. The median age of patients was 37 (range 30–45), and three were of advanced maternal age (>35 years) at the time of delivery. Two deliveries were complicated by a breech presentation. All patients presented with stage IV disease at diagnosis. All had left-sided primary tumors, three with primary tumors in the rectum and two with primary tumors in the sigmoid colon. The most common metastatic site was to the liver (three of five). None of the patients had known risk factors for CRC including IBD or a history of CRC in a first-degree relative. Oral hormonal therapy was reported by two patients. None had previously undergone a colonoscopy prior to CRC diagnosis. Of the five women, four were alive at time of this report; thus, median overall survival was not calculable.

**Molecular features**

The results of ERα, ERβ and PR IHC performed on tumor specimens and normal tissue are reported in Table 2. All the tumors were negative for ERα and ERβ, and only one was weakly positive (1%) for PR. As a comparison, all normal tissue was negative for ERα, ERβ and PR. The results of NGS are summarized in Table 3. An APC mutation was detected in four tumors; TP53 mutations were detected in four tumors; both APC and TP53 mutations were detected in three tumors. KRAS mutations were detected in three tumors, and both KRAS and TP53 mutations were detected in two tumors. A full summary of the types of somatic alterations is provided in Fig. 3.

**Discussion**

CRC diagnosed during the peri-partum period is rare. However, all patients in this series presented with metastatic disease, raising the question of whether their clinical presentations could be related to unique disease biology or whether the presentations with advanced stage were due to delays in diagnosis, with symptoms masked by pregnancy.

Of all patients diagnosed with CRC, only 21% have stage IV disease at diagnosis (1). However, in a systematic review of 119 case reports of CRC diagnosed during pregnancy, 48% of women presented with metastatic disease.
disease at diagnosis, and stage-for-stage overall survival was worse when compared to nonpregnant women (18). Because symptoms of CRC overlap with those of pregnancy, women may be subject to diagnostic delays. In our cohort, initial signs and symptoms of CRC included nausea, vomiting, malaise, abdominal distension, altered bowel habits and rectal bleeding – all of which are common symptoms during pregnancy. Four out of the five women in this study experienced rectal bleeding, which was attributed to hemorrhoids typically associated

Table 1  Demographic and clinical characteristics.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>34</td>
<td>30</td>
<td>39</td>
<td>37</td>
<td>45</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>Asian</td>
<td>White</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>BMI</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>40 w 4 d</td>
<td>37 w 2 d</td>
<td>39 w 5 d</td>
<td>NSVD</td>
<td>NSVD</td>
</tr>
<tr>
<td>Type of delivery</td>
<td>NSVD</td>
<td>C-section</td>
<td>NSVD</td>
<td>C-section</td>
<td>NSVD</td>
</tr>
<tr>
<td>Complications during pregnancy or delivery</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Obstetric history</td>
<td>G1P1</td>
<td>G1P1</td>
<td>G3P2</td>
<td>G3P1</td>
<td>G2P2</td>
</tr>
<tr>
<td>Conception (IUI vs IVF)</td>
<td>Spontaneous</td>
<td>Spontaneous</td>
<td>IUI</td>
<td>Spontaneous</td>
<td>Spontaneous</td>
</tr>
<tr>
<td>Existing medical conditions</td>
<td>Anemia, anxiety, bulimia, depression, patent foramen ovale, herpes genitalis</td>
<td>Anemia, anxiety, bulimia, depression, patent foramen ovale, herpes genitalis</td>
<td>Gastritis</td>
<td>Anemia, anxiety</td>
<td>Anemia, anxiety</td>
</tr>
<tr>
<td>IBD (UC or CD)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>Former smoker</td>
<td>Never smoker</td>
<td>Administrative worker</td>
<td>Never smoker</td>
<td>Current smoker</td>
</tr>
<tr>
<td>Occupation</td>
<td>Artist</td>
<td>Nurse</td>
<td></td>
<td>Teacher</td>
<td>Horse trainer</td>
</tr>
<tr>
<td>Past colonoscopy</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>History of oral hormone use</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Personal history of cancer</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>First-degree relative with cancer</td>
<td>Skin (father)</td>
<td></td>
<td>Brain (mother)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration between delivery and diagnosis (months)</td>
<td>10.7</td>
<td>3.4</td>
<td>15</td>
<td>5.5</td>
<td>17.1</td>
</tr>
<tr>
<td>Disease stage at diagnosis</td>
<td>IV</td>
<td>IV</td>
<td>IV</td>
<td>IV</td>
<td>IV</td>
</tr>
<tr>
<td>Histologic grade</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Primary tumor location</td>
<td>Sigmoid colon</td>
<td>Rectum</td>
<td>Splenic flexure</td>
<td>Rectum</td>
<td>Rectum</td>
</tr>
<tr>
<td>Metastatic sites</td>
<td>Liver</td>
<td>Peritoneum, lungs</td>
<td>Liver</td>
<td>Liver, bone</td>
<td>Lungs</td>
</tr>
<tr>
<td>CEA at diagnosis</td>
<td>726.6</td>
<td>5.7</td>
<td>1</td>
<td>582.4</td>
<td>78.6</td>
</tr>
<tr>
<td>MMR status&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Proficient</td>
<td>Proficient</td>
<td>Proficient</td>
<td>Proficient</td>
<td>Proficient</td>
</tr>
</tbody>
</table>

<sup>a</sup>Done by IHC testing for MLH1, MSH2, MSH6 and PMS2.

CD, Crohn’s disease; IBD, inflammatory bowel disease; IUI, intrauterine insemination; IVF, in vitro fertilization; NSVD, normal spontaneous vaginal delivery; UC, ulcerative colitis.

Table 2  Estrogen receptor alpha (ERα), estrogen receptor beta (ERβ) and progesterone receptor (PR) status of CRC tumors and matched normal tissue.

<table>
<thead>
<tr>
<th>IHC expression</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER&lt;sub&gt;α&lt;/sub&gt; status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Normal tissue</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>ER&lt;sub&gt;β&lt;/sub&gt; status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Normal tissue</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>PR status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive (1%)</td>
<td>Negative</td>
</tr>
<tr>
<td>Normal tissue</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
with increased pressure in the rectal venous plexus during pregnancy. These findings are consistent with clinical observations from a case series of 119 cases of pregnancy-associated CRC, which also reported that bleeding and abdominal pain were the most common presenting symptoms, occurring in 47 and 38% of the women, respectively (18). Because early diagnosis is one of the most important contributing factors to the prognosis of CRC (25), efforts to increase awareness of the possible concurrence of CRC during pregnancy among obstetric providers could possibly reduce diagnostic delays.

Expanding upon the existing literature which only described the clinical features of CRC during pregnancy, our study sought to improve our understanding of etiologic factors that might contribute to the development of CRC in pregnant women. It is notable that all the women in our series lacked any known predisposition to CRC, including IBD, alcohol consumption, hereditary CRC syndromes and a family history of CRC (26, 27, 28, 29, 30, 31, 32). No germline mutations were detected, which is consistent with a study that found low probability of having a known germline mutation in patients with CRC <40 years old (33). However, numerous VUS were identified in all of the women, which may merit further exploration.

Etiologic mechanisms associated with pregnancy have been the subject of extensive speculation, including the overexpression of pregnancy-related growth factors and human placental growth hormones and the immunosuppressive state of pregnancy. Other cancers have been shown to be hormonally reactive to elevated estrogen and progesterone levels that occur during gestation (19); however, the absence of ER alpha, ER beta and PR expression in the tumors we examined

<table>
<thead>
<tr>
<th>Patient</th>
<th>APC</th>
<th>SOX9</th>
<th>TP53</th>
<th>KRAS</th>
<th>TP53</th>
<th>ARID2</th>
<th>CDH1</th>
<th>SETD2</th>
<th>TP53</th>
<th>BRCA1</th>
<th>p.R496C, GNAS</th>
<th>p.I304D</th>
<th>TGF-β pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>WNT pathway</td>
<td>Genome maintenance</td>
<td>RAS/MAK pathway</td>
<td>PI3K pathway</td>
<td>Other</td>
<td>Chromatin modulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td>WNT pathway</td>
<td>Genome maintenance</td>
<td>RAS/MAK pathway</td>
<td>PI3K pathway</td>
<td>Other</td>
<td>Chromatin modulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 3</td>
<td>WNT pathway</td>
<td>Genome maintenance</td>
<td>RAS/MAK pathway</td>
<td>PI3K pathway</td>
<td>Other</td>
<td>Chromatin modulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 4</td>
<td>WNT pathway</td>
<td>Genome maintenance</td>
<td>RAS/MAK pathway</td>
<td>PI3K pathway</td>
<td>Other</td>
<td>Chromatin modulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 5</td>
<td>WNT pathway</td>
<td>Genome maintenance</td>
<td>RAS/MAK pathway</td>
<td>PI3K pathway</td>
<td>Other</td>
<td>Chromatin modulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3  Next-generation sequencing of tumors.
revealed no evidence that these tumors were mediated by the elevated estrogen and progesterone levels that occur during pregnancy. These results are supported by a study done on 156 CRC specimens from nonpregnant individuals which used the same antibodies and found that none of the 156 tumors stained positive for ER, and only one was PR positive (22). That study and our current study used standard antibodies and contemporary techniques which determine ER status of breast cancers in the modern era. However, an earlier study, which in contrast used a dextran-coated charcoal assay, reported that both normal colon tissue and CRC express ER (40 of 60 were ER-positive) and PR (7 of 50 were PR positive) (20). The dextran-coated charcoal assay is a method that is not currently used clinically, but future studies should compare the efficacy of the different techniques for determination of ER status in colon tissue. Given the lack of detectable ER and PR expression in our five cases, elevated estrogen and progesterone levels during pregnancy seem unlikely to be driving the pathogenesis of CRC during pregnancy. The lack of ER and PR expression is further supported by The Cancer Genome Atlas RNA sequencing data for CRC (34).

In evaluating the molecular profiles of the tumors, the most common mutations were in TP53, APC, and KRAS. Notably, all patients had mutations in the WNT pathway which may be relevant due to crosstalk with estrogen signaling (35); however, the specific mutations in this pathway were heterogeneous among the patients. Beyond these mutations, which are characteristic of CRC tumors (34, 36, 37), there were no clear commonalities between the mutational profiles of the five tumors in our case series.

Without clear support for our hypothesis that tumor pathogenesis was driven by pregnancy-related estrogen or progesterone, alternate hypotheses were considered. One alternate hypothesis for the aggressive pathogenesis of CRC during pregnancy is that prostaglandins and growth factors that are elevated during pregnancy could possibly incite transformation of a precursor lesion or rapid growth and metastasis. Relevant molecules include cyclooxygenase-2 (COX-2) and the insulin-like growth factor (IGF) family members. One study found that COX-2 mRNAs were abundant in CRCs and were low to undetectable in normal colon tissue (38). The IGF family has been shown to have mitogenic and anti-apoptotic effects on proliferation in various epithelial cells, including the colon (39). In addition, high blood levels of IGF-I and IGF-II have been shown to be associated with increased risk of CRC (40).

Another possible hypothesis involves elevated placental production of angiogenic factors that enter maternal blood. By the end of the first trimester of pregnancy, placental cells have invaded the uterus and initiated the flow of maternal blood to the placenta. As a result, the architecture of the maternal–fetal interface promotes the release of the vascular endothelial growth factor (VEGF) family into maternal blood. This is supported by the fact that placental growth factor (PIGF) and soluble fms-like tyrosine kinase 1 (sFlt-1) levels increase as gestation progresses (41). The VEGF family is of particular interest because it has been shown to be angiogenic and tumor promoting (42). Other molecules that the placenta releases into maternal blood include fibroblast growth factor (FGF) and transforming growth factor beta (TGF-β) (43, 44). One study showed that PIGF is overexpressed in tumor types and the circulating form is elevated in patient sera (45, 46). Given this information, it would be reasonable to explore whether the increase in VEGF family molecules and other growth factors during pregnancy could contribute to rapid tumor development.

Lastly, the immunosuppressive state of pregnancy has been postulated to accelerate tumor growth during pregnancy. Exact mechanisms of immunosuppression during pregnancy are not well understood, and the most convincing evidence is more anecdotal than experimental. Infection with the influenza virus during pregnancy is more likely to be severe (47, 48). This phenomenon has also been observed with Ebola (49, 50); however, reasons why pregnant women respond poorly to these viruses is unknown. Additionally, autoimmune diseases have dichotomous effects during pregnancy – some worsen (systemic lupus erythematosus), while others improve (rheumatoid arthritis) (51). This paradoxical response of the maternal immune system could be involved in a lowering of host defenses against CRC and perhaps other tumor types, allowing unbridled growth and rapid spread.

Several limitations of this retrospective case series should be acknowledged. This study was exploratory in nature, and our small convenience sample reflects the rarity of this condition. Although the evaluation of these tumors was notable for the absence of hormone receptor expression or genetic predisposition, these factors cannot be entirely excluded for other similar patients. Additionally, the cancer specimens available were preserved in FFPE, a process that may damage DNA (52), such that robust DNA extraction and sequencing library construction protocols must be applied.
Also, many archived samples available for testing were limited in cellularity; therefore, the specimens used for our analyses may not be representative due to the known possibility of intra-tumoral heterogeneity (53). Moreover, because all patients were diagnosed by biopsies obtained after pregnancy, the ER and PR expression of the tumor during pregnancy could not be analyzed. Theoretically, the ER and PR expression profiles of the tumors could have evolved following delivery, with the tumor hormonally reactive during pregnancy but then downregulated at the time of biopsy in the post-partum period.

Conclusion
These five patients each presented with advanced stage CRC in the peri-partum period, without genetic predisposition or known risk factors. The long periods of symptoms prior to diagnosis raise concern that the symptoms of CRC are often masked by symptoms of pregnancy, resulting in diagnostic delays. This series is notable for consistent presentations with all left-sided tumors; however, there were no other obvious consistencies in molecular and clinicopathologic features among the cases. Weak positivity for PR was detected in only one tumor, and none demonstrated positive staining for ERα or ERβ. Further investigation is warranted to elucidate if and why pregnancy-related hormones and growth factors in the immunosuppressive stage of pregnancy alter the disease biology of CRC and to more completely characterize the molecular features of CRC diagnosed in the peri-partum period.

Supplementary data
This is linked to the online version of the paper at https://doi.org/10.1530/EC-19-0063.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
Jordyn Silverstein received support from the UCSF Resource Allocation Program for Trainees (RAPTr) Program. Dr Chloe Atreya is supported by the NIH and NCI under award number K08CA175143.

Acknowledgments
The authors are grateful to the patients and their family members for their generous contributions to this research study.

References
17 Steinberg L, Nisenbaum HL, Horit SC & Salhaniy KE. Post-cesarean section pain secondary to intussuscepting colonic adenocarcinoma.
Post-partum colorectal cancer


47 Rasmussen SA, Jamieson DJ & Breeze JS. Pandemic influenza and pregnant women. Emerging Infectious Diseases 2008 14 95–100. (https://doi.org/10.3201/eid1401.070667)

48 ANZIC Influenza Investigators and Australasian Maternity Outcomes Surveillance System. Critical illness due to 2009 A/H1N1 influenza in pregnant and postpartum women: population based cohort study. BJM 2010 340 c1279. (https://doi.org/10.1136/bmj.c1279)


Received in final form 22 May 2019
Accepted 10 July 2019
Accepted Preprint published online 10 July 2019