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Systematic diagnostic evaluation for immune-related colitis: a single institutional review of advanced melanoma patients treated with ipilimumab

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Thesis

SYSTEMATIC DIAGNOSTIC EVALUATION FOR IMMUNE-RELATED COLITIS: A SINGLE INSTITUTIONAL REVIEW OF ADVANCED MELANOMA PATIENTS TREATED WITH IPILIMUMAB

by

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SYSTEMATIC DIAGNOSTIC EVALUATION FOR IMMUNE-RELATED COLITIS: A SINGLE INSTITUTIONAL REVIEW OF ADVANCED MELANOMA PATIENTS TREATED WITH IPILIMUMAB

MARLENE GARCIA-NEUER

Abstract

Colitis can be a life-threatening immune-related adverse event (irAE) for patients with metastatic melanoma treated with immune checkpoint blockade, a new anti-cancer immunotherapy. With the increasing use of PD-1/PD-L1 and CTLA-4 inhibitors, particularly in combination in melanoma and other cancers, timely and accurate diagnosis of colitis will become increasingly important for oncologists. The main goal of this study is to understand the clinical presentation of ipilimumab-induced colitis and to validate the use of CT scans as a safe and effective diagnostic tool. We analyzed a cohort of 303 patients who received ipilimumab at Dana Farber Cancer Institute on an expanded access protocol or standard of care between the years of 2008 and 2015. Age, number of doses and frequency of ipilimumab doses were found to be clinical characteristics which could help differentiate patients who develop ipilimumab-induced colitis from those who only present with diarrhea and other gastrointestinal symptoms. Of the 303 patients, 100 (33%) developed diarrhea and 43 (14%) received treatment with corticosteroids for ipilimumab-induced colitis. For all patients with suspected immune-related colitis, an effort was made to firmly establish the diagnosis prior to or immediately after initiation of treatment. Forty-one of 43 patients (95%) who received steroids for presumed immune-related colitis had a colonoscopy and 27 of 43 (63%) patients had both
computed tomography (CT) of the abdomen/pelvis and a colonoscopy including biopsy. In the 31 patients with a CT and biopsy, CT was highly predictive of the presence of colitis on biopsy (sensitivity 85%, specificity 75%, PPV 96%) and the absence of CT findings was predictive of a negative biopsy (negative LR 0.2). In the 44 patients who had symptoms and CT evaluation, CT was highly predictive of the need for steroids to reach resolution of symptoms (sensitivity 85%, specificity 88%, PPV 92%, positive LR 7.3). Fifteen of the 17 patients with negative CT findings did not require steroids to reach resolution of symptoms. In conclusion, CT of the abdomen/pelvis is a fast, reliable, and non-invasive mode of diagnosing ipilimumab-induced immune-related colitis, whereas colonoscopy may not be needed to firmly establish that diagnosis.
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LIST OF ABBREVIATIONS

AJCC……………………………………………………American Joint Committee on Cancer
CMV Colitis……………………………………………. Cytomegalovirus Colitis
CT Scan………………………………………………. Computed Tomography Scan
CTLA-4………………………………………………..Cytotoxic Lymphocyte Antigen 4
IrAE……………………………………………………Immune Related Adverse Event
PD1……………………………………………………Programmed Death 1
PDL-1………………………………………………….Programmed Death 1 Ligand
TCR……………………………………………………T-Cell Receptor
INTRODUCTION

Melanoma is the deadliest form of skin cancer and its incidence is rising faster than any other solid tumor\textsuperscript{1}. In the United States, there were 76,000 new cases in 2014 and approximately 10,000 deaths\textsuperscript{1}. Melanoma develops by malignant transformation of melanocytes, which are cells that make the pigment melanin and are typically associated with the skin but can occur on the eyes, ears, meninges, bone, heart and intestines\textsuperscript{1,2}. The greatest risk of developing melanoma comes from UVA and UVB exposure from the sun or tanning beds. However, genetics also play a role. Lighter skinned people have higher rates of melanoma; non-Hispanic whites are more likely to develop melanoma than those of other races. Melanoma also disproportionately affects men over women and can occur at a wide spectrum of ages. Thickness, ulceration status of the primary skin tumor and the presence or absence of regional lymph node or distant metastases are the major elements of staging and determine the prognosis of melanoma\textsuperscript{1}.

Melanoma accounts for 75\% of skin cancer deaths. Prevention and early diagnosis are still the best treatments for melanoma and, until 2010, distant metastatic melanoma (AJCC stage IV) had a median survival of 6–12 months compared with early Stage 1 melanoma which has a 3-year survival rate of 90\%. Before the advent of the anti-CTLA-4 monoclonal antibody ipilimumab, dacarbazine, a single agent chemotherapy approved by the FDA in 1975, was the first line treatment for patients with metastatic melanoma\textsuperscript{3}. Dacarbazine has a response rate of 6–15\% and only 2\% of patients treated with dacarbazine were alive 6 years after treatment\textsuperscript{2,4}. The overall 10 year survival rate of
patients with metastatic melanoma was 10% in 2009\(^3\).

In 2010, ipilimumab was the first treatment to show improvement in overall survival for metastatic melanoma. Inhibitory checkpoint signals expressed on T cells and other immune cells modulate the immune response after initial activation to prevent damage to healthy self tissues \(^5\). The cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) and programed cell death protein 1 (PD-1) are examples of immune checkpoint points. CTLA-4 acts during the activation of T-cells in the lymph node, while the role of PD-1 is to shut down already activated T-cells in peripheral tissues (further detail below). Immune checkpoint inhibitors are antibody treatments that reduce the ability of specific immune cells to produce inhibitory signals and thus produce a greater immune response to low stimulatory antigens and antigens present in immune suppressive environments. In simpler terms, immune checkpoint inhibitors are a type of cancer treatment which can reactivate attenuated immune cells, increasing the potential for an immune reaction against the cancer\(^5\).

Ipilimumab was the first immune check point inhibitor approved by the FDA (2011) and until recently was the only therapy with improved overall survival for metastatic melanoma. Ipilimumab is an an antibody against CTLA-4 which binds to CTLA-4 and prevents its binding to the ligand CD80/86 as well as preventing the inhibition of T-cell activation. Ipilimumab has a response rate of 10 to 15% and a long term survival of 20% in advanced melanoma\(^6,7,8\). In 2014 two PD-1 immune checkpoint inhibitors were approved by the FDA: nivolumab and pembrolizumab\(^4\). These new therapies were associated with a 30 to 40% response rate in patients with metastatic
The immunological complementary functions of CTLA-4 and PD-1 have lead to clinical trials using combination of ipilimumab and nivolumab to treat metastatic melanoma, showing response rates of 54% in patients with negative PDL-1 status, leading to FDA approval of this regimen in 2015.\textsuperscript{4,9,10}

The blocking of inhibitory immune checkpoint signals also carries the risk of de-regulating self-tolerance leading to immune related adverse events (irAE), which are different from those adverse events typically seen with chemotherapy.\textsuperscript{11} Self-tolerance is the ability of the immune system to recognize and not attack the host’s cells. Immune checkpoints modulate the ability for the immune system to recognize self-cells and to turn off immune reactions when it encounters these cells. IrAE’s arise from the breaking of self-tolerance and thus mimic many known autoimmune diseases.\textsuperscript{5} The irAE’s can include adverse gastrointestinal, dermatological, endocrine and hepatic events. Ipilimumab has been associated with several irAE’s due to the infiltration of normal tissues with active CD4 and CD8 T cells and inflammatory cytokines.\textsuperscript{5,12} Most of the irAE’s are mild to moderate, affecting 70–88% of patients and some association between irAE’s and number of ipilimumab doses have been found but are not directly correlated.\textsuperscript{6,13,14} Toxicities can occur at any point during therapy. Symptoms suggestive of irAE’s such as diarrhea and rash can have infectious or allergic etiologies, which should be ruled out prior to starting treatment with immunosuppressive agents, which could potentially counteract the treatment effect of immune checkpoint blockade. Given the potential life-threatening nature of some irAE’s, a timely and accurate diagnosis is
Validating fast and non-invasive diagnostic tests to identify irAE’s is therefore desirable.

**Ipilimumab Mechanisms of Action**

The immune system has a dual function, which balances tumor surveillance and self-tolerance to identify and eliminate nascent tumors without causing damage to healthy tissues. Tumor surveillance is the continual process by which the immune system identifies and eliminates cancerous cells. The tumor surveillance process is hypothesized to happen continuously throughout life, eliminating many nascent cancers. This is a delicate balance since the immune system must be able to differentiate healthy self-cells from cancerous self-cells. Cancer cells can create environments which up-regulate self-tolerance mechanisms to escape notice of the immune system.\textsuperscript{15,16} This immune suppressive environment can be complex and highly variable. The tumor microenvironment can foster helper T cells which express the CTLA-4 receptor, creating a highly immune suppressive environment.\textsuperscript{17,18,19} CTLA-4 inhibits T-cell activation as part of the primary T cell activation cascade. For a T cell to recognize a tumor as ‘foreign’ and become activated, two steps must occur. Firstly the T cell receptor (TCR) must bind with a tumor associated peptide bound to the MHC receptor of an antigen presenting cell (APC). After the coupling of the TCR with the potential tumor antigen it receives either a positive signal through the CD28 receptor or inhibitory signal through the CTLA-4 receptor. Both receptors bind the same ligand (B7/CD80/CD86) however CTLA-4 binds B7/CD80/CD86 with greater affinity, skewing T cell activation towards tolerance\textsuperscript{15,16}. The importance of CTLA-4 is highlighted by CTLA-4 knockout mice and
CTLA-4 polymorphisms found in humans. Complete blocking of CTLA-4 in murine models leads to massive lymph proliferation and death within 2–3 weeks of major organ failure due to lymphproliferative disease.\textsuperscript{20} No CTLA-4 negative phenotype exists in humans, however, studies in humans have shown that polymorphisms in CTLA-4 receptors are linked to early onset type 1 diabetes, rheumatoid arthritis, autoimmune thyroid disease and endocrinopathies as well as Addison’s disease.\textsuperscript{21,22,23,24} Partially blocking CTLA-4 via an antibody, such as ipilimumab, which binds the CTLA-4 receptor, blocks its ability to receive negative signals and allows for a greater number of T cells to become or stay activated via CD28 receptor binding. Using ipilimumab to partially block CTLA-4 enhances the endogenous immune response to induce tumor regression.\textsuperscript{25} The partial blockade of this T cell inhibitor causes a large range of nonspecific off-target immune-based adverse events.\textsuperscript{13,26} The triggers of the ipilimumab...
induced irAE’s are unknown, but appear to be T cell mediated with characteristic T-cell-rich tissues and inflammatory infiltrates without circulating antibodies. Therefore many of the irAEs mimic known autoimmune inflammatory conditions such as inflammatory bowel disease but show different etiologies which will be discussed later.

**Ipilimumab Induced Colitis: Presentation, Identification and Treatment**

Ipilimumab associated gastrointestinal symptoms are the second most common reported irAE, with about 30–35% of patients having mild symptoms and 5–8% having moderate to severe symptoms. Colitis, which is characterized as inflammation of the colon, can occur at any time during treatment, presentation can be subtle and other causes must be ruled out prior to treatment. Ipilimumab induced colitis can be severe and life-threatening, especially if diagnosis is delayed. Most patients respond to treatment but some become refractory to treatment and have recurrence of symptoms with worsened severity.

The primary symptom is diarrhea but can also include abdominal cramping, nausea, vomiting, GI bleeding, fever, fatigue, dyspepsia, leukocytosis, hypoalbuminemia, and serum electrolyte abnormalities. In any patient receiving ipilimumab, the occurrence of diarrhea is concerning for immune related colitis. The timing of onset can vary even though it typically does not present until after the second dose. Procedures have been developed for the identification and treatment for gastrointestinal adverse events. Firstly, other etiologies, such as other medication-induced diarrhea, infectious diarrhea, such as bacterial (Clostridium difficile) or viral infection (CMV colitis, particularly in cases of reoccurrence after prolonged steroids use) should be ruled out.
through blood and stool studies. A flexible sigmoidoscopy or colonoscopy should be considered for persistent grade 2 diarrhea and any grade 3–4 diarrhea to rule out other diarrhea etiologies and confirm severity of colitis\textsuperscript{29,30,28}. During the workup to confirm ipilimumab-induced colitis, treatment should be held. Grade 1 and uncomplicated grade 2 diarrhea are treated symptomatically and should be carefully monitored. If grade 2 diarrhea persists, ipilimumab should be held and oral steroids should be administered. Patients with grade 3 or 4 diarrhea may need hospitalization during the workup with intravenous hydration, bowel rest, and high-dose IV steroids\textsuperscript{30}.

Figure 2: irAE Gastrointestinal Management Algorithm
Monitoring of patients is critical as bowel perforations can develop\textsuperscript{28}. For patients who have received maximal medical support and are refractory to steroid therapy, treatment with the TNF-alpha inhibitor infliximab should be considered. Partial or complete colectomy may be necessary as a last resort or if bowel perforation occurs\textsuperscript{30,28}. Currently there are no markers to predict who will develop colitis, therefore monitoring and rapid identification of symptoms and administration of corticosteroids are key for an effective treatment strategy\textsuperscript{30}.

**Mechanisms of Ipilimumab Induced Colitis**

The etiology of ipilimumab-induced colitis is believed to be the disruption of gut mucosal immunity. The complex intestinal mucosal immune system dynamically interacts with trillions of bacterial and food antigens. It has a dual purpose of protecting commensal bacteria and creating tolerance to food antigens while also mounting a strong inflammatory response to invading pathogens over a 400m\textsuperscript{2} area\textsuperscript{31}. The gut immune system is comprised of lymphocytes, gut epithelium, connective tissue in the lamina propria, and Peyers patches (small lymphoid tissues). There are specialized subsets of T cells, which mediate different parts of immune response and tolerance. Broadly they are divided into two groups: killer or cytotoxic T- cells and T-helper cells. Generally the two sets of T cells are referred to by their co-receptor expression — cytotoxic T cells express the CD8 co-receptor and T helper cells express the CD4 co-receptor, hence T cells are referred to as CD8\textsuperscript{+} T cells and CD4\textsuperscript{+} T cells. CD8\textsuperscript{+} T cells have the ability to lyse cells that are infected or damaged, whereas CD4\textsuperscript{+} T cells modulate the immune response. A subset of CD4\textsuperscript{+} T cells called T regulatory cells (T\textsubscript{reg}) have immune suppressive
properties. T regulatory cells are identified by the presence of the forkhead transcription factor Foxp3 and the expression of the co-receptors CD4\(^+\)CD25\(^+\). CTLA-4 plays an important part in T\(_{reg}\) suppression of the immune system in a variety of ways\(^{32}\). CTLA-4 on T\(_{reg}\) cells transduces a co-stimulatory signal with the TCR to activate other T\(_{reg}\) cells to increase suppression\(^{33,34,35}\). There is also evidence that CTLA-4 on T\(_{reg}\) mediates suppression directly by triggering the induction of indolamine 2, 3-dioxygenase, an enzyme that catalyzes the conversion of tryptophan to kynurenine, which has immunosuppressive effects in the immediate environment. This induction is achieved by the binding of CTLA-4 with CD80/CD86 receptor on dendritic cells\(^{36,37}\). T\(_{reg}\) cells also constitutively express CTLA-4 unlike their naive T cell counterparts which only express CTLA-4 after activation\(^{34,35}\). Mouse models have shown that antibodies directed at the CTLA-4 receptor abolish the protective effect that CD25\(^+\)CD4\(^+\) T\(_{reg}\) cells provide in inflammatory bowel disease model in mice\(^{34}\). Anti-CTLA-4 antibodies are also used in other mouse models to elicit autoimmune diseases similar to those produced by depletion of CD25\(^+\)CD4\(^+\) T\(_{reg}\) cells without depleting their numbers\(^{35}\). This emphasizes the role of CTLA-4 on T\(_{reg}\) cells as an important part of the immunosuppressive environment, tolerance and ipilimumab-induced colitis.

With respect to ipilimumab-induced colitis, the disruption of the immune-suppressive environment of the gut is thought to result in nonspecific and cross-reactive tissue damage to the intestines caused by activated T cells. Concurrently, there is some evidence that patients with previous autoimmune inflammatory conditions of the bowel are more likely to develop ipilimumab induced colitis\(^{30}\). Other biological markers that
predict susceptibility to ipilimumab-induced colitis have fallen short and currently no established predictive markers exist.

Patients with suspected ipilimumab-induced colitis often undergo both a CT scan of the abdomen/pelvis and a colonoscopy to confirm the diagnosis. Intestinal perforation is a rare but serious complication of colonoscopy, but the risk increases in patients with ipilimumab induced colitis because of the inflamed tissue (0.5–1%)\(^{38,29,6}\). Radiologic findings have not been previously correlated with colonoscopy or biopsy findings for ipilimumab induced colitis. Using CT scans alone as a diagnostic tool to identify ipilimumab-induced colitis would be preferable given its non-invasive nature compared to colonoscopy. The majority of patients with ipilimumab-induced colitis exhibit CT findings of mesenteric vessel engorgement (81.3%), and bowel wall thickening (75%). Some patients have CT findings of fluid-filled distended colon, suggestive of diarrhea, (25%), underlying diverticulosis (25%) and increased mucosal enhancement (18.8%)\(^{39}\). Validating the effectiveness of CT scans for diagnosing ipilimumab-induced colitis could simultaneously decrease time to diagnosis and reduce the risk of bowel perforation associated with colonoscopy.

**Purpose of Study**

The Purpose of this study is to understand the clinical course of patients presenting with gastrointestinal symptoms after ipilimumab and to assess the clinical characteristics that improve diagnosis of immune related colitis after ipilimumab.
Primary Study Question

Can the clinical characteristics of gender, age, body mass index and the timing, frequency and number of doses and symptoms differentiate patients who present with gastrointestinal symptoms from those who go on to develop ipilimumab-induced colitis?

Primary and Secondary Objectives

Primary Objective

1) To understand if the clinical characteristics of gender, age, body mass index and the timing, frequency and number of doses and symptoms can help differentiate patients who present with gastrointestinal symptoms from those who develop ipilimumab-induced colitis.

Secondary Objectives

2a) To evaluate if CT scans are an effective diagnostic tool for the identification of ipilimumab induced colitis, in the setting of finding a low risk diagnostic tool for rapid identification of ipilimumab-induced colitis in patients who present with diarrhea and associated gastrointestinal symptoms.

2b) To assess if the clinical characteristics of gender, age, body mass index and the timing, frequency and number of doses and symptoms can help identify patients with ipilimumab induced colitis who develop bowel perforation.
Study Rationale

Reports on the clinical course of ipilimumab induced colitis, from presentation of symptoms to resolution, have previously been limited to a small number of case studies with varying diagnosis and treatment protocols\(^{40,41}\). There have been no previous studies comparing CT and colonoscopies as diagnostic tools for ipilimumab-induced colitis. Comparative studies between CT and colonoscopy in the general population focus on colorectal cancer screening using CT colonography for the identification of pre-cancerous adenomas whereas in the detection of ipilimumab-induced colitis a CT of the abdomen and pelvis is used\(^{42}\). Of note, the colorectal cancer screening studies are used as preventative diagnostic tools where time is not typically a major concern. A more relevant, though less direct, comparison was studied in inflammatory bowel disease.

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), arises from the deregulation of GI mucosal immunity and thus similarities between ipilimumab-induced colitis and IBD are of interest\(^{43}\). Diagnosis and assessment of IBD was historically achieved via endoscopy however the field has moved towards combined PET/CT scans. A 2007 study found that there was a 100% sensitivity for the detectability of severe lesions in IBD and 70% sensitivity for the detectability of all lesions in IBD with combined PET/CT compared to endoscopy\(^{44}\). Ipilimumab-induced gastrointestinal symptoms and adverse events have been thoroughly documented in the clinical trials of ipilimumab although the diagnostic workup has not been studied systematically. At our institution the majority of patients with suspected immune-related colitis were evaluated with both CT scans and colonoscopy/biopsy. This allows for a
well-documented clinical course of patients who have been treated with ipilimumab and who experienced irAE’s. We hypothesize that better understanding of the clinical course, the validity of our diagnostic tools, and the response to treatment for colitis allows for improved diagnosis and treatment of patients with immune-related colitis in the future.

Methods

Study Design and Subject Population

This retrospective cohort study was conducted at Dana Farber Cancer Institute. Subjects were identified through the electronic medical record system. All patients were diagnosed with metastatic melanoma and had received at least one dose of ipilimumab on an expanded access protocol or as standard of care between the years of 2008 and 2015. To be included, patients must have had at least 1 episode of diarrhea (Table 1). To be placed in the category of having developed ipilimumab induced colitis patients must have had a diagnostic test confirming ipilimumab induced colitis or received steroid treatment for resolution of symptoms.

<table>
<thead>
<tr>
<th>Table 1: Inclusion and Exclusion Criteria</th>
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<tbody>
<tr>
<td><strong>Inclusion</strong></td>
</tr>
<tr>
<td>General</td>
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<tr>
<td>Patient receiving treatment at Dana Farber Cancer Institute and/or Brigham and Women’s Hospital</td>
</tr>
<tr>
<td>Received at least 1 dose of ipilimumab</td>
</tr>
<tr>
<td>Received care on expanded access protocol or as standard of care during 2008–2015</td>
</tr>
<tr>
<td>Gastrointestinal Symptoms</td>
</tr>
<tr>
<td>Had at least 1 episode of diarrhea</td>
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<tr>
<td>Ipilimumab Induced Colitis</td>
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<tr>
<td>Confirmatory diagnostic test or received steroid treatment for resolution of symptoms.</td>
</tr>
<tr>
<td>Exclusion</td>
</tr>
<tr>
<td>Received the majority of care at a clinic not pertaining to Dana Farber Cancer Institute and/or Brigham and Women’s Hospital</td>
</tr>
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</table>
Statistical Analysis

Medical records were reviewed for suspected immune related colitis and the method used for diagnosis (CT scan and/or colonoscopy and biopsy). Other information extracted from the medical record included demographic data: age and sex, but mostly relied on clinical data related to the course of symptoms, treatment and diagnostic tools (Table 2). The primary endpoint for the first objective of the study was to identify criteria that were different between the group of patients who only had gastrointestinal symptoms compared to the group of patients who developed ipilimumab-induced colitis in one or more of the categories extracted from the medical record (Table 2). The primary endpoint for aim 2b was the same. To test statistical differences between the two groups for aims 1 and 2b a two-sided Welch T-test with a 95% confidence interval was used for the continuous variables in Table 1 and a chi-squared test was used for the categorical variables in Table 2. For gender in aim 2b a chi-squared test with Yates correction was used because of the small sample size. These tests were calculated in R along with the mean and standard deviation for each variable or N (%) for categorical variables.

The primary endpoints for objective 2 are to assess the sensitivity, specificity and positive and negative likelihood ratios of the CT scans. Two methods were used to identify the validity of the CT scan results. Firstly, for those patients who received both a CT scan and colonoscopy/biopsy (27 patients), the biopsy results were used to validate the CT findings. For those patients who received a CT scan (43) the CT findings were validated by their need to receive steroid treatment for resolution of symptoms. Patients were identified as having ipilimumab-induced colitis if they had a confirmation biopsy or
needed steroid treatment to manage their symptoms. The analysis for sensitivity, specificity, positive and negative predictive value and positive and negative likelihood ratio was performed in R. Sensitivity is the probability of a patient having ipilimumab-induced colitis and also having positive CT findings. Specificity is the probability of a patient who does not have ipilimumab-induced colitis also having a negative CT. The positive predictive value (PV+) is the probability of a patient who receives a positive CT result actually having ipilimumab-induced colitis and the negative predictive value (PV-) is the probability of a patient with a negative CT result not having ipilimumab-induced colitis. Likelihood ratio (LR) tells us how much of an increase or decrease there is in the probability of finding ipilimumab-induced colitis when we do not know whether the patient has or does not have ipilimumab-induced colitis. There are two types of LR, positive likelihood (LR+) and negative likelihood (LR-). LR+ is the probability of a patient having ipilimumab-induced colitis with a positive CT result over the probability of a patient who does not have ipilimumab-induced colitis having a positive CT. A large LR+, for example between 5 and 10, gives a moderate increase in the likelihood that there is presence of ipilimumab-induced colitis if the CT scan is positive. LR – is the probability of a patient with ipilimumab-induced colitis having a negative CT results over the probability of a patient without ipilimumab-induced colitis having a negative CT results. A very small number in this case (0.1–0.02) gives a moderate increase in the likelihood of not having ipilimumab-induced colitis if CT findings are negative.
Confounders

For objective 1 and 2b, the naturally disproportionate number of men in the study could mask clinically relevant gender differences because of the small sample size of women. Previous chemotherapies of patients were not recorded and could potentially factor into clinical characteristics analysis, especially if they were on chemotherapies or radiation, which could affect their immune system.

For objective 2a, the prevalence and administration of diagnostic tools could be confounding. Colonoscopies and CT scans are administered at the physician’s discretion when the patient presents with severe enough gastrointestinal symptoms to be suspect of ipilimumab-induced colitis. Therefore, sicker patients may receive more diagnostic evaluation skewing the data to show an increase in the ability for the diagnostic tool to detect disease.

Results

<table>
<thead>
<tr>
<th>Table 2: Prevalence of gastrointestinal symptoms, ipilimumab induced colitis and the number of patients who received CT scans and or colonoscopies.</th>
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<tbody>
<tr>
<td>Subgroup</td>
</tr>
<tr>
<td>---------------------------------------------------------</td>
</tr>
<tr>
<td>Received ipilimumab</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
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<tr>
<td>Ipilimumab induced colitis</td>
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<tr>
<td>Steroid treatment for colitis</td>
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<tr>
<td>Bowel Perforation</td>
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<td><strong>Diagnostic Tools Used</strong></td>
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<tr>
<td>CT</td>
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<tr>
<td>Colonoscopy</td>
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</table>
Of the 303 patients who received ipilimumab 33% reported diarrhea and 15.18% developed ipilimumab-induced colitis (Table 2). In both the diarrhea only group and ipilimumab induced colitis group there were fewer women 20 (37%), and 15 (32.6%), respectively, compared to men 34 (63%) and 31 (67.4%), respectively (Table 3). There was no difference in the occurrence of gastrointestinal symptoms or developing ipilimumab induced colitis based on gender (Table 3).

<table>
<thead>
<tr>
<th>Table 3: Demographic and clinical patient characteristic analysis.</th>
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<td>Subgroup</td>
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<tr>
<td>Gender, n (%)</td>
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<td>Male</td>
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<tr>
<td>Female</td>
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<tr>
<td>Age</td>
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<tr>
<td>BMI</td>
</tr>
<tr>
<td>LDH</td>
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<tr>
<td>Number of doses before onset of symptoms</td>
</tr>
<tr>
<td>Days to onset of symptoms from first dose of ipilimumab</td>
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<tr>
<td>Days to resolution of symptoms</td>
</tr>
<tr>
<td>Days before onset of symptoms per doses of ipilimumab</td>
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</tbody>
</table>
Patients who developed ipilimumab-induced colitis were older (65.14, ±13) compared to patients who reported diarrhea (58.13, ±13.4) \((p=0.01289)\). BMI and LDH were not different between the two groups (BMI: 28.4, ±4.9 and 28.8, ± 5.9; LDH: 244.9, ±203 and 275, ±254) (Table 3). Patients who developed ipilimumab-induced colitis had significantly more doses of ipilimumab (3.1, ± 1.4) than did patients with diarrhea, but not colitis (2.5, ± 1.2, \(p=0.04162\)). Patients who developed ipilimumab-induced colitis did not have a statistically significant difference between time of onset of symptoms after the first dose of ipilimumab (53.8 ± 35) than did those who only developed diarrhea (71.3 ± 78, \(p=.1247\)). Patients with ipilimumab-induced colitis had significantly longer time to resolution of symptoms than patients with diarrhea only (37.7, ±29 and 6.2, ± 7.9,
respectively $p=1.225 \times 10^{-8}$) (Table 3). The frequency of days per dose of ipilimumab was shorter for patients who developed ipilimumab-induced colitis than for those who only developed gastrointestinal symptoms ($17.9 \pm 9.1$ and $24.7 \pm 21.2$ $p= 0.0387$, respectively) (Table 3).

Bowel perforation was an uncommon complication of ipilimumab-induced colitis with a prevalence of 1.65% and only occurred in patients who also received a colonoscopy. Patients with bowel perforation were significantly older ($72, \pm 5.45, = 5$) compared with those who did not have a bowel perforation ($64.2 \pm 13.61, = 41$) with a p-value of less than 0.05 ($p= 0.01257$) (Table 4).

Of the patients who were treated with ipilimumab and developed symptoms typical of ipilimumab induced colitis 43 and 47 had symptoms severe enough to result in a CT scan or a colonoscopy with biopsy, respectively (Table 2). Common findings from the CT include bowel wall thickening (55%) and air or fluid filled bowel indicative of diarrhea (23%) (Table 5). Only 3% of the patients who were diagnosed with colitis did not have defined findings on their CT scans.

<p>| Table 5: Sensitivity and specificity analysis of CT scans by biopsy and treatment with steroids. |
|-----------------------------------------------|-----------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Biopsy</th>
<th>Steroid Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>31</td>
<td>44</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>85.19%</td>
<td>85.19%</td>
</tr>
<tr>
<td>Specificity</td>
<td>75.00%</td>
<td>88.24%</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>95.83%</td>
<td>92.00%</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>42.86%</td>
<td>78.95%</td>
</tr>
<tr>
<td>Positive Likelihood Ratio (LR+)</td>
<td>3.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Negative Likelihood Ratio (LR-)</td>
<td>.19</td>
<td>.16</td>
</tr>
</tbody>
</table>
The diagnostic performance of the CT scan was validated using two methods. All patients who received a colonoscopy also underwent a colon biopsy. Therefore, of the 31 patients who received both diagnostic tools (CT and Colonoscopy) the CT findings were compared to the biopsy results from the colonoscopy (Table 5: Biopsy group). The sensitivity and specificity of CT to detect colitis was 85 and 75%, respectively.

The positive and negative predictive value of the CT was 95.8 and 42.9, respectively. The negative likelihood ratio showed a moderate increase in the probability of ruling out disease (0.19) and the positive likelihood ratio showed a small increase in the probability of ruling in disease (3.3)\(^45\).

The second approach to validate the use of CT for the diagnosis of immune-related colitis was to correlate the CT results with the use of steroids to achieve resolution of colitis symptoms (Table 5, Treatment group). With this approach, the sensitivity and specificity of CT was of 85.2 and 88.2%, respectively. The positive and negative predictive value of the CT was 92 and 79, respectively. The negative likelihood ratio showed a moderate increase in the probability of ruling out disease (0.16) and the positive likelihood ratio also showed a moderate increase in the probability of ruling in disease (7.2)\(^45\).

**Discussion**

Colitis can be a life-threatening immune-related adverse event (irAE) for patients treated with immune checkpoint blockade. With the increased use of immune checkpoint blockade, particularly the combination of anti-CTLA-4 and PD-1 inhibition in melanoma
and other cancers, timely and accurate diagnosis of colitis will become increasingly important for oncologists.

Clinical Presentation and Characteristics of Ipilimumab-Induced Colitis in the Clinical Setting

Of the 303 patients who received ipilimumab at Dana Farber Cancer Institute, 97 developed diarrhea and, 46 developed ipilimumab induced colitis, and 43 required corticosteroids for resolution of their symptoms (Table 2). The study cohort had more men, 67% in the ipilimumab-induced colitis groups and 63% in the group that only had symptoms. There was no difference in the frequencies of diarrhea or ipilimumab-induced colitis based on gender (Table 4). The frequencies of diarrhea (33%) and colitis (15%) in our cohort were similar to those reported in other studies and were similar in the patients treated on expanded access protocol and as standard of care 6,26,13,39 (Table 2). Bowel perforation was slightly more common (1.65%) in our cohort compared to the frequencies reported from clinical trials (0.5–1%) which could reflect a sicker patient population 28,11 (Table 2). Advanced age seems to be a potential risk factor for the development of ipilimumab-induced colitis (65.14 and 58.13, \( p=0.01 \)) and bowel perforation (72.8 and 63.3, \( p=0.012 \))(Tables 4, 5). This could be due to an aging gut: some research has shown that both the physiology and the microbiome of the gut change with age and may contribute to increased inflammation and immune deregulation 46,47. It may be interesting to investigate fecal inflammatory markers such as calprotectin, lactoferrin, M2-PK and S100A12 from patients before the first dose of ipilimumab and
see if there are any elevated markers that correlate to developing ipilimumab-induced colitis\textsuperscript{48}. Concurrently, a microbiome panel can be established from patients before the start of treatment to see if any specific composition of bacteria can lead to an increased risk of developing ipilimumab induced colitis\textsuperscript{46}. The increased risk of bowel perforation in older patients highlights the need for non-invasive diagnostic methods. The number of days per dose of ipilimumab (or ipilimumab-days) was lower for patients who developed ipilimumab-induced colitis than for those who only developed gastrointestinal symptoms (17.9 and 24.7, respectively)(Table 3). This indicates that patients who received ipilimumab in a shorter time period or had less time between each dose of ipilimumab had a greater chance of developing ipilimumab-induced colitis. This could be due to two situations: either the dosing scheduling does not allow enough time between doses to lower toxicity or it could be that for the ipilimumab-colitis-susceptible patient population having more spacing between doses could limit the development of ipilimumab induced colitis.

The clinical course of gastrointestinal symptoms may also help in predicting which patients will develop colitis requiring steroids for resolution. Patients who do not develop colitis have a significantly shorter course of diarrhea (6 days) compared with those who develop colitis (37.6 days) (Table 3). Another noteworthy clinical characteristic, of those patients who develop colitis after presenting with diarrhea is that they have received significantly more doses of ipilimumab (3.2), than those who have diarrhea but do not develop colitis (2.5) (Table 3). Previous studies have shown that there is some correlation of ipilimumab-induced toxicity affirming the results seen here. The
duration of diarrhea and the number of ipilimumab doses received may help to risk stratify patients in a way that can help the clinician decide which patients require further diagnostic evaluation.

**CT Findings and Validation**

The most common findings on CT scans in patients with ipilimumab-induced colitis were bowel wall thickening (55%) and air or fluid filled bowel (23%) (Table 4). This is similar to previously documented CT findings in ipilimumab induced colitis. Bowel wall thickening and air or fluid filled bowel may be characteristics of ipilimumab-induced colitis. To help increase the accuracy of CT scan findings further, better understanding of the CT findings reflecting pathology are desired. A follow-up experiment that has potential to yield useful information would be the prospective gathering and evaluation of CT scans of a greater number of patients with ipilimumab induced colitis and those without to establish precise pathology characteristics of CT-findings that can help identify ipilimumab-induced colitis.

Colonoscopy and CT scans were performed commonly but not universally in patients who presented with symptoms suggestive of colitis (Table 5). In the first assessment of the predictability of the CT scan, 31 patients were analyzed who had received both a CT scan confirmed with colonoscopy and biopsy (Table 5, Biopsy group). The CT could correctly identify 85% of patients who had ipilimumab-induced colitis and 75% of patients without colitis. The negative likelihood ratio was small (0.19) which means there was a moderate increase in the probability that the test accurately
ruled out disease or that the CT scan had a large to moderate ability to identify the absence of disease. The positive likelihood ratio was not very large (3.3) and therefore there was only a small increase in the probability that the CT scan could correctly identify the presence of colitis.

For the patients who only received a CT scan without colonoscopy and biopsy we confirmed the CT results with the need for steroids to achieve resolution of colitis. The second approach to validate the CT results was to correlate the CT results with the use of steroids to achieve resolution of colitis symptoms (Table 7, Treatment group). The CT could correctly identify 85% of patients who had ipilimumab-induced colitis and 88% of patients without disease. The negative likelihood ratio was small (0.16) which means there was a moderate increase in the probability that the test accurately ruled out disease or that the CT scan had a large to moderate ability to identify the absence of colitis. The positive likelihood ratio was large (7.2) and therefore there was moderate increase in the probability that the CT scan could correctly identify the existence of colitis (Table 7).

Given these findings, we propose that colonoscopy poses an unnecessary risk and may not be needed to firmly establish the diagnosis of ipilimumab-induced colitis in the presence of diarrhea and CT findings of colitis.

**Limitations**

Fifteen of the 17 patients with negative CT findings did not need steroids to reach resolution of symptoms. Not all patients received a CT scan or colonoscopy. Those who underwent both procedures may have had more severe symptoms and therefore may have been more likely to have positive results on CT or colonoscopy. This could lead to an
over exaggeration of the accuracy of CT scan. However, since we assessed all patients who developed gastrointestinal symptoms we likely captured the milder cases as well in our analysis. Additionally, given the retrospective nature of this evaluation the patients who went on to get further diagnostic evaluation are the patients that were deemed clinically worrisome to the provider and therefore the patient population for which we hope to find a safe and accurate diagnostic tool. Another limitation of this study was that no multivariable analysis was performed to identify risk factors for colitis. Therefore, potential confounders may be present that were not detected in the general analysis. A multivariable analysis could not be calculated for perforation since the sample size was too small (5 events).

**Future Direction**

To minimize confounding in aim 2 of the overexaggeration of the accuracy of CT scan because sicker patients may receive more CT scans, a prospective study could be initiated where all patients receive a CT scan when they report diarrhea or other gastrointestinal symptoms suggestive of colitis. The CT evaluation could then be evaluated by the need for steroid therapy to resolve symptoms. Since all patients would be receiving a CT scan, a more accurate assessment of the accuracy of CT scan at identifying ipilimumab-induced colitis could be achieved.

Age has not previously been reported as risk factor for adverse events stemming from ipilimumab treatment, however our approach was unique since we looked at the difference between gastrointestinal symptoms and development of ipilimumab-induced colitis as one group which separates into two. Previous studies have assessed age, but
only within the separate groups and found no difference\textsuperscript{6} The aging gut is known to be physiologically different as well as to harbor a different microbiome, which may contribute to increased inflammation and immune deregulation\textsuperscript{46,47}. Several follow up experiments could be done; primarily it would be interesting if differences in the microbiome would be supported in other patient populations. Several retrospective cohort studies could be performed at varying institutions looking at patients who develop gastrointestinal symptoms and those who continue on to develop colitis with age being the primary endpoint. To understand the biological mechanisms and also potentially elucidate a predictive marker for development of ipilimumab-induced colitis, a prospective cohort study could be performed which analyzes fecal samples from patients who have gastrointestinal symptoms and compares them to patients who develop-ipilimumab induced colitis. The endpoints would be inflammatory markers such as calprotectin, lactoferrin, M2-PK and S100A12 as well as a microbiome panel analysis to look at difference between patient groups\textsuperscript{48,46}.

\textbf{Conclusion}

In conclusion, CT of the abdomen/pelvis is a fast, reliable, and non-invasive mode of diagnosing ipilimumab induced immune-related colitis, whereas colonoscopy may not be needed to firmly establish that diagnosis. Given these findings, we propose that colonoscopy poses an unnecessary risk and may not be needed to firmly establish the diagnosis of ipilimumab-induced colitis in the presence of GI symptoms and CT findings of colitis.
References


CURRICULUM VITAE

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Year of Birth: 1992

Education

MSc
May 2016 Masters of Science in Clinical Investigation Boston University Medical School.
GPA 3.62 Thesis: Systematic diagnostic evaluation for immune-related colitis: a single institution review of advanced melanoma patients treated with ipilimumab. (Thesis work performed at Dana Farber Cancer Institute)

BSc
May 2014 Bachelor of Science in Microbiology, Cum Laude, Barrett, the Honors College at Arizona State, GPA 3.41.
Thesis: The Public Versus Private Nature of Naïve T Cell Receptor Gene Recombination Profiling

Research Experience

Graduate Research January 2015– May 2016
Retrospective Review of Patients with Ipilimumab-Induced Colitis, Dana Farber Cancer Institute, Protocol 15-045 (20 hrs/wk 2015). Advisor: Dr. Patrick Ott.
Responsibilities: creation, analysis and interpretation of data set from patients.

Undergraduate Research January– May 2014
B Cell Receptor DNA Origami Project, Center for Infectious Disease and Vaccinology, Biodesign, (20 hr/wk). Advisors: Drs Joseph Blattman, Hao Yan.
Responsibilities: designed and performed experiments, analysis of data.
October 2012–December 2013

**T Cell Receptor DNA Origami Project**, Center for Infectious Disease and Vaccinology, Biodesign, (20 hr/wk) Advisors: Drs. Joseph Blattman, Hao Yan

**Responsibilities:** assisted with experiments.

October 2011–September 2012

**T Cell Receptor Recombination Diversity Project**, Center for Infectious Disease and Vaccinology, Biodesign, Arizona State University (20 hr/wk.) Advisor: Dr. Joseph Blattman. **Responsibilities:** designed, performed and analyzes experiments. Manuscript in preparation.

**Presentations**

SMR Annual Meeting November 2015


AAI Annual Meeting May 2014


AAAS Annual Meeting February 2014


Arizona/Nevada Branch ASM March 2012

**Using Single-Chain TCR Transgenic Mice to Estimate Total TCRab Pairing Diversity** Marlene Garcia-Neuer, Philip Johnson, Harlan Robins, Rustom Antia, & Joseph Blattman. Local Arizona Nevada Branch of American Society of Microbiology Arizona State University, Tempe, AZ 2012.
Leadership
Research Mentor 2013–2014

Research Mentor, Gamma Delta T Cell Receptor Project, CIDV (Center for Infectious Disease and Vaccinology), Biodesign, Arizona State University, Tempe, AZ and Hamilton High School, Chandler, AZ

(ca. 6 hr/wk) Advisor: Dr. Joseph Blattman

Responsibilities: Helped design, perform, analyze and prepare project for presentation at a national science fair competition alongside three high school students.

BIOARTISTRY Mini Lecture Series 2014

Initiator and Organizer, BIOARTISTRY Mini Lecture Series, Arizona State University, Tempe AZ. 2014.

Description: Hosted faculty talks from both the School of Life Sciences and the Herberger School of Art, bringing together faculty and students from both disciplines to engage in meaningful discussion about the interface of science and art.

Undergraduate Research Association 2014

Founding Member, Undergraduate Research Association, Arizona State University, Tempe, AZ. 2014

Goals: Provided a supportive community for undergraduates in research. Aided students in finding and continuing research. Organized and managed an undergraduate research journal and poster symposium.

ASU Flying Samaritans Club 2013–2014

Vice President, Arizona State University Flying Samaritan’s Club, Tempe, AZ. 2013–2014

Responsibilities: Coordinated meetings, trips and fundraisers. Directed the Medical Spanish Series. Worked with club officers to assess and achieve club goals.
Honors and Awards

Travel Grant 2015
Society for Melanoma Research Travel Grant, awarded from Boston University Graduate Medical School. Amount: $250

SOLUR Research Fellow 2014
School of Life Science Undergraduate Research Grant, Arizona State University, Tempe AZ. Amount: $2,000

Travel Grant 2014
AAAS National Conference Travel Grant Award from Barrett the Honors College and the Center for Biology and Society. Amount: $1,000

Scholarship 2010–2014
Dean’s Award Scholarship, 4 years, Arizona State University, Tempe, AZ. Amount: 2,500 per year.