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### Checkpoint and Cyclooxygenase Inhibition in Microsatellite Stable Metastatic Colorectal Cancer

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CHECKPOINT AND CYCLOOXYGENASE INHIBITION IN MICROSATELLITE  
STABLE METASTATIC COLORECTAL CANCER

A Thesis Presented to The Faculty of the School of Medicine  
Yale University

In Candidacy for the Degree of  
Master of Medical Science

August, 2019

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## **ABSTRACT**

Traditionally, metastatic colorectal cancer has been treated using cytotoxic chemotherapy but the development of immunotherapeutic agents has afforded higher durable remission rates and more tolerable side effect profiles in a small subset of patients. Immunotherapy treatments are currently approved for the treatment of microsatellite instability high subgroup that comprises four percent of metastatic colorectal cancer. However, immunotherapy treatments have little clinical activity in the microsatellite stable subgroup, which encompasses the majority of colorectal cancers. In this phase II trial, we propose to study the efficacy and safety of a three-drug regimen comprised of two immunotherapy treatments, programmed death 1 and cytotoxic T-lymphocyte associated protein 4 blockade, and a cyclooxygenase inhibitor in the microsatellite stable subgroup. This combination aims to increase the treatment eligible proportion of colorectal cancers by establishing a viable immunotherapy option for the microsatellite stable subgroup.

## **CHAPTER 1: INTRODUCTION**

### **1.1 BACKGROUND**

Immuno-oncology is a new paradigm in cancer treatment that has the potential to provide unprecedented and robust tumor regression as well as increased survival compared to traditional chemotherapeutic agents in several cancers even at an advanced stage. The first immune checkpoint inhibitor (ICI), a human cytotoxic T-lymphocyte antigen 4 (CTLA-4)-blocking antibody named ipilimumab (Yervoy; Bristol Myers Squibb) was approved in 2011 and many others began development based on its unmatched success.<sup>1</sup> ICIs are now a standard of care for various cancer types such as metastatic melanoma, non-small cell lung cancer (NSCLC) and renal cell carcinoma.<sup>2</sup>

In colorectal cancer (CRC), immunotherapies have achieved remarkable efficacy shown by significantly prolonged progression free survival (PFS) and safety profiles that are favorable compared to traditional chemotherapy, but only in a small subset. Three ICI therapies were approved recently for a small subset of metastatic colorectal cancers (mCRC): pembrolizumab (Keytruda), nivolumab (Opdivo) and the combination treatment nivolumab and ipilimumab (Yervoy).<sup>3-5</sup> This subset is comprised of patients with the biomarker called microsatellite instability high (MSI-H) or deficient mismatch repair (dMMR) that makes up 15-20% of the CRC and about 4% of the mCRC population.<sup>6</sup>

### **1.2 STATEMENT OF THE PROBLEM**

While immune checkpoint inhibitors are capable of causing tumor regression and improved overall survival with fewer side effects compared to traditional chemotherapy, only a minority of the CRC patients responds to treatment. The importance of increasing

the response rate of ICIs in CRC and specifically in microsatellite stable/proficient mismatch repair (MSS/pMMR) colorectal cancer is paramount.

### **1.3 GOALS AND OBJECTIVES**

Prior preclinical data suggest that the combination of checkpoint inhibition and COX inhibition might achieve a higher response rate in a subset of MSS CRC patients.

The aim of this study is to find whether the combination treatment of anti-PD-1/anti-CTLA4 and anti-COX treatment will enhance the response rate in MSS mCRC, while maintaining an adequate safety profile. We will also evaluate the efficacy measures of PFS and OS, toxicity profiles and associations with known biomarkers.

### **1.4 HYPOTHESIS**

This parallel assignment, randomized allocation phase II trial is designed to study the hypothesis that the combination of PD-1 and CTL4 blockade and COX-2 inhibition or COX-1 and 2 inhibition significantly increases the objective response rates (ORR) (23.7%) compared to the historical response rate of 8.7% with PD-1/CTLA4 blockade alone in MSS/pMMR mCRC that has progressed on previous treatment with cytotoxic chemotherapy with irinotecan, fluorouracil and oxaliplatin. The expected effect size of 15% will be tested for statistical significance.

### **1.5 DEFINITIONS**

PD-1: Programmed death 1 receptor, co-inhibitory receptor found on effector T cells

PD-L1: Programmed death ligand 1 found on tumor cells

CTLA-4: Cytotoxic T-lymphocyte associated protein 4, co-inhibitory receptor found on recently activated T cells

CD80/CD86: Ligand for CTLA-4, found on tumor-associated antigen presenting cells (APCs)

Pembrolizumab (Keytruda): Humanized anti-PD-1 antibody (Merck & Co.)

Nivolumab (Opdivo): Fully human anti-PD-1 antibody (Bristol-Myers Squibb)

Ipilimumab (Yervoy): Fully human anti-CTLA-4 monoclonal antibody (Bristol-Myers Squibb)

Celecoxib (Celebrex): A selective COX-2 inhibitor (Pfizer)

ASA: Aspirin, a dual COX-1 and COX-2 inhibitor

FOLFOX: Combination chemotherapy containing oxaliplatin with fluorouracil (5FU) and folinic acid

FOLFIRI: Combination chemotherapy containing fluorouracil (5FU), folinic acid and irinotecan

FOLFOXIRI: Combination chemotherapy containing oxaliplatin with fluorouracil (5FU), folinic acid and irinotecan

Capecitabine (Xeloda): Antimetabolite chemotherapy agent (generic)

5FU(Adrucil): fluorouracil, an antimetabolite chemotherapy agent (generic)

Irinotecan (Campostar): Topoisomerase inhibitor chemotherapy agent

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## **CHAPTER 2: REVIEW OF THE LITERATURE**

### **2.1 INTRODUCTION**

We searched relevant clinical trials; articles and conference abstracts published in the English language in PubMed and Ovid MEDLINE, Cochran Review and ClinicalTrials.gov between the years 1980 and 2018. The searches were conducted with the MeSH subject headings of “immunotherapy”, “colorectal cancer”, “metastatic”, “COX-2 inhibitors” and “microsatellite stable”. Other non-MeSH terms we searched were “deficient mismatch repair”, “proficient mismatch repair”, “checkpoint inhibitor”, “COX inhibition” and “combination therapies”.

#### **2.1a Incidence of Colorectal Cancer**

Colorectal cancer (CRC) is the third most common cancer as well as third leading cause of cancer mortality in the US<sup>7</sup> and the second leading cause of cancer mortality worldwide.<sup>8</sup> Although the recent advances in screening improved both incidence and mortality rates, The National Cancer Institute estimates that there will be 140,250 new cases and 50,630 deaths associated with CRC in the United States in 2018. CRC comprises 8.1% of new cancer diagnoses and the median age at diagnosis is 67. mCRC or Stage IV CRC that is defined by spread of the disease to distant sites in the body, comprises 21% of the CRC population. The incidence is slightly higher in men than women, and in African Americans, compared to other races.<sup>9</sup>

#### **2.1b Prognosis**

Based on data from Surveillance, Epidemiology and End Results (SEER) from 2008 to 2014, the overall 5-year survival rate for CRC is 64.5% in the US. The 5-year survival rate for localized CRC is 89.8%, for regional is 71.1%, and for distant metastases is 13.8%.<sup>9</sup> Prognostic biomarkers are lymph node involvement, presence of metastases,

right- vs. left-sided tumor, microsatellite instability (MSI-H)/deficient mismatch repair (dMMR) vs. microsatellite stable (MSS)/proficient mismatch repair (pMMR), KRAS, BRAF and NRAS mutation status.<sup>10</sup>

### **2.1c Current Treatment Guidelines**

As metastatic tumors are rarely resectable or treatable solely by radiation and generally require systemic treatment. The main systemic treatment options for mCRC are traditional cytotoxic chemotherapy, anti-VEGF or EGFR therapy.<sup>10</sup>

First line chemotherapeutic agents such as FOLFOX, FOLFIRI, Capecitabine and 5FU/leucovorin have been used with or without the anti-angiogenic agent bevacizumab. For high tumor burden or rapidly progressing disease the quadruple chemotherapy FOLFOXIRI can be considered. These agents can be used as neo-adjuvant therapy or post-resection and are generally administered every 2-3 weeks for several cycles. The efficacy and response rate of chemotherapy varies based on the regimen. In a representative study looking at chemotherapy versus chemotherapy with an anti-angiogenic agent, overall survival (OS) in mCRC was 15.6 months with IFL (irinotecan, 5FU and leucovorin) and 20.3 months with IFL plus bevacizumab, corresponding to a hazard ratio of 0.66 for death ( $p < 0.001$ ). The response rates were 44.8% and 34.8% respectively.<sup>11</sup>

For refractory mCRC the drugs regorafenib or trifluridine/tipiracil are recommended and offer a modest survival benefit.<sup>10</sup> A meta-analysis evaluating data for 702 patients in 12 studies, assessing the efficacy of regorafenib found summary progression free survival rates of 3.34 months and overall survival rates of 7.27 months but the response rate was a modest 2%.<sup>12</sup> The side effects of chemotherapy vary based on the combination but the most commonly reported are diarrhea, fatigue, myelosuppression, neuropathy, hair loss

and cold sensitivity. Some side effects, such as neuropathy can be treatment limiting and irreversible.<sup>10</sup>

Recent advances in immunotherapy afforded new checkpoint blockade options for a small subset of mCRC patients, discussed in Section 2.2.

## **2.2 REVIEW OF EMPIRIC STUDIES**

### **2.2a Immune Checkpoint Inhibitors in the Treatment of Colorectal Cancer**

The immune system recognizes foreign invaders such as bacteria and viruses but it can also mount an attack against our own deviant cells, such as cancer cells. Attributed to the number of mutations they accumulate, cancer cells can display abnormal epitopes called neo-antigens on their surface. Neo-antigens recognized by the immune system can trigger an immune attack leading to the destruction of the cancer cells. Checkpoints built into the system to help curb aberrant autoimmune reactions toward healthy cells. The co-receptor portion of his double system, consisting of the antigen presented by an antigen presenting cell (APC) and a co-receptor whose activation can prevent immune system activation is a frequent target of cancer immune evasion. Some neoplasms acquire mechanisms to evade destruction by T-cells by hijacking these checkpoints leading to the dampening or curbing of the immune attack despite neo-antigen recognition. This immune evasion generally takes place by negative regulation of T-cell activation either during primary activation or during the memory forming immune response phase.<sup>1</sup>

The two most well studied of these checkpoints are cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) and its ligand programmed death-ligand 1(PD-L1). CTLA-4 is a co-inhibitory receptor found on recently activated T cells whose ligand is found on tumor associated APCs. PD-1 receptors work as co-inhibitory receptors on effector T cells. Their ligand, PD-L1,

programmed death ligand 1 is found on tumor cells. Many other newly discovered checkpoints are now being investigated as future therapeutic targets.

Immune checkpoint inhibition (ICI) is a strategy that aims to stimulate immune cells, mainly T cells. Several ICI agents have emerged, commonly targeted pathways being PD-1 using the humanized antibody pembrolizumab, or the fully human antibody nivolumab. Anti-CTLA-4 antibodies such as the fully human antibody ipilimumab are also available.

Releasing these breaks to aid tumor destruction via checkpoint inhibition has achieved unprecedented success in treating certain tumor types. A meta-analysis of 13 studies with 3,513 immunotherapy treated small cell lung cancer patients and 3,072 chemotherapy/placebo treated patients found greatly improved progression free survival (PFS)(odds ratio 1.81, [95% CI 1.36, 2.42;  $P < 0.0001$ ]) and overall survival (OS) ( $P < 0.0001$ ).<sup>13</sup>

While ICIs can cause sustained responses in selected patients, based on cancer type and specific host tumor markers, there are several obstacles to be overcome. One of the most pressing concerns is the low response to immune checkpoint inhibitor monotherapies. The response rates have been inconsistent both amongst different types of cancers, and histologically different cancers within a specific cancer type. The overall response rate to immune checkpoint therapy ranges from 15% with ipilimumab<sup>14</sup> to up to 40% with PD-1/PD-L1 blockade, including those patients whom only achieved a partial response to treatment.<sup>15</sup> The response rates can be as high as 61% in melanoma with PD-1/CTLA-4 (nivolumab/ipilimumab) treatment<sup>16</sup> however, melanoma has been showing higher objective response rates compared to other types of cancer. Thus, with the

exception of melanoma, ICI response rates are between one quarter to one half of recipients.

It is vital to improve both the response rate to immune checkpoint inhibitor treatments in mCRC as well as to increase the efficacy of the treatment signified by extended progression free survival (PFS) and overall survival (OS). The discovery of biomarkers that can identify patients who are likely to respond to checkpoint inhibitor treatment is crucial to achieving this goal.

Mismatch repair (MMR) or microsatellite instability (MSI) are biomarkers that have a large impact on ICI treatment response rates and prognosis. Mismatch repair is a mechanism cells use to repair DNA damage during cellular replication. Mismatch repair deficient (dMMR) or Microsatellite Instability High (MSI-H) is a small subset of CRC that has a high mutation rate. A higher mutation burden translates into a larger number of neo-antigens displayed in cancer cells' MHC I molecules. Antigen presentation renders these tumors easier to be recognized by the adaptive immune system, higher density of TIL (tumor infiltrating lymphocytes) leading to a more efficient elimination by ICIs.<sup>17</sup> This increased immunogenicity in MSI-H tumors is termed as being “hot”, versus MSS tumors that are labeled “cold” tumors based on their diminished immunogenicity attributed to the lack or paucity of TILs.<sup>18</sup>

Historically, the MSI-H/dMMR and MSS/pMMR subsets of CRC respond very differently to single agent immunotherapy. While in MSI-H/dMMR CRC the objective response rate was 40%, to the anti-PD-1 antibody pembrolizumab (Keytruda Merck & Co.), there was no objective response for MSS/pMMR CRC.<sup>5</sup>

Generally, the dMMR/MSI-H subset comprises only 15-20% of all colorectal cancers, and as the stage of the disease increases, the portion of the dMMR/MSI-H CRC decreases. Only 4% of Stage IV CRC is dMMR/MSI-H.<sup>6</sup> Thus, only a very small portion of mCRC lends itself to ICI treatment, with the majority of mCRC that is MSS/pMMR failing to demonstrate meaningful clinical activity.

As the MSI-H/dMMR tumor biomarker is predictive of response to PD-1 checkpoint inhibitor therapy<sup>6</sup>, guidelines published by the American Society of Clinical Pathology, College of American Pathologists, Association of Molecular Pathology and the American Society of Clinical Pathology recommends universal MSI and mismatch repair (MMR) testing in all mCRC patients since 2017.<sup>19</sup> See Appendix B for MMR testing and MSI classification. As only 4% of the mCRC population possesses this biomarker, the majority of mCRC patients are excluded from ICI treatment options. It is paramount to further elucidate the differences, especially regarding molecular patterns, cytokine expression and tumor infiltrating lymphocyte (TIL) content between MSS and MSI-H tumors.<sup>17</sup>

Although mutation burden and in turn the number of neo-epitopes cancer cells display in a specific tumor seem to be the most important factor in predicting response to ICIs, a new paradigm in immune-oncology recognizes that other factors are at play, especially given that the MSI-H/MSS classification does not always correlate with response to ICI therapy. In fact, genomic profiling of tumors from over 6000 patients found that about 3% of MSS CRC have high mutation burden (HMB MSS).<sup>20</sup> Of note, some MSS/pMMR tumors have high T-helper cell infiltration, cytotoxic gene content, cytokine and

chemokine expression similarly to their MSI-H/dMMR counterpart and importantly, have similar prognosis.<sup>17</sup>

The best ICI response rates were seen in MSI-H/dMMR CRC, but even with the combination of ipilimumab and nivolumab, only a little more than half of this population responds. Although microsatellite instability is not a perfect marker for ICI response, it is the strongest one we have. The challenge is to coax the MSS tumors to behave more like their MSI-H counterparts.<sup>17</sup> Current advances in using Immunoscore as a new biomarker might yield a better indicator for checkpoint therapy success. If we accept Immunoscore as a valid predictor of response rate, the challenge will become to increase a certain tumor's Immunoscore.

Immunoscore is a scoring system based on the density of CD3+ and CD8+ T-Cell effectors within the tumor and its invasive margin.<sup>21</sup> It was validated for Stage I-III CRC by a large international consortium.<sup>21</sup> Another study found that Immunoscore is also predictive of DFS (disease free survival) and OS (overall survival) in mCRC.<sup>22</sup> Refer to Appendix C for the description of the Immunoscore scoring system.

The two ICI monotherapies approved for chemotherapy pre-treated MSI-H/dMMR mCRC are pembrolizumab and nivolumab.<sup>3-5</sup> The first agent to be approved was the anti-PD-1 antibody pembrolizumab, based on studies conducted on eleven cancer types, including CRC. In this study 52% of CRC patients responded to pembrolizumab and the estimated 1-year PFS was 64%. As per protocol, the patients stopped therapy at 2 years and none had recurrence at the median follow up of 8 months.<sup>5</sup> The extended durable response suggests conceivable curative potential that must be followed up for a longer duration.

Nivolumab, another PD-1 blocker was approved for MSI-H/dMMR CRC patients who progressed on traditional chemotherapy after the Nivolumab monotherapy arm of the CheckMate 142 trial found a 31.1% response rate in MSI-H/dMMR CRC with a corresponding 1-year survival of 50%.<sup>3</sup>

### **2.2b Clinical Efficacy of Ipilimumab and Nivolumab**

The complexity and redundant nature of cellular signaling often favors combinatorial treatments. Ongoing efforts aim to test combinations of ICI and radiation, targeted therapy, chemotherapy and anti-angiogenic agents.<sup>2</sup> These combinations can either block a single checkpoint pathway more effectively such as concurrent PD-1/PD-L1 blockade or simultaneously target distinct pathways. Attempts to combine different ICIs such as anti-CTLA-4 ipilimumab and the anti-PD-1 nivolumab (Opdivo; Bristol-Myers Squibb) have been increasingly effective in several cancer types including colorectal cancer<sup>2</sup>.

Just recently, based on the results of the CheckMate 142 study, ICI therapy using the anti-PD-1/anti-CTLA4 nivolumab and low dose ipilimumab was approved for MSI-H/dMMR CRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.<sup>23</sup> The response rate using this combination improved to 55% over 31.1% with nivolumab only and the 1-year survival increased to 71% from 50%. The price of higher response rate and efficacy was higher frequency of CTCAE Grade 3 and 4 adverse events that increased from 20% with nivolumab only, to 32% with the combination. These adverse events, however, were overall manageable.<sup>4</sup>

While the Phase II study CheckMate 142 focused on the combination of CTLA-4 blockade and PD-1 blockade in the MSI-H mCRC patient population, responses were also seen amongst the much smaller arm consisting of MSS patients. 23 MSS patients were enrolled of which two patients responded with a median PFS of 1.4 months (1.2 and

1.9 months). The median PFS was minimal compared to the MSI-H/dMMR cohort's that was not reached after 33 PFS events. It is notable that the responses were not associated with tumor PD-L1 expression levels in either cohort. Furthermore, responses were not associated with *BRAF/KRAS* mutation or history of Lynch syndrome in the MSI-H/dMMR cohort.<sup>4</sup>

While these results are promising, addition of an immunomodulating drug could increase ORR and improve survival in both subgroups.

### **2.2c Immune Mediated Adverse Events using Nivolumab and low-dose Ipilimumab**

Treatment related adverse events (TRAEs) associated with nivolumab and low dose ipilimumab treatment, are mainly immune mediated adverse events (IMAE). IMAEs can affect any of the main organ systems and have a diverse range of manifestation. During the CheckMate 142 trial the most common TRAEs found were diarrhea (22%), fatigue (18%) and pruritus (17%) followed by pyrexia and elevated AST (15%).<sup>4</sup> The in depth safety analysis was published separately.<sup>24</sup> TRAEs resolved in the majority of the patients (71-96%), except for endocrine adverse events which only resolved in 40% of patients. The discontinuation rate due to an adverse event (AE) remained low at 13% and there were no treatment related deaths reported.

The addition of COX-inhibitors in the current study might modulate these adverse events, which will be closely monitored and recorded throughout the study with the goal of early recognition, as early recognition and treatment of IMAEs with or without the need for steroids, could improve outcomes in ICI treatment.<sup>25</sup> See Appendix D for Common Terminology Criteria for Adverse Events (CTCAE) grades.

## **2.2d Management of Immune-related Adverse Events**

Immune related adverse events (irAE) will be managed by the recommendations set forward by the American Society of Clinical Oncology (ASCO) Clinical Practice Guideline, briefly outlined in Appendix R. For further information and organ specific management, refer to the published guidelines.<sup>25</sup>

## **2.2e The Role of the COX Pathway in Colorectal Cancer**

In this section we will discuss the role of COX pathway in colorectal cancer and specifically in MSS CRC and few of the mechanisms that are thought to be at play.

COX-2 is overexpressed in many human cancers such as colorectal, pancreatic, lung, breast and stomach cancers.<sup>27</sup> Additionally, research in different cancer types show that microsatellite instability has an inverse relationship with COX-2 expression levels. In gastric cancer, microsatellite instability is inversely proportional to COX-2 expression.<sup>28</sup> Karnes et al. found that a subset of colorectal carcinomas with microsatellite stable phenotype (MSS) shows increased expression of COX-2 compared to colon cancer with the MSI-H phenotype.<sup>29</sup> Furthermore, response to COX-2 inhibitors is reduced in cell lines with reduced COX-2 expression in in vitro experiments.<sup>30</sup> The COX-2 overexpression in MSS CRC therefore could be predictive of COX-2 inhibitor treatment success.

The COX-2 enzyme can be inhibited both by steroids (by inhibiting the release of the COX substrate arachidonic acid) and non-steroidal anti-inflammatory drugs (NSAIDs) that either inhibit COX-1 and COX-2 enzymes or selectively inhibit COX-2.<sup>31</sup> COX-1/2 (Aspirin, Bayer) or selective COX-2 inhibitors such as celecoxib (Celebrex, Pfizer) are readily available on the US market.

The product of the COX-2 pathway is prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), a vital homeostatic factor that has a wide range of functions in many body systems, including several roles in modulating immune responses.<sup>31</sup> The COX-2 pathway plays a dual role in cancer. Paradoxically, while PGE<sub>2</sub> is considered to be a mediator of inflammation via activation of dendritic cells, it has also been shown to suppress innate and antigen-specific immune responses by suppressing the dendritic cells' ability to interact with naïve, effector and memory T-cells, and suppressing other effector functions associated with cytotoxic T-cells (CTL) and natural killer cells (NK). Thus while PGE<sub>2</sub> stimulates acute local inflammation, it suppresses immune function by effector cells such as CTL, T-helper 1(Th1) and NK cells, especially in the later stages of inflammation.<sup>31</sup> This is thought to prevent the tissue-damaging actions of nonspecific inflammation during chronic inflammatory conditions such as chronic infections or cancer.<sup>32,33</sup> PGE<sub>2</sub> is also implicated in promoting angiogenesis.<sup>33</sup> Together PGE<sub>2</sub>'s angiogenic effect, T-cell suppressive and pro-inflammatory activities contribute to immune evasion and are advantageous for tumor formation.<sup>31</sup>

Another proposed function that may contribute to the COX pathway's T-cell suppressive action is its direct involvement with checkpoint molecules such as PD-L1 an important target of ICI therapy. Botti et al. found that COX-2 expression positively correlates with PD-L1 expression in both primary tumors and metastases in melanoma in vitro.<sup>34</sup> Furthermore, inhibition of COX-2 by celecoxib down-regulated the expression of PD-L1 in two different human melanoma cell lines.<sup>34</sup>

Another proposed mechanism for the COX pathway's involvement in immune checkpoint blockade is the inhibition of the indoleamine 2,3-dioxygenase 1 (IDO1)

pathway. IDO1 has an essential role in regulating tryptophan catabolism by degrading tryptophan and therefore represses tryptophan-dependent T-cell populations,<sup>35</sup> a mechanism that is at least in part responsible for the tumor immune escape. Hennequart and colleagues showed that COX-2 expression drives constitutive expression of IDO1 which in turn represses the T-cells in human tumor cells in seven human cell lines, including colorectal cancer. COX-2 inhibition by celecoxib treatment promoted rejection of IDO1 expressing human tumor xenografts. Of note, the reduction of IDO1 was associated with infiltration by CD3+ and CD8+ T cells.<sup>35</sup>

The most compelling argument for using anti-inflammatory drugs such as COX inhibitors (alone or in combination with ICIs) is the elimination of chronic inflammation that leads to immunosuppression, by inhibiting myeloid-derived suppressor cells (MDSCs). MDSCs are myeloid precursors that are unique to cancer, and less frequently to autoimmune diseases,<sup>38</sup> thus they are an excellent target for anti-cancer drug development. Their exclusive role is to provide a protective environment to cancer cells and they were found to be the most important mediator of chronic inflammation, which in turn, leads to immunosuppression.<sup>39</sup>

The tumor microenvironment (TME) that includes the stroma and infiltrating cells is gaining attention as an important site of immune modulation. The TME has several cell types that help protect cancer cells by secreting cytokines and chemokines thereby altering myelopoiesis. Out of these tumor harboring cell types MDSCs seems be the most crucial, by promoting the formation of Tregs and other cell types that help protect cancer cells and are instrumental in promoting immune tolerance.<sup>40</sup>

Targeting MDSCs, is a complex problem. The candidate drug needs to be an anti-inflammatory drug without the immune-suppressive effects.<sup>41</sup> COX inhibitors, such as celecoxib or aspirin fit this description in that they decrease inflammation and are not immunosuppressive but do not target cytokines of chronic inflammation as a monotherapy.<sup>42</sup> However in combination with ICI they were shown to decrease both MDSCs, Tregs and immunosuppressive cytokines. The preclinical murine study that explored the effect of celecoxib plus PD-1 blockade in melanoma and breast cancer cells found reduced MDSC and Treg population in the TME, as well as reduced cytokines IL-1 $\beta$  and IL-6. The other preclinical murine study also reported reduced cancer promoting growth actors and chemokines, including IL-1, IL-6 and IL-8.<sup>43</sup> Importantly, MDSCs, the chronic inflammation marker C-reactive protein and the immune suppressive cytokines can be measured.<sup>38</sup>

In summary, COX inhibitors decrease inflammation and are not immunosuppressive but do not target the cytokines of chronic inflammation as a monotherapy,<sup>42</sup> thus are not thought to be effective in preventing tumor progression. In the murine study conducted by Zelenay et. al. COX inhibition had no effect on the progression of implanted COX-competent melanoma cells.<sup>27</sup> COX inhibitors have been used as primary or secondary prophylaxis or as an adjunct to other modalities in colorectal adenomas with moderate efficacy, discussed in the next section.

### **2.2f Clinical Efficacy of COX Inhibition**

Celecoxib and aspirin have both been used in colon adenocarcinomas as chemopreventive agents. A large study that followed up four randomized trials and pooled individual patient data of over 14,000 patients showed that the long-term use (mean duration of 6 years) of an at least 75mg daily dose of aspirin reduced the long-term

incidence and mortality due to CRC. The 20-year risk of colon cancer was reduced in the cohort taking aspirin (incidence hazard ratio [HR] 0.76, 0.60-0.96,  $p=0.02$ ), and also reduced mortality (HR 0.65, [95% CI, 0.48-0.88,  $p=0.005$ ]).<sup>44</sup> Although the studies being analyzed differed in many characteristics including median duration of treatment, methods of post trial follow up or proportion of current smokers, they were all double blinded placebo controlled trials. This analysis demonstrates the degree to which aspirin monotherapy was able to affect patients even after years of treatment.

A much larger meta-analysis of 281,063 CRC patients in 37 RCTs found that aspirin reduced CRC incidence and mortality in a dose dependent manner (risk ratio [RR], 0.74 [95% CI, 0.57-0.97]) for high-dose ( $\geq 325$  mg daily dose) and (RR, 0.81 [95% CI, 0.67-0.98]) for very-low-dose ( $\leq 100$  daily dose)).<sup>45</sup> Although the previous two meta-analyses used different statistical methods, they both showed association of chemopreventive aspirin use and reduced mortality and the second meta-analysis showed a dose dependent relationship.

Similarly, celecoxib monotherapy was shown to be efficacious in secondary prevention. A systematic review that examined three RCTs and three post-trial studies of 6,559 patients found that celecoxib doses between 400-800mg/day, when used for the duration of 1-3 years showed statistically significant reduction of the recurrence of advanced colorectal adenomas (RR 0.42 [95% CI, 0.34-0.53]) compared to placebo. Subgroup analysis using 400mg/day celecoxib demonstrated very similar effects on advanced adenomas (RR 0.45, [95% CI, 0.35-0.58]) compared to placebo, regardless of the dosing regimen of 200mg twice or 400mg once daily.<sup>46</sup>

Celecoxib is also used as an adjunct in addition to anticancer agents in the treatment of several cancer types. A large meta-analysis that analyzed data from 11 trials found that when used in combination with cytotoxic chemotherapeutic agents in advanced colorectal, non-small cell lung cancer (NSCLC), prostate, breast and ovarian cancer, celecoxib significantly increased the objective response rate (ORR) (RR 1.20, [95% CI, 1.06-1.36, P=0.005]) but had no effect on 1-year mortality (RR 1.02; [95% CI, 0.92-1.13; P = 0.68]).<sup>47</sup>

These studies provide evidence for aspirin or celecoxib use for primary and secondary prevention or as an adjunct in CRC. The new 2016 USPSTF guideline now suggests the use of <100mg daily dose of aspirin for primary CRC prevention for patients without bleeding risk.<sup>48</sup> In summary, COX inhibition offers some protection against colorectal cancer but is not intended as a monotherapy for treatment.

### **2.2g Drug-Related Adverse Events Associated with COX Inhibitors**

The most common side effects of aspirin are GI disorders such as heartburn, nausea, vomiting and abdominal pain. Elevated liver enzymes were also reported as well as very rare cases of renal impairment and acute renal failure. Bleeding time is also prolonged, leading to increased risk of bleeding.<sup>78</sup>

The most serious adverse events associated with the use of aspirin are gastrointestinal bleeding, hemorrhagic stroke<sup>53</sup> and less frequently aspirin-exacerbated respiratory disease. A meta-analysis found the incidence of GI bleeds in 65,987 patients taking 50-1500 mg daily dose of aspirin for the mean duration of 28 months to be 2.47% compared to 1.42% in patients taking placebo.<sup>49</sup> Thus, the incidence of GI bleed associated with aspirin use remains low. Furthermore, bleeding risk with low to high doses of aspirin currently approved was found to minimal and the regimen was deemed safe for

chemoprevention in CRC. A large systematic review and meta-analysis examining 281,063 patients in 37 RCTs taking aspirin for chemoprevention found the dose range of 75-325 mg/day safe regarding cardiovascular mortality and major GI bleeding among patients of average risk.<sup>45</sup>

The most common side effects of celecoxib are also GI symptoms, such as dyspepsia, abdominal pain, nausea and diarrhea.<sup>79</sup> Celecoxib has a more favorable safety profile compared to other non-selective NSAIDs regarding the risk of gastrointestinal injury. Celecoxib was found to have significantly lower incidence of endoscopically observed duodenal ulcers compared to 500mg twice-daily Naproxen. Doses from 50mg up to 400mg twice daily were studied and scientists found no correlation between celecoxib dose and the incidence of duodenal ulcers.<sup>50</sup> Concerns remain regarding the increased cardiovascular risk of thromboembolism associated with selective COX-2 inhibitors. A large randomized controlled trial (PRECISION) that recruited 24,081 arthritis patients found that 100mg twice daily celecoxib was non-inferior to other NSAIDs such as ibuprofen or naproxen regarding cardiovascular risk.<sup>51</sup> The cardiovascular risks were found to be similarly low in the CRC population. A meta-analysis that included randomized controlled trials and post-trial studies of 6579 CRC patients investigated the efficacy and safety of various celecoxib doses (200mg twice daily, 400mg daily or 400mg twice daily) versus placebo for secondary chemoprevention in CRC found the 400mg once daily dosing to be optimal without increasing cardiovascular risks. Cardiovascular risk compared to placebo with the 400mg once daily dose was 1.01 [95% CI, 0.70-1.46]. The 400mg twice-daily doses lead to as significant increase of serious

cardiac events (3.42 [95% CI, 1.56-7.46]).<sup>46</sup> Of note, the currently approved doses of celecoxib are 200-400mg daily for osteoarthritis and rheumatoid arthritis.<sup>52</sup>

### **2.2h Management of Drug-Related Adverse Events associated with COX Inhibitors**

Patients who are considered high risk for aspirin associated GI bleed, such as patients with previous GI bleed or ulcer or concomitant use of clopidogrel will be excluded from the study. Patients enrolled in the study will be closely monitored for signs and symptoms of GI bleed.

The most effective strategies for reducing the risk of aspirin related upper GI bleed is switching to an alternative antiplatelet therapy or concomitant use of proton pump inhibitor.<sup>54</sup> Proton pump inhibitors will be offered to patients as a prophylactic measure. As switching to an alternative therapy is not an option in this case, aspirin use might have to be discontinued in cases of serious GI bleeding.

A possible adverse effect associated with both aspirin and selective COX-2 inhibitors is aspirin-exacerbated respiratory disease (AERD) that presents with moderate to severe asthma symptoms and rhinosinusitis. Patients with asthma or asthma known to be exacerbated by aspirin use are excluded from the study. Management of AERD includes discontinuing aspirin or COX-2 inhibitor use and pharmacological management of asthma symptoms and chronic rhinosinusitis.<sup>55</sup>

Interruption of treatment with aspirin or celecoxib will be permitted in case of the need for elective surgery or biopsy. Aspirin and celecoxib will be discontinued 7 days prior to the date of surgery and will be resumed at the surgeon's discretion.

## **2.2i Preclinical and Clinical Data Regarding Combination Checkpoint Inhibition and COX Inhibition**

Several pre-clinical works using *in vivo* animal models and *in vitro* human cell cultures and some clinical data of ad hoc data or tissue marker analysis of large prospective cohort studies shows promise for the combination of PD-1 blockade and COX-2 inhibition in CRC and multiple other cancer cell lines.<sup>27,43,56</sup> Accumulating evidence indicates that the combination of checkpoint inhibition and COX-2 inhibition could be a viable treatment option for several cancer types as an adjunct to ICI. The combination of COX inhibitors and PD-1 blockers resulted in decreased tumor growth compared to PD-1 monotherapy alone in murine melanoma models.<sup>27</sup> The inhibition of the COX-2 pathway combined with anti-PD-1 therapy improved the eradication of tumors and increased the number of tumor-specific cytotoxic T lymphocytes (CTLs) in human cell cultures from advanced ovarian cancer patients.<sup>56</sup>

The seminal work by Zelenay et al. presented compelling pre-clinical evidence for the use of COX inhibition as an adjunct to ICIs. His team found that COX inhibition works synergistically with checkpoint inhibition via anti-PD-1 blockade inducing eradication of tumors in *in vivo* experiments using the CT26 syngeneic murine colorectal tumor model. Of note, the CT26 murine colorectal cells were found to share molecular features with MSS colorectal tumors.<sup>57</sup> In their immunomic, genomic and transcriptomic characterization study, Castle and colleagues found that CT26 cells lack mutations in the Mlh1 and Msh2 mismatch repair genes that are associated with microsatellite instability. CT26 cells were also found to lack a *Braf* gene mutation that is also frequently associated with the MSI-H phenotype. Overall CT26 cells were found to have a molecular makeup that resembles aggressive, undifferentiated, refractory human colorectal cancer cells.<sup>57</sup>

Zelenay and colleagues showed that COX induces PGE<sub>2</sub> in tumors that disrupts myeloid function and propose that PGE<sub>2</sub> dependent suppression of myeloid cell activation could be an additional mechanism of tumor immune escape in addition to the immunoediting process whereby the immune system selects for less immunogenic tumor cells. Furthermore, they showed that COX ablation in tumors re-establishes immune control. A possible mechanism investigated by the researchers was the reduction of tumor promoting factors such as *Interleukin 6 (Il6)* and *Il1β* expression by COX ablation leading to increased levels of antitumor pathway mediators.

Importantly, similar effects were achieved by pharmacological reduction of PGE<sub>2</sub> levels. They propose that COX inhibition synergizes with checkpoint blockade therapy via anti-PD-1 blockade, and its efficacy is higher than either PD-1 blockade or COX inhibition alone. This synergistic effect of COX inhibition and ICIs could revolutionize ICI therapy in colorectal cancer. Since the COX inflammatory signature and signaling pathway is conserved in humans<sup>27</sup>, and its inhibitors are readily available, it is a viable target of investigation in clinical studies.

Another pre-clinical study explored the effect of local and systemic delivery of celecoxib and PD-1 blockade on melanoma and breast cancer in tumor-bearing mice. Researchers found that the treatment elicited potent and sustained antitumor effect by enhancing T cell immunity, reduced immunosuppression and reducing inflammation and tumorigenesis.<sup>43</sup> The drug combination acted synergistically, to enhance the presence of CD4<sup>+</sup> interferon (IFN) $\gamma$ <sup>+</sup> and CD8<sup>+</sup> IFN $\gamma$ <sup>+</sup> T cells both within the tumor and in the immune system. Importantly, the combination treatment reduced myeloid derived suppressor cells (MDSC) and Tregs in the tumor environment. MDSC promote Tregs and

is a marker for chronic inflammation. Once again, *Il-6* and *Il-1 $\beta$*  were shown to be suppressed, leading to diminished angiogenic and pro-inflammatory tumor microenvironment. Thus, combination PD-1 blockade and COX-2 inhibition simultaneously targeted both the immunosuppressive network and the chronic inflammation in the tumor environment, both of which are thought to dampen ICI therapeutic efficacy.<sup>39</sup> Of note, PD-1 blockade caused an increase of PGE<sub>2</sub>, which was subsequently completely abolished by celecoxib treatment.

Aspirin (a dual COX-1 and COX-2 inhibitor and antiplatelet agent) may be more effective in synergizing with ICIs than selective COX-2 inhibition by celecoxib. Zelenay and colleagues found that aspirin was more effective than celecoxib in tumor eradication when combined with PD-1 inhibition in mouse experiments.<sup>27</sup> The increased efficacy of dual COX inhibition is thought to be due to the inhibition TGF- $\beta$  (released by platelets) associated with COX-1 inhibition and anti-platelet activity. TGF- $\beta$  is thought to attenuate tumor response to PD-L1 blockade by contributing to the exclusion of CD8<sup>+</sup> T-cells.<sup>58</sup> TGF- $\beta$  is even thought to be one of the main drivers of immune evasion in colon cancer metastasis.<sup>59</sup> In mouse experiments, COX inhibition alone using aspirin had no effect on the progression of implanted melanoma cells, compared to the effect with combination with anti-PD-1 therapy where COX inhibition promoted a much more rapid tumor regression than anti-PD-1 alone, and notably was more efficacious than selective COX-2 inhibition.<sup>27</sup>

While both studies were carried out in mouse models, the COX pathway and the COX-dependent inflammatory signature is remarkably conserved in humans. In addition to mouse models, scientist also examined human biopsy samples from cutaneous

melanoma and found very similar mRNA expression levels of tumor promoting cytokines.<sup>27</sup>

While the previous studies were carried out mainly in murine models, the same principles have been demonstrated using human tissue cultures. Combined PD-1 blockade and disruption of COX-2 signaling lead to the eradication of tumors and increased the number of tumor-specific cytotoxic T lymphocytes (CTLs). Furthermore, the study showed that tumor-derived prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) plays an important role in depressing CTL function.<sup>56</sup>

Clinical evidence for the synergistic effect of aspirin (a COX-1 and COX-2 inhibitor) and immune checkpoint blockade comes from the analysis of data collected in two large prospective cohort studies.<sup>60</sup> Hamada and colleagues examined data collected using questionnaires and analyzed tumor samples from the participants of the Nurses' Health Study that followed 121,701 women and Health Professional's Follow Up study that included 51,529 men. 621 records were chosen for analysis based on the availability of tumor samples, data of PD-L1 expression level measured by immunohistochemistry and aspirin use. Patients were observed from 1976 and 1986 respectively, to death or January 1<sup>st</sup> 2012, whichever came first. Regular aspirin use was defined as either standard-dose or low-dose, or both taken two or more times per week. Post-diagnosis aspirin use with colorectal cancer-specific survival differed by PD-L1 (CD274) expression status ( $P_{\text{interaction}} < .001$ ) and overall survival ( $P=0.004$ ) compared with aspirin nonusers. In patients with low PD-L1 expression, the multivariable-adjusted hazard ratios (HR) for regular aspirin users were 0.16 (95% CI, 0.06 to 0.41). Patients with high level of PD-L1 expression did not seem to benefit from post-diagnosis aspirin use ( $P=0.85$ , HR

1.01 95% CI 0.61 to 1.67). Of note, this differential prognostic association of aspirin use and PD-L1 expression status seemed consistent among MSS and MSI-H colorectal tumors. A weakness of this study was that chemotherapy regimens were not available for most participants. Although data collected on the patients who did have available chemotherapy descriptions, post-diagnosis aspirin used was not associated with chemotherapy use, the use of different regimens could introduce a confounder.

We were interested in the dose-response relationship of which we could model our aspirin experimental arm. Unfortunately, secondary analysis was lacking statistical power to establish a dose-response relationship, although there seemed to be a prognostic association by PD-L1 expression status.

In summary, while this study followed patients who underwent cytotoxic chemotherapy or received no treatment and did not include patients whom underwent ICI therapy, it reveals an important association between the efficacy of post-diagnosis aspirin use and PD-L1 expression level in patients with CRC. This data points to a stratum of CRC that will benefit from post-diagnostic aspirin use and may have very important implications in corroborating added aspirin use in combination with ICI.

Several phase I/II trials investigating ICI + COX inhibition that are either preparing to or are currently actively enrolling patients with various solid tumors, three of which are carried out with colorectal cancer patients specifically or include them in their eligible cancer type. The choice of ICI is either nivolumab or nivolumab and ipilimumab at standard doses and aspirin at the doses 200-325mg daily or celecoxib at the doses of 200-400mg daily. As these are ongoing studies, there are no published results. We will briefly

discuss the similarities and differences in proposed rationale. See Appendix P for a summary of these trial designs.

The current phase II study titled Nivolumab, Ipilimumab and COX2-inhibition in Early Stage Colon Cancer: an Unbiased Approach for Signals of Sensitivity (NICHE) [NCT03026140] is set out to investigate the combination of nivolumab, ipilimumab and celecoxib as a neoadjuvant therapy in early stage CRC in both the MSS and MSI-H subgroups. This neoadjuvant approach administers the drug combination for the short period of 6 weeks in anticipation of shrinking the tumor prior to surgery. The regimen for the active comparator group (Group 1) consists of a single dose of ipilimumab 1mg/kg on day 1 and two cycles of nivolumab 3mg/kg on day 1 and 15, respectively. The experimental group (Group 2) is given a single dose of ipilimumab 1mg/kg on day 1, two cycles of nivolumab 3mg/kg on day 1 and 15 and 200mg celecoxib daily until the day before surgery. While all MSI-H patients are allocated to Group 1, MSS patients are randomized to Group 1 or Group 2. Compared to this trial design, our proposed study will recruit CRC patients with metastatic disease that this trial excludes. Notably, we will use a double the dose (400mg) of celecoxib proposed here. Although the study design of neoadjuvant treatment might warrant a lower dose. Furthermore, our study avoids randomizing MSS participants into an ipilimumab/nivolumab treatment group only that has previously shown an inadequate response by using a cross trial comparison arm. While our approach might have decreased validity due to slight institutional differences in how variables are operationalized, it will expose participants to the least possible harm.

Another current Phase II study, titled PD-1 Antibody Combined With COX Inhibitor in MSI-H/dMMR or High TMB Colorectal Cancer [NCT03638297] investigates the

combination of nivolumab and COX inhibition in MSI-H/dMMR CRC. As mentioned earlier, about 3% of MSS lesions are high TMB (tumor mutation burden), thus they would be included in this study. This trial differs from ours in that it restricts its participants to high TMB that is generally associated with a faulty DNA repair mechanism and adds celecoxib to modulate the inflammatory tumor environment. This would theoretically increase the proportion of CRC that responds to ICI treatment but continues to exclude the majority of CRC patients.

The current Phase II study titled An Open Label Phase II Study Combining Nivolumab and Celecoxib in Patients With Advanced " Cold " Solid Tumors (NICE-COMBO) [NCT03864575] investigates the combination of nivolumab and celecoxib in late stage solid tumors. Although this study did not specify CRC as an eligible cancer type, the characteristics of the cancers treated, e.g. "cold", "advanced solid tumors" "with an indication of treatment with anti-PD1 antibodies" technically include MSI-H mCRC, for which the nivolumab/ipilimumab combination is FDA approved. This trial is similar to ours in that it aims to treat "cold" tumors.

The phase Ia/Ib trial investigates safety in a dose escalation of radiation 0.5-3Gy in addition to a fixed ICI and proposed immunomodulatory regimen (aspirin, cyclophosphamide) dose. The ipilimumab/nivolumab regimen is combined with a 300mg daily aspirin dose and cyclophosphamide. This is the only Phase I study examining ICI plus immunomodulation, due to the radiation dose escalation component, while the drug doses remain fixed.

The Phase II study titled PRIMMO aims to treat refractory cervical and endometrial cancer using a combination of radiation, ICI (pembrolizumab) and immunomodulation

(Vitamin D, aspirin, cyclophosphamide and lansoprazole). The aspirin dose used was 325mg daily. This study also examines the combination of ICIs and immunomodulation including aspirin in a stage IV solid tumor type.

In conclusion, there is mounting evidence to support translational continuation of pre-clinical work with ICI and COX inhibition combination.

### **2.3 REVIEW OF POSSIBLE CONFOUNDING VARIABLES**

There are well-studied prognostic markers that pertain to the general CRC population. The prognostic markers within the MSS mCRC population are less well studied, especially regarding to ICIs treatment. In this section, we will discuss prognostic markers as they pertain to confounding study data.

The general prognostic markers of CRC are lymph node involvement, presence of metastases, right- versus left-sided tumor, microsatellite instability (MSI-H)/deficient mismatch repair (dMMR) vs. microsatellite stable (MSS)/proficient mismatch repair (pMMR), KRAS, BRAF and NRAF mutation status.<sup>10</sup> As we will be restricting our study population to MSS metastatic CRC, the known prognostic markers that remain as possible confounders are right- versus left-sided tumor, KRAS, BRAF and NRAF mutation status. KRAS confers poor prognosis in the MSS CRC according to the guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology, thus it will be considered a confounder.

Myelosuppression is a known adverse event associated with chemotherapeutic agents<sup>10</sup> that is theoretically a negative predictor of ICI therapy response, although not well studied in CRC. Thus, previous and especially recent cytotoxic chemotherapy might be a negative predictor of response and a confounder.

Right- versus left-sided tumor was found to be a strong prognostic marker of survival. A large meta-analysis and systematic review of 1,347,846 patients found that left-sided primary tumor location was associated with a markedly reduced risk of death (HR, 0.82; 95% CI, 0.79-0.84;  $P < 0.001$ ) independently of stage, race, use of chemotherapy, year of study (1995-2016), number of participants and quality of study.<sup>61</sup> While this study did not perform a sub-group analysis based on MSI status and didn't explore ICI use, its results must be considered. As a strong prognostic predictor in the general CRC population, the location of the primary tumor should be considered a confounder and be factored into the final data analysis.

Age and sex were both found to be prognostic factors specifically for ICI use.<sup>62</sup> Increased age is associated with immunosenescence that leads to decreased lymphocyte function and exhaustion of T-cell function.<sup>63</sup> Male patients seem to respond better and demonstrate significantly increased survival when treated with ICI therapy, but the role of sex in this phenomenon is largely unknown.<sup>62</sup>

Another strong prognostic predictor specifically in the mCRC population we will be restricting is the presence of brain or leptomeningeal metastases. While a rare type of metastases with an incidence in CRC are 0.6-3.2%,<sup>64</sup> it is a strong prognostic predictor of morbidity and mortality. The median survival after brain metastases diagnosis is 2.6-7.4 months<sup>64</sup>, compared to any distant metastases that are associated with a median survival of approximately 20 months.<sup>65</sup> Due to its paucity, restriction of brain metastases will not likely to have a significant effect on external validity, while increasing internal validity.

In summary, we will take several precautions in treating variables that can have an effect on the outcomes in our study. We have restricted some variables, such as brain

metastases, that were rare enough to pose threat to the external validity. Possible confounders discussed here and proposed mediators discussed in Section 2.4i will all be considered in the post-trial exploratory analysis.

## **2.4 REVIEW OF METHODOLOGY**

This section will provide evidence to support methodology proposed in this study. Please refer to Chapter 3 for detailed description of methodology and study protocols.

### **2.4a Study Design**

The proposed study will be a single-center, open label, Phase II exploratory study with randomized parallel assignment of two arms investigating the effects of nivolumab/ipilimumab + celecoxib or aspirin compared to nivolumab/ipilimumab alone in patients with MSS/pMMR mCRC. We will report efficacy, safety, biomarker association and patient reported outcomes (PRO) in both MSS/pMMR cohorts. All participants will receive the same weight-based dose nivolumab/ipilimumab + fixed dose celecoxib or aspirin regimen. The MSS/pMMR cohort will not receive the nivolumab/ipilimumab only regimen due to the low objective response rate (2/23) and short PFS (1.4 months) observed in this cohort in the CheckMate 142 trial.<sup>4</sup> The mean PFS associated with the current standard of care therapy of regorafenib is 3.34 months and the associated response rate is 2%.<sup>12</sup> The MSS/pMMR cohort will be compared to historical control of the CheckMate 142 MSS/pMMR cohort with an identical ICI regimen in combination with COX inhibition and comparable follow up time. This cross-trial comparison will allow for sparing the MSS/pMMR cohort from being randomized into a treatment group that has shown less than optimal results previously.

Although each treatment component separately has well known and tolerable safety profiles, our aim is to closely monitor the safety of the combination treatment.

#### **2.4b Patient Selection and Study Population**

This study will compare two single treatment arms to the historical control of the CheckMate 142 study MSS cohort and will use the same patient selection criteria to increase the validity of this cross-trial comparison.<sup>4</sup> This study will recruit adults of age  $\geq$  18 years with histologically confirmed distant metastatic spread of CRC (Stage IV as defined by TNM staging criteria), confirmed MSS/pMMR genotype who has progressed on previous treatment with cytotoxic chemotherapy using fluoropyrimidine, oxaliplatin and irinotecan. In the CheckMate 142 study, the restriction of previously treated patients was necessary to conform to the FDA approved indication for the low dose ipilimumab and nivolumab combination, although patients who declined the use of chemotherapeutic agents were included. In this study, we are using this regimen for a population (MSS mCRC) for whom it is not FDA approved, but will keep the restriction of previous treatment to increase the validity of the cross-trial comparison. The population of interest will have ECOG performance status of 0 or 1, evaluated within one month before randomization.

The exclusion criteria include high dose prednisone ( $> 10\text{mg}$  daily dose) required for autoimmune disease. As prednisone reverses the effect of ICIs, it would dampen treatment effect. Patients with prior treatment with the anti-PD1, anti-PD L1/L2 and anti CTL-4 immune checkpoint or other agent targeting T-cell co-stimulation or immune checkpoint pathways are excluded from this study. Patients with serious uncontrolled medical disorders are also excluded, as these confounding disorders would affect morbidity and mortality. Some organ function parameters are also dictated by the added aspirin or celecoxib treatment, as these medications are contraindicated for patients with

acute or severe hepatic or renal failure or bleeding diatheses.<sup>66</sup> Patients must demonstrate adequate organ function as defined in Appendix O.

Patients must have a lesion that is accessible for a biopsy or has had a biopsy with accessible tissue in the past 6 months and the patient is willing to provide this tissue for study purposes.

Patients with brain metastases will be excluded from this study. Brain metastases are rare in the CRC population. A systematic review found that the incidence of brain metastases in CRC are 0.6-3.2%.<sup>64</sup> They, however, carry increased mortality and morbidity. The median survival after brain metastases diagnosis is 2.6- 7.4 months<sup>64</sup> compared to about 20 months with any metastases<sup>65</sup> and thus brain metastases diagnosis must be excluded from our study as a potential confounder.

Patients must not be taking Non-steroidal Anti-inflammatory Drugs (NSAIDs), aspirin or COX-2 inhibitors at the time of registration and they must not have a documented allergic reaction or hypersensitivity to them. Aspirin is a non-reversible anti-platelet drug, which is also contraindicated for patients with bleeding diatheses. Patients with bleeding diatheses will also be excluded due to the increased bleeding risk. Patients with a history of peptic ulcer disease or gastrointestinal bleeding will be excluded from the study due to the increased risk of bleeding seen with NSAIDs, especially aspirin.<sup>66,52</sup>

Female participants must not be pregnant or breastfeeding and both sexes must agree to use effective contraception for the duration of the study and up to 90 days after the last study drug administration.

Refer to Appendix E for ECOG Performance Status Criteria. Please see Appendix B form MSI criteria and testing options. Of note, the three MSI testing options are deemed

to be equally effective thus there is no specific testing option is recommended by the FDA.<sup>17</sup> Please see the criteria for TNM staging in Appendix A and the RECIST criteria in Appendix K.

#### **2.4c Dosing Regimen of Nivolumab and Ipilimumab**

The FDA approved dosage of nivolumab and ipilimumab for MSI-H/dMMR CRC is nivolumab 3 mg/kg followed by ipilimumab 1 mg/kg on the same day every 3 weeks for 4 doses, then nivolumab 240 mg every 2 weeks until disease progression or unacceptable toxicity. This regimen was determined based on safety data from the CheckMate 142 trial.<sup>4</sup>

FDA dosage recommendations were revised in March 2019 to a non-weight-based regimen as follows: Adult and pediatric patients  $\geq 40$  kg: 240 mg every 2 weeks or 480 mg every 4 weeks. Adult and pediatric patients  $\geq 40$  kg: 3 mg/kg followed by ipilimumab 1 mg/kg on the same day every 3 weeks for 4 doses, then 240 mg every 2 weeks or 480 mg every 4 weeks.<sup>67</sup>

The recently revised Opdivo (nivolumab) package insert advises 30 minute infusion times, however, in the CheckMate 142 study the drug infusions of nivolumab took place over 60 minutes and the infusions of ipilimumab took place over 90 minutes, thus we will be using this infusion timeline as well as the dosing regimen to keep study parameters identical increase the validity of the cross-trial comparison.

#### **2.4d Dosing Regimen of Celecoxib**

The fixed dosing regimen of celecoxib was determined based on safety data and the intent of providing the highest dosage with tolerable toxicities to avoid sub-therapeutic treatment dosages. The 400mg once daily regimen was based on safety data obtained in a meta-analysis that examined celecoxib doses in secondary chemoprevention in CRC

patients.<sup>46</sup> Celecoxib safety is discussed further in Section 2.2g. There will be no dose adjustments made to keep study variables consistent.

#### **2.4e Dosing Regimen of Aspirin**

As the highest dose of aspirin that is effective in producing a synergistic effect with ICIs is not known, the dose of aspirin was also determined based on safety data. Our intent was to use the highest tolerable dose to avoid subtherapeutic dosages. We will use the 325mg daily dose based on safety data discussed in Section 2.2g. No dose adjustments will be made to keep study variables constant.

#### **2.4f Intervention**

The intervention consists of celecoxib or aspirin that will be administered in addition to the nivolumab plus low dose ipilimumab combination. The timing, mode of administration and dose of nivolumab and ipilimumab will be modeled after the CheckMate 142 trial to ensure increased validity of the cross-trial comparison. The celecoxib and aspirin doses were determined based on safety studies referenced in Section 2.4d and 2.4e as there are no safety or efficacy data from clinical trials regarding the combination of COX inhibitors and ICIs.

#### **2.4g Primary Outcome Measures**

The primary outcome measure will be objective response rates (ORR) radiologically examined and evaluated by the RECIST v1.1 criteria.

Refer to SUPPLEMENT Q for definitions of outcome measures as per RECIST v1.1.

This outcome measure was chosen to match the CheckMate 142 trial to ensure the validity of the cross-trial comparison.

#### **2.4h Secondary Outcome Measures**

The secondary outcome measures were also chosen to match the secondary outcomes of the historical control of the CheckMate 142 trial, except for the blinded

independent central review (BCIR) ORR and PFS, as our study is a single center study and does not require central review. Secondary endpoints are disease control rate (DCR), progression free survival (PFS), overall survival (OS), safety and tolerability, association between biomarker expression (BRAF mutation status, KRAS mutation status, PD-L1 expression, clinical history of Lynch syndrome) and efficacy and changes from baseline in patient reported outcomes (PRO).

The patient reported outcomes (PRO) will consist of two questionnaires. These will be conducted at baseline, and at every 6 weeks based on the European Organisation for Research and Treatment of Cancer (EORTC) Core Quality of Life Questionnaire (QLQ-C30) and three-level five-dimensional EuroQol instrument (EQ-5D). The EORTC QLQ-C30 has three components: symptoms, functioning and quality of life (QOL). The EuroQol instrument is a non-disease specific questionnaire that is intended to complement the QLQ-C30 that was designed to evaluate quality of life measures in patients participating in clinical studies.<sup>68,69,70</sup>

#### **2.4i Other Variables**

Although there are no validated independent prognostic predictors of survival or response specifically in MSS mCRC regarding ICI treatment, there are several promising emerging biomarkers such as Immunoscore and MDSC level.

Immunoscore is a novel cancer classification system with a focus on immune infiltration in the tumor microenvironment. The Immunoscore system sums up the density of CD3<sup>+</sup> and CD8<sup>+</sup> T-cell effectors both within the tumor and within the invasive margin, scored 0 (no T-cell effectors in either tumor or invasive margin) to I4 (T-cell effectors in both tumor tissue and invasive margin).

There is accumulating evidence that Immunoscore could be predictive of ICI efficacy in both the MSS and MSI-H populations. In fact, a multivariate analysis showed that Immunoscore is a stronger predictor of patient survival and disease specific recurrence than microsatellite instability.<sup>71</sup>

An international consortium conducted a study to assess the prognostic value of Immunoscore found that in TNM stage I-III colorectal cancer, Immunoscore association for time to recurrence was independent of patient age, sex, T stage, N stage, microsatellite stability and other existing prognostic factors studied.<sup>21</sup> The study that examined tissue samples from 3539 patients also found significant positive correlation between densities of CD3<sup>+</sup> and CD8<sup>+</sup> tumor cells and survival and negative correlation with the risk of recurrence. The difference in risk of recurrence reported as hazard ratio (HR) in high vs. low Immunoscore was 0.20, (95% CI 0.10-0.38; p<0.00010) translating to an 80% reduction in recurrence risk.<sup>21</sup> Of significance, 21% of patients with MSS colorectal tumors have high Immunoscore<sup>21</sup> and about 50% had I3-I4.<sup>71</sup> Thus, it is possible that Immunoscore could identify a subset of MSS CRC that can be targeted with immunotherapies. Although Immunoscore was found to be a more effective predictor than age and sex, the results have not been validated for mCRC (only TNM stage I-III), thus age and sex will have to be considered as factors for stratification in the mCRC population in our study.

A negative predictor of survival is MDSC level. Importantly, MDSCs found in the blood stream predict higher TNM stage and increased mortality,<sup>72</sup> thus it is a useful marker of survival. A meta-analysis found cancer mortality doubled upon the detection of MDSCs with associated HR for OS, 1.94; 95% confidence interval [CI], 1.42-2.66; P <

0.0001) in patients with solid tumors, including CRC.<sup>73</sup> Since increased COX-2 expression leading to increased PGE2 levels may be one mechanism that allows tumor cells to evade host immune surveillance through accumulation of myeloid-derived suppressor cells (MDSCs) and evasion from T cell-mediated immune attack, we anticipate that MDSC level be a better marker of objective response rate than COX-2 expression level. Thus, instead of COX-2 levels, we will use its downstream effector MDSC as a stratum for random allocation.

Of note, Immunoscore and MDSC level are both hypothesized to be in the CRC causal pathway, thus are considered to be mediators rather than confounders.

Biomarker assessment will include genotyping the oncogenes KRAS, NRAF, BRAF (mutated vs. wild type), clinical history of Lynch syndrome, tumor PD-L1 expression level, tumor burden and COX-2 expression. We will measure MDSC levels and C-reactive protein as a novel marker of chronic inflammation, as well as the immune suppressive cytokines sTNF, *Il-1 $\beta$* , *Il-10* and TGF- $\beta$ .

The CheckMate 142 trial did not find any association between objective response rates in MSI-H/dMMR mCRC and history of Lynch syndrome, BRAF or KRAS mutation status or PD-L1 expression status<sup>4</sup>. These associations, except for no association of responses with PD-L1 expression status, were not reported and perhaps would not have been statistically meaningful in the small (23 patients) MSS/pMMR population examined in their trial. In this study we aim to recruit a larger sample of MSS patients to direct us toward more clinically meaningful data on biomarker association with objective response rate within the MSS/dMMR population.

#### **2.4j Sample Size and Statistical Significance**

Simon's two-stage design will be used. The primary objective of phase II trials is to determine whether the intervention (here a drug combination) has sufficient activity against a disease state, which here is MSS mCRC. In a traditional single stage study design, this determination could take months or years, exposing patients to treatments that might have no benefit for them, and might even expose them to undue risk. The goal of the Simon's two-stage design is to minimize sample size in case the intervention has low or no activity.<sup>74</sup>

In the first stage, 14 patients will be enrolled. If there are 1 or fewer responses in these 14 patients, the study will be stopped. Otherwise, 27 additional patients will be enrolled for a total of 41. The null hypothesis will be rejected if 7 or more responses are observed in 41 patients. This design yields a type I error rate of 5% when the true response rate is 23.7%. Refer to Appendix N for more information.

#### **2.5 CONCLUSION**

Several lines of evidence have shown that the combination of checkpoint inhibition and COX-2 inhibition act synergistically to reduce tumor promoting inflammation and increase immunogenicity in the tumor environment. The success of checkpoint inhibition via the combination of PD-1 blockade and COX-2 inhibition in preclinical studies using murine and human in vitro models suggests a possible role for this combination therapy in the treatment of mCRC in the clinic. The association of lower PD-L1 expression and survival in post hoc analysis of large prospective cohort studies further strengthens the case for the addition of COX inhibitors as an immunomodulatory agent to ICIs for the treatment of MSS CRC, that historically responds poorly to ICI treatment.

## 2.6 DEFINITIONS

*KRAS*: Kirsten Rat Sarcoma gene. Oncogene that normally controls cell proliferation. Mutated *KRAS* confers unchecked cell proliferation.

*NRAS*: Neuroblastoma Rat Sarcoma gene, proto-oncogene.

*BRAF*: Murine oncogene viral sarcoma Homolog B, a proto-oncogene

Interferon  $\gamma^+$  A cytokine that functions in immune processes

Tregs Tumor-infiltrating regulatory T-cells

*Interleukin 6,8 and 1* tumor promoting cytokines

CTLs Cytotoxic T lymphocytes, lymphocytes responsible for attacking viral invaders and cancer cells or other damaged cells

CD274 A gene that encodes programmed death ligand one (PD-L1)

*Mlh1* and *Msh2* mismatch repair genes often mutated in the MSI-H phenotype

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## **CHAPTER 3: STUDY METHODS**

### **3.1 STUDY DESIGN**

The proposed study will be a single center, open label, randomized allocation, parallel assignment, Stage II clinical trial investigating the effects of nivolumab/ipilimumab plus celecoxib or aspirin in patients with MSS/pMMR mCRC compared to a historical control of nivolumab/ipilimumab alone in MSS/pMMR mCRC arm of the CheckMate 142 trial. Assignment to the celecoxib or aspirin treatment group will be determined by random assignment. We will also follow the biomarkers BRAF/KRAS/NRAS mutation status (at baseline), PD-L1 expression, IDO1 expression, MDSC level and COX-2 expression level, cytokines Il-6 and Il-1 $\beta$  evaluated at baseline and at the follow up biopsy.

#### **3.1a Study Groups**

Our interventional groups consist of patients who are  $\geq 18$  years of age and have mCRC that is histologically confirmed as MSS or pMMR mCRC, evaluated by local guidelines. (See Appendix B for MSI criteria evaluation options.) These patients either have progressed on or were intolerant of previous treatment with cytotoxic chemotherapy with irinotecan, fluorouracil and oxaliplatin. Patients who declined systemic chemotherapy are also eligible. Patients are permitted to have participated in curative-intent or palliative radiation therapy, chemotherapy, biological therapy or other investigational therapy but all therapies must be completed by  $>28$  days before treatment initiation and all palliative radiation treatments must be completed  $\geq$  two weeks before treatment initiation. All patients must have an Eastern Cooperative Oncology Group (ECOG) performance status of  $\leq 1$  and measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST version 1.1). See Appendix E for ECOG performance status and Appendix K for RECIST criteria classifications.

Exclusion criteria includes any active, known or suspected autoimmune disease, requiring corticosteroid treatment >10mg prednisone daily or other immunosuppressive medication  $\leq$  14 days prior to beginning treatment. Patients with prior treatment with the anti-PD1, anti-PD L1/L2 and anti CTL-4 immune checkpoint or other agent targeting T-cell co-stimulation or immune checkpoint pathways are excluded.

Also excluded are patients with serious uncontrolled medical disorders. Patients with active brain or leptomeningeal metastases or prior malignancy within the previous 3 years except for cured select localized cancer are also excluded from the study.

Patients must not be taking Non-steroidal Anti-inflammatory Drugs (NSAIDs), Aspirin or COX-2 inhibitors at the time of registration and they must not have a documented allergic reaction or hypersensitivity to them. Patients with a history of peptic ulcer disease or GI bleeding are excluded. Patients with bleeding coagulopathies, such as Von Willebrand disease, liver failure, antiphospholipid syndrome, glucose-6 phosphate dehydrogenase (G6PD) deficiency, and hemophilias will be excluded from the study.

Female participants must not be pregnant or breastfeeding and both sexes must agree to use effective contraception for the duration of the study and up to 90 days after the last study drug administration.

### **3.1b Treatment Administration**

Nivolumab will be dosed at 3mg/kg IV, administered over 60minutes followed by ipilimumab 1mg/kg IV infusion administered over 90minutes once every 3 weeks for four doses and then nivolumab 3mg/kg IV once every 2 weeks until disease progression, discontinuation because of toxicity, death, withdrawal of consent, or study end. No dose modifications are permitted. See Appendix H for Nivolumab and Ipilimumab dose calculations. Dose interruptions due to treatment related adverse events (TRAEs) are

permitted and documented. See Appendix M for Criteria for Treatment Delay and Resumption. Treatment beyond the initial progression will be permitted if the patient tolerates and deemed to benefit from the study treatment at the investigator's discretion.<sup>4</sup>

### **3.2 PATIENT SELECTION AND STUDY POPULATION**

The source population includes adults of at least 18 years of age with previously treated microsatellite stable (MSS) mCRC. The study population is drawn from this pool over a 6-month enrollment period at Yale New Haven Hospital. All participants must have a completed eligibility criteria checklist (Appendix F Eligibility Criteria Checklist). The eligibility criteria are described in detail in Section 3.1. After confirmation of eligibility, signing of the Consent for Participation in the Research Project (Appendix G) by both the patient and the investigator and completing the Patient Registration Form (Appendix I), the patients will be assigned an identifying number (Patient Registration Number) by a regulatory staff member. This identifying number will be recorded on every document in addition to the patient's name and serve as a patient identifier.

### **3.3 STUDY REGULATION AND SUBJECT CONFIDENTIALITY**

Yale cancer trials are sponsored by the National Cancer Institute (NCI) and follow NCI guidelines outlined in the Investigator's Handbook.<sup>75</sup> The protocol application along with the Compound Authorization and Consent for Participation in a Research Study form (Appendix G) will be submitted to the Yale Institutional Review Board (IRB). All study personnel will obtain Health Insurance Portability and Accountability Act (HIPAA) certification prior to the beginning of recruitment. Research staff must also complete the Yale HIC training. Although all four drugs used in the trial are commercially available, because they are used off label, the study will be required to file the investigational new drug (IND) form with the FDA. The study protocol and amendments will be approved by

the Yale Human Investigation Committee (HIC). The study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and the informed consent form that explains the study in an easily understandable language will be supplied to eligible participants.

### **3.4 RECRUITMENT**

Study participants will be recruited from the pool of patients undergoing treatment for mCRC at Yale Cancer Center (YCC), which is a National Cancer Institute (NCI) designated comprehensive cancer center in Southern Connecticut. YCC is a collaboration between nationally and internationally renowned researchers, physicians, physician assistants, nurse practitioners and medical staff at the Yale School of Medicine and Smilow Cancer Hospital. Participants will be recruited from the YCC group sites over a 6 months period via flyers and advertisements placed on clinical trial websites.

### **3.5 STUDY VARIABLES AND MEASURES**

The independent variables of the study are the drug regimen used in the two arms of the study. Arm A will be given ipilimumab 1mg/kg IV, nivolumab 3mg.kg IV and aspirin 325mg daily orally, while Arm B will be given ipilimumab 1mg/kg IV, nivolumab 3mg/kg IV and celecoxib 400mg daily orally. The dependent variable examined as the primary outcome is objective response rate (ORR). ORR is defined as best response or complete response (CR) or partial response (PR) divided by the number of treated patients as per RECIST v1.1 guideline. To determine the ORR, tumor burden will be measured at baseline and throughout the treatment period. Secondary endpoints (dependent variables) were disease control rate (DCR), safety and tolerability, progression free survival (PFS), overall survival (OS), association between biomarker expression (COX-2 expression level, MDSC level, PD-L1 level, C-reactive protein level,

cytokine *Il-1β* and *Il-6* expression) and efficacy (ORR, PFS, OS) and changes from baseline in patient reported outcomes (PRO). Secondary outcome measures will also be measured at baseline and followed throughout the study, as discussed in Section 3.7.

### **3.6 METHODOLOGY CONSIDERATIONS**

#### **3.6a Assignment of intervention**

Upon the completion of the eligibility checklist and registration form, the patients will be randomly assigned using permuted block randomization to either study Arm A or B of by a statistician. Neither patients, nor research staff will be blinded to allocation.

#### **3.6b Adherence**

Adherence will be monitored and recorded throughout the duration of the study. Study medications administered by the treatment team such as nivolumab and ipilimumab infusions will be logged at each visit. Patients will be reminded of infusion appointments via telephone 24 hours prior to the appointment time and will require a verbal confirmation. Patients will be given the oral medications in study issued bubble-packaging with dates for each dose and patients will be required to return the empty packaging at the subsequent appointment time. Adherence to oral medication will be logged by Research Staff.

#### **3.6c Monitoring of Adverse Events**

All adverse events (AEs) will be reported to the Yale IRB at the time of the continuing review. Serious adverse events (SAE) will be reported to the Yale IRB within 5 business days using the Form 710 FR4: UPIRSO, Including AEs Reporting Form through the Yale IRES (See Appendix Q for definitions of AE and SAE).<sup>76</sup>

Adverse events will be further analyzed based on the published in-depth safety evaluation of the CheckMate 142 trial<sup>24</sup> and will include frequency of treatment related adverse events (TRAEs), select TRAEs (sTRAEs), and immune related adverse event

incidences, time to onset (TTO), time to resolution (TTR), immune modulating medication (IMM) use, dose delay, and sTRAE occurrence after resuming therapy. Refer to Appendix N for definitions of TRAE and IMAE (Immune mediated adverse events).

### **3.7 DATA COLLECTION**

Efficacy and safety data will be collected on any patient who received at least one treatment dose. The data will be collected by the study Research Staff and by administrative staff at Smilow Cancer Hospital at Yale. Subjects will be enrolled during a six months enrollment period and the data collection phase will take 18 months for the total study duration of two years.

Tumor burden will be measured at baseline using computed tomography (CT) or magnetic resonance imaging (MRI) evaluated by the RECIST criteria (Version 1.1)  $\leq 28$  days before the first dose (Treatment Start Date). Subsequent assessments will be carried out every 6 weeks for 24 weeks and every 12 weeks thereafter until either disease progression or discontinuation. All responses will need to be confirmed by a second scan  $\geq 4$  weeks later. The primary endpoint of objective response rate (ORR) will be based on these imaging studies. Patients will be observed for survival every three months up to the two-year mark of the study to evaluate the secondary endpoint of overall survival (OS).

Patient reported outcome (PRO) analyses will be conducted at baseline, and at every 6 weeks based on the European Organisation for Research and Treatment of Cancer (EORTC) Core Quality of Life Questionnaire (QLQ-C30) and three-level five-dimensional EuroQol instrument (EQ-5D).<sup>69, 68</sup>

The EORTC QLQ-C30 has three components: symptoms, functioning and quality of life (QOL), each using a scale of 0-100, with 100 corresponding to the best functioning

and 0 corresponding to the worst functioning. For each of these scales an at least 10-point change from baseline was deemed clinically meaningful.<sup>70</sup>

The EQ-5D will analyze problems in five health dimensions: mobility, self-care, usual activities, pain or discomfort, and anxiety or depression and will rate them on a scale of “None”, “Some” or “Extreme”. We will also utilize the EQ-5D visual analog scale that patient will use to rate their health on a scale of 0-100, with higher values corresponding to better health. An at least 7-point change from baseline will be regarded as clinically meaningful.

Participants will be assessed for signs and symptoms of ICI related adverse events and blood samples will be collected at baseline and before each ICI dose to evaluate clinical chemistries including liver function tests (LFTs), adrenocorticotrophic hormone (ACTH) level, and thyroid function tests.

Tissue samples will be collected from all participants. Patients will be able to provide data from a previous biopsy to determine the baseline biomarker status but a second sample will be collected to determine changes from baseline. Tissue samples will be collected according to the Yale IRB Policy 440: Collection and Banking of Data, Biological Specimens and Other Materials in Human Research.<sup>77</sup>

Safety will be evaluated using the Common Terminology Criteria for Adverse Events (CTCAE Version 5.0 See Appendix D) throughout the duration of the treatment.<sup>50</sup>

### **3.8 SAMPLE SIZE CALCULATION**

Simon’s two-stage design will be used.<sup>74</sup> The null hypothesis that the true response rate is 8.7% observed in the CheckMate 142 trial will be tested against two one-sided alternatives. In the first stage, 14 patients will be enrolled. If there are 1 or fewer responses in these 14 patients, the study will be stopped. Otherwise, 27 additional

patients will be enrolled for a total of 41. The null hypothesis will be rejected if 7 or more responses are observed in 41 patients. This design yields a type I error rate of 5% when the true response rate is 23.7%. See Appendix J for additional calculations.

### **3.9 ANALYSIS**

Data analysis will be modeled after the CheckMate 142 trial. The baseline demographics data such as age, sex, race, ECOG performance status, primary tumor location, number of prior systemic treatments, prior therapies received, prior radiotherapy received, mutation status (*BRAF/KRAS* wild type, *BRAF* mutation, *KRAS* mutation, unknown), tumor PD-L1 expression quantifiable at baseline and clinical history of Lynch syndrome of the study sample will be described and compared by treatment group and historical control group. The data will be reported using descriptive statistics with standard deviation and means, medians and ranges for continuous variables.

Data from all enrolled patients will be analyzed regarding efficacy and safety. The secondary outcomes of safety and tolerability and patient reported outcomes (PRO) will also be measured using descriptive statistics. The 95% CI for the dichotomous measure of objective response rates (ORR) will be estimated using the Clopper and Pearson method. ORR (proportion of patients with a predefined amount of tumor reduction) in the two treatment arms will be compared using the chi-square test. If the observations are rare, Fisher Exact Test will be used. The Kaplan-Meier product-limit method will be used to determine medians for time to event measures such as progression-free survival (PFS), overall survival (OS), duration of response (DOR) and the corresponding 95% CI will be calculated based on log-log transformation.

The primary outcomes of objective response rate (ORR) and the secondary outcomes of disease control rate (DCR), progression free survival (PFS), overall survival

(OS) will be measured based on the RECIST v1.1 guideline. Data will be analyzed based on the per protocol analysis. We will consider P-values less than 0.05 as statistically significant for all analyses.

### **3.10 TIMELINE AND RESOURCES**

The study time period is planned to be 24 months, with a 6-month period of rolling enrollment followed by 18 months of treatment administration. Statistical analysis or objective response rate will be performed on a rolling basis, but majority of the data analysis will take place after the conclusion of the treatment of the last participant.

The full-time study personnel will include the principal investigator (PI), Dr. Michael Hurwitz, Co-PI, Angela Preda, PA-SII, Research Staff (administrative) who will be tasked with patient recruitment, data collection, and data entry. The study will seek to recruit part time pathologist to assist with analysis of tumor samples and a statistician to aid with data analysis. Standard oncological care will be provided by the patients' oncologist at Yale New Haven Hospital who will have frequent communication with the study research personnel. All clinical tasks such as phlebotomy, imaging and laboratory analysis will be performed by hospital resources.

The full-time personnel will require office space in the Oncology Department at Yale New Haven Hospital equipped with a computer with appropriate software for statistics, data storage and analysis.

The study drugs ipilimumab and nivolumab will be supplied by Bristol-Myers Squibb and Celebrex will be supplied by Pfizer Inc. No other resources will be utilized from these pharmaceutical companies.

## REFERENCES

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## **CHAPTER 4: CONCLUSION**

### **4.1 Study Advantages**

Although our Phase II study sample is smaller than a Phase III trial's, we aimed to reduce the effect of confounders by the randomization of subjects into the two arms.

The use of a cross-trial control arm intended to reduce potential harm to subjects by eliminating randomization to an arm with suboptimal response rates and response durations. This trial design also reduced the number of subjects need to be recruited.

The Simon's two-stage design introduces an element of safety as the trial will only go to second stage if there are adequate number of responding patients. This practice avoids exposing patients to undue risk in case the intervention has low or no activity.

The relative ease of recruitment is another advantage. The low prevalence of the MSI-H mCRC forced the CheckMate 142 trial to be carried out as a multi-center study. With 96% of mCRC being MSS,<sup>6</sup> our study anticipates greater ease in recruiting. Given the 21% prevalence of mCRC is in the CRC population, the MSS mCRC portion of all CRC patients presenting is about 20%. Thus, theoretically, one fifth of all Yale New Haven Hospital CRC patients are eligible to participate.

### **4.2 Study Limitations**

To reduce the possibility of confounders, we used restriction and randomization during the design phase of the study while also keeping the external validity in mind.

The study has moderately permissive exclusion criteria, a practice that could lend itself to introduction of confounders. For example, previous treatment with biological or other investigational treatments were not a reason for exclusion. As we are uncertain of the long-term effects of these treatments, we cannot rule out them out as confounders.

Although more strict restriction would have increased internal validity, it would have decreased the external validity and generalizability.

Comparing the treatment arms to a historical control can also present as a drawback since we are comparing patient populations at different institutions. For the measure of the effect, namely objective response rate (ORR) we modeled our trial after the CheckMate 142 study's arm that recruited non-MSI-H mCRC patients (MSS). This arm of the study was rather modest, recruiting only 23 patients but showed an ORR of 8.7% (2 responses in 23 patients). Demographics and biomarker data (except for PD-L1) was not reported for this cohort, thus we will not be able to compare the control arm and our experimental arms regarding these variables.

While our study is designed to run for 18 months, follow up of disease recurrence at later time points is crucial. Our 18-month study duration will be sufficient for elucidating responses and immediate adverse events but it is essential that patients will be followed for a longer period of time to measure long-term effects of these drugs.

Another limitation of our study is that although it permits the participation of patients who decline the use of cytotoxic chemotherapy, the majority of patients will have had previous treatment with one or more first or second line agents. To recruit sufficient number of patients in a single center setting, we cannot restrict this variable. However, the response rates might be increased in treatment naïve patients.

#### **4.3 Clinical Significance**

This trial aims provide an ICI option to the MSS subgroup, which currently relies on traditional cytotoxic chemotherapy. Thus, an immune therapy option for this population would signify a paradigm shift. We also anticipate gaining insight into the biomarkers identifying future responders.

## REFERENCES

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## APPENDICES

### APPENDIX A TNM STAGING OF COLORECTAL CANCER

**General Definition of Colorectal Cancer:**

Adenocarcinoma, high-grade neuroendocrine carcinoma and squamous carcinoma of the colon and rectum are covered by this staging system

Excluded are appendiceal carcinoma, anal carcinoma and well-differentiated neuroendocrine tumor (carcinoid)

**Stage grouping**

Stage 0:	Tis	N0	M0
Stage I:	T1 - T2	N0	M0
Stage IIA:	T3	N0	M0
Stage IIB:	T4a	N0	M0
Stage IIC:	T4b	N0	M0
Stage IIIA:	T1 - T2	N1 / N1c	M0
	T1	N2a	M0
Stage IIIB:	T3 - T4a	N1 / N1c	M0
	T2 - T3	N2a	M0
	T1 - T2	N2b	M0
Stage IIIC:	T4a	N2a	M0
	T3 - T4a	N2b	M0
	T4b	N1 - N2	M0
Stage IVA:	any T	any N	M1a
Stage IVB:	any T	any N	M1b
Stage IVC:	any T	any N	M1c

**Primary tumor (pT)**

- **TX:** primary tumor cannot be assessed
- **T0:** no evidence of primary tumor
- **Tis:** carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
- **T1:** tumor invades submucosa (through the muscularis mucosa but not into the muscularis propria)
- **T2:** tumor invades muscularis propria
- **T3:** tumor invades through the muscularis propria into the pericolorectal tissues
- **T4:**

- **T4a:** tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
- **T4b:** tumor directly invades or adheres to other adjacent organs or structures

**Regional lymph nodes (pN)**

- **NX:** regional lymph nodes cannot be assessed
- **N0:** no regional lymph node metastasis
- **N1:** metastasis in 1 - 3 regional lymph nodes
  - **N1a:** metastasis in 1 regional lymph node
  - **N1b:** metastasis in 2 - 3 regional lymph nodes
  - **N1c:** no regional lymph nodes are positive but there are tumor deposits in the subserosa, mesentery or nonperitonealized pericolic or perirectal / mesorectal tissues
- **N2:** metastasis in 4 or more regional lymph nodes
  - **N2a:** metastasis in 4 - 6 regional lymph nodes
  - **N2b:** metastasis in 7 or more regional lymph nodes

**Distant metastasis (pM)**

- **M0:** no distant metastasis by imaging; no evidence of tumor in other sites or organs (this category is NOT assigned by pathologists)
- **M1:** distant metastasis
  - **M1a:** metastasis confined to 1 organ or site without peritoneal metastasis
  - **M1b:** metastasis to 2 or more sites or organs is identified without peritoneal metastasis
  - **M1c:** metastasis to the peritoneal surface is identified alone or with other site or organ metastases

**Reference:**

Weisenberg E. TNM staging of colorectal carcinoma (AJCC 8th edition).

PathologyOutlines.com website.

<http://www.pathologyoutlines.com/topic/colontumorstaging8ed.html>. Accessed July 13th, 2019.

**APPENDIX B MICROSATELLITE INSTABILITY CLASSIFICATION**

Testing for microsatellite instability in CRC can be accomplished via three methods:

1. Immunohistochemical staining for the complete loss of the four most common mismatch repair (MMR) proteins: MLH1, MLH2, MSH6 and PMS2.
2. Testing the length of five specific microsatellites (BAT25, BAT26, D2S123, D5S346, D17S250) via polymerase chain reaction (PCR). MSI-H classification is

conferred to tumor samples with instability (defined as length variation between normal and tumor samples) in greater than 30% of microsatellites.

3. MSI sensor which is a next-generation sequencing that evaluates a large number of microsatellites throughout the genome.

Of note, the FDA does not recommend a specific testing methodology to establish MSI-H status.

**Reference:**

Overman MJ, Ernstoff MS, Morse MA. Where We Stand With Immunotherapy in Colorectal Cancer: Deficient Mismatch Repair, Proficient Mismatch Repair, and Toxicity Management. *American Society of Clinical Oncology educational book. American Society of Clinical Oncology. Annual Meeting. 2018(38):239-247.*

**APPENDIX C IMMUNOSCORE**

Quantitative immunohistochemistry to determine the density of CD3+ and CD8+ cells (as markers of TH1/cytotoxic memory T lymphocytes CD8 and CD45RO) in CRC tumors. Immunohistochemistry staining of CD3+ and CD8+ is performed in two regions, CT (center of tumor) and IM (invasive margin) and is followed by automated quantification of whole slide sections. The Immunoscore utilizes the numeration of cells in the CT and the IM of resected tumors to provide a score ranging from 0-4, Immunoscore 0 (“I” 0), when low densities of both cell types are found in both regions, to Immunoscore 4 (“I” 4), when high densities are found in both regions.

- I 0** – – – –
- I 1** Hi – – –
- I 2** Hi Hi – –
- I 3** Hi Hi Hi –
- I 4** Hi Hi Hi Hi

Current Immunoscore procedure and reagents

Procedure	Current recommended steps
Tumor selection	Block which is the most infiltrated by the immune cells and containing the core of the tumor (CT) and the invasive margin (IM)
Sample preparation	2 paraffin sections of 4-microns of the tumor block deposited in deionized water on Superfrost-plus slides
Immuno-histochemistry (IHC)	2 single stainings using IVD certified antibodies
Antigen retrieval	CC1 tris-based buffer pH8
Primary antibody	CD3 (2GV6, Ventana) and CD8 (C8/144, Dako)
Primary antibody	
Diluent	K 004 (Clinisciences) for CD8

Secondary reagents	Ultraview TM DAB (Ventana)
Counterstaining	Hematoxylin II (Ventana)
Autostrainer	Benchmark XT (Ventana)
Scanner	NanoZoomer 2.0-HT (Hamamatsu)
Digital pathology	Architect XD software (Definiens)
Immunoscore	
Quantification	Immunoscore Plug-in (INSERM / AP-HP)

**Reference:**

Galon J, Pagès F, Marincola FM, et al. Cancer classification using the Immunoscore: a worldwide task force. *Journal of translational medicine*. 2012;10(1):205.

**APPENDIX D NATIONAL CANCER INSTITUTE COMMON  
TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (CTCAE) Version  
5.0**

**Grade 1**

Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

**Grade 2**

Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL\*.

**Grade 3**

Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL\*\*.

**Grade 4**

Life-threatening consequences; urgent intervention indicated.

**Grade 5**

Death related to AE.

\*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

\*\*Self care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

**Reference:**

National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) v5.0

[https://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/docs/CTCAE\\_v5\\_Qwick\\_Reference\\_5x7.pdf](https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Qwick_Reference_5x7.pdf) Published November 27, 2017. Accessed December 6, 2018.

## APPENDIX E ECOG PERFORMANCE STATUS SCALE

### GRADE

- 0 Fully active, able to carry on all pre-disease performance without restriction
- 1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
- 2 Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
- 3 Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
- 4 Completely disabled; cannot carry on any self-care; totally confined to bed or chair
- 5 Dead

### Reference:

Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol.* 1982;5:649-655.

## APPENDIX F ELIGIBILITY CHECKLIST

The following form must be filled out completely and must be signed by both the patient and the provider. Patients must meet all criteria to be eligible for this study.

Proposed treatment start date: \_\_\_\_\_

- 1.  Age  $\geq$  18 years of age. DOB \_\_\_\_\_ Today's date: \_\_\_\_\_
- 2.  Recurrent CRC or mCRC. Histologic confirmation \_\_\_\_\_  
Sites of metastases \_\_\_\_\_
- 3.  No active brain or leptomeningeal metastases or prior malignancy within the previous 3 years except for cured select localized cancer
- 4.  Histologically confirmed MSS or pMMR  Histochemistry or  PCR or  MSIsensor Date performed \_\_\_\_\_
- 5.  Use of previous chemotherapy regimens of irinotecan, fluoroacil or oxaliplatin or refused chemotherapeutic agents
- 6.  Completed curative-intent radiation therapy, chemotherapy, biological or other investigational treatment  $>$  28 days before treatment start date
- 7.  Completed palliative radiation therapy  $\geq$  two weeks before treatment start date
- 8.  ECOG Performance Status 0 or 1
- 9.  Measurable disease as per RECIST v1.1 criteria

10.  No active autoimmune disease or other disease requiring high dose immunosuppression
11.  No prior treatment with anti PD-1, anti-PD-L1/2 or anti-CTLA-4 immune checkpoint agent or other agent targeting T-cell co-stimulation or immune checkpoint pathways
12.  No serious uncontrolled medical disorder
13.  Not taking NSAIDs, aspirin or COX-2 inhibitors at the time of the registration.
14.  No documented allergic reaction or hypersensitivity to COX-2 inhibitors, aspirin or NSAIDs
15.  No history of peptic ulcers or gastrointestinal bleeding
16.  No bleeding diathesis
17.  Absolute leukocyte count  $\geq 2500$  /mcL
18.  Absolute lymphocyte count (ALC)  $\geq 500$  /mcL
19.  Absolute neutrophil count (ANC)  $\geq 1500$  /mcL
20.  Platelets  $\geq 100,000$  / mcL
21.  Hemoglobin  $\geq 9$  g/dL or  $\geq 5.6$  mmol/L without transfusion or EPO dependency (within 7 days of assessment)
22.  No asthma exacerbated by aspirin or NSAID use
23.  No acute or severe hepatic failure
24.  Serum total bilirubin  $\leq 1.5$  X ULN  
or  
 Direct bilirubin  $\leq$  ULN for subjects with total bilirubin levels  $> 1.5$  ULN
26.  AST and ALT  $\leq 2.5$  X ULN OR  $\leq 5$  X ULN for subjects with liver metastases
27.  Albumin  $> 2.5$  mg/dL
28.  No acute or severe renal failure
29.  Serum creatinine  $\leq 1.5$  X upper limit of normal (ULN)  
or  
Measured or calculated creatinine clearance  $\geq 60$  mL/min for subject with creatinine levels  $> 1.5$  X (GFR can also be used in place of creatinine or CrCl) institutional ULN
30.  Agrees to use effective contraception (both male and female)

Women only:

31.  Not pregnant or breastfeeding determined by  $\beta$ -Hcg level 72 hours prior treatment start date

\_\_\_\_\_ Date: \_\_/\_\_/\_\_\_\_  
Patient signature

All criteria met.

\_\_\_\_\_ Date: \_\_/\_\_/\_\_\_\_  
Provider signature

**APPENDIX G COMPOUND AUTHORIZATION AND CONSENT FOR PARTICIPATION IN A RESEARCH STUDY**

IRB Protocol#

**COMPOUND AUTHORIZATION AND CONSENT FOR PARTICIPATION IN A RESEARCH STUDY**

**YALE UNIVERSITY SCHOOL OF MEDICINE  
YALE-NEW HAVEN HOSPITAL  
YALE-NEW HAVEN HOSPITAL: SMILOW CANCER CENTER**

**Study Title:** Checkpoint and Cyclooxygenase Inhibition in Microsatellite Stable Metastatic Colorectal Cancer

**Principal Investigator (the person who is responsible for this research):**

Dr. Michael Hurwitz, MD, PhD 20 York Street, New Haven, CT, 06510

**Phone Number:** (203) 400-4822

**Research Study Summary:**

We are asking you to join a research study.

- The purpose of this research study is to investigate how effective and how safe is a new drug combination for the treatment of metastatic colorectal cancer.
- Study procedures will include: a screening visit, physical examination, blood testing, biopsy of metastatic tumor site, CT or MRI imaging studies, infusion of immunotherapy drugs and a medication taken by mouth.
- The number of visits required cannot be determined in advance. You will be required to complete a screening visit and if you qualify for participating in the study, you will

be receiving a dual immunotherapy infusion every three weeks for the total of four doses followed by only one of the drug infusions every other week until we determine that your cancer is not responding or you need to discontinue the treatment for another reason.

- These visits will take between 1- and 3.5-hours total.
- There are some risks from participating in this study. You may experience side effects that can range from uncomfortable to serious and can even lead to death while taking the study medications.
- The study may have no benefits to you. As the effectiveness of combination of medications was not previously studied, we cannot tell if you will personally benefit from participating in this study.
- There are other choices available to you outside of this research. You may opt for the standard of care treatment prescribed by your oncologist, participating in another study or seek comfort measures only.
- Taking part in this study is your choice. You can choose to take part, or you can choose not to take part in this study. You can also change your mind at any time. Whatever choice you make, you will not lose access to your medical care or give up any legal rights or benefits.
- If you are interested in learning more about the study, please continue reading, or have someone read to you, the rest of this document. Take as much time as you need before you make your decision. Ask the study staff questions about anything you do not understand. Once you understand the study, we will ask you if you wish to participate; if so, you will have to sign this form.

#### **Why is this study being offered to me?**

We are asking you to take part in a research study because you are an adult  $\geq 18$  years of age who has been diagnosed with colorectal cancer that has spread to distant sites (metastasized).

We are looking for 82 participants to be part of this research study that will take place at Smilow Cancer Hospital at Yale New Haven Hospital.

#### **Who is paying for the study?**

This study is funded by the National Institute of Health (NIH).

#### **Who is providing other support for the study?**

Study medications ipilimumab (Yervoy) and nivolumab (Opdivo) were the generous gift of Bristol-Myers Squibb. Celecoxib (Celebrex) was the generous gift of Pfizer.

#### **What is the study about?**

The purpose of this study is to investigate the effects of the combination of three drugs in two separate groups, either nivolumab, ipilimumab and celecoxib or nivolumab, ipilimumab and aspirin on colon cancer that has spread to distant organs (metastatic colon cancer). Our study seeks to investigate whether either of these combinations are more effective in the treatment of metastatic colorectal cancer than nivolumab and ipilimumab alone. We also seek to investigate the safety of these drug combinations as well as find biomarkers (specific markers found in the blood or in a tissue sample) that can identify patients who respond well to treatment.

### **What are you asking me to do and how long will it take?**

If you agree to take part in this study, this is what will happen: In order to determine whether your participation in this study is appropriate, you will be asked to complete medical screening visit. The testing is routine and can be completed by your medical provider in one visit. To be considered eligible to participate in this study, you must be  $\geq$  18 years of age and have a diagnosis of colorectal cancer that has spread to distant sites but not to the brain or leptomeninges (the covering of the brain). All patients will have a magnetic resonance imaging (MRI) to confirm the absence of brain metastases. The diagnosis of colorectal cancer and distant spread must be confirmed by a tissue sample (histologic diagnosis). You must be able to supply this sample and authorize our trial to run tests on this sample. The tests run on this sample or previous testing must classify you as microsatellite stable (MSS) and/or proficient mismatch repair (pMMR). If no sample is available, you must authorize the study to collect a biopsy sample from an available site. If there is no accessible biopsy site available, you will not be eligible to participate in the study. Your provider will complete a full medical history to gather information about your health, medications and allergies. Your provider will also complete a thorough physical examination. You must have normal organ function prior to starting this study. Your provider can assess this with routine blood work, which will check your blood counts, kidney function and liver function. If any of these routine blood tests are not within normal limits, you will not be eligible to participate. As part of the blood testing, you will have blood testing for infectious diseases such as Hepatitis B, Hepatitis C and HIV. If any of these tests are positive, you will not be able to participate in the study.

A small blood sample will be taken for routine blood testing for electrolytes, blood counts and to measure liver and kidney function and monitor for infection. This routine blood testing will be repeated before each ipilimumab dose.

You will not be able to participate in this study if you were diagnosed with any autoimmune disease that requires you to take more than 10mg of prednisone daily. Your provider will determine if you are eligible. Your performance status will be assessed and you can only participate if you are fully active or if you are restricted only in strenuous activity.

You are eligible for this study if you have used chemotherapy of irinotecan, fluorouracil or oxaliplatin previously and your cancer did not respond to this treatment or if you did not want to use chemotherapy. Prior treatment with any immune checkpoint therapy is not permitted. Prior radiation treatment is allowed, but must be completed by  $>28$  days before treatment start date and all palliative radiation (not curative intent) treatments must be completed  $\geq$  two weeks before treatment initiation. Prior treatment with any checkpoint inhibitor is not permitted.

If you are a female of childbearing age, you must have documented negative pregnancy test within 72 hours prior to the first dose of study medication. This can be a blood or urine test and should be done in all women unless you have had your uterus removed (hysterectomy) or are postmenopausal (no menstruation in the preceding 12 months). During the study, both female and male participants must agree to use adequate birth control.

The size, number and location of your tumors will be assessed using computerized tomography (CT) or magnetic resonance imaging (MRI) scan of your chest, abdomen and pelvis. You will also have an MRI to check for tumors in your brain. Both of these imaging studies will be performed within 28 days of starting study protocol. An ECG will be performed to determine heart function. If your provider deems necessary, additional imaging might be performed. The information gathered during screening visits will be recorded on the Eligibility Checklist that will be stored in your file.

If you decide to participate in this study and your provider determines that you are eligible, you will have additional testing throughout the study period including medical history, physical examination and imaging. This additional care is equivalent to standard cancer care you would receive if you were not enrolled in the study. Computerized tomography (CT) will be performed throughout the study duration to monitor for response to the drug regimen and tumor size. CT imaging might be done more frequently during the study than you would receive if you received standard care. At the beginning of the treatment you will have imaging studies to evaluate tumor size every 6 weeks for 24 weeks and every 12 weeks thereafter until either disease progression or discontinuation. If you respond to treatment, there will be another confirmatory scan  $\geq$  4 weeks later.

A small blood sample will be taken for routine blood testing for electrolytes, blood counts and to measure liver and kidney function and monitor for infection throughout the study duration. This routine blood testing will be repeated before each ipilimumab dose.

If you are a female participant, you will be asked for a urine sample for pregnancy testing at every visit. Both female and male participants will be asked to use adequate birth control method throughout the duration of the study and 3 months after the administration of the last dose of nivolumab/ipilimumab. You will be asked to report all side effects to your provider as soon as they occur during study duration and up to 3 months after the administration of the last nivolumab/ ipilimumab dose.

This study is planned to run for a total of 18 months. During this trial, you must agree not to take other anti-cancer medication (chemotherapy or immunotherapy) or supplements that has not been prescribed by your study provider. You also cannot be taking immunosuppressant medications, the most common of which is corticosteroids. Inhaled and topical forms of corticosteroids are allowed during the trial period.

Participants will be randomly assigned to Arm A (nivolumab/ipilimumab plus aspirin) or Arm B (nivolumab/ipilimumab plus celecoxib) by study administrators. As a participant, you must agree to adhere to the assignment and take the home medications as prescribed. Dose adjustment of these medications will not be allowed. If dose adjustment is necessary for any reason, you will no longer be able to participate in the study. The treatment will begin within 5 business days of randomization. If you are in Arm A, you will receive weight-based infusions of nivolumab and ipilimumab and a fixed dose of aspirin. If you are in Arm B, you will receive weight-based infusions of nivolumab and ipilimumab and a fixed dose of celecoxib. You will receive nivolumab and ipilimumab every 3 weeks in the beginning for a total of four doses followed by nivolumab only infusions every other week. The total number of infusions will be decided based on whether you respond to treatment and how well you respond to treatment. You might receive the nivolumab infusions until the 18-month mark. Each visit to Yale New Haven Hospital will last between 1 and 3 hours and a half hours. The doses and allocation to each group are not determined by your clinician and cannot be changed once assigned. An equal number of participants will be assigned to either arm. Once assigned, you will be required to adhere to the medication regimen in the assigned arm. You will be asked to keep a log of the home medication portion of the study (aspirin or celecoxib) and bring the log to every appointment. You will also fill out two questionnaires in the beginning and every 6 weeks that will ask you about your overall wellbeing. These questionnaires generally take about 11 minutes to complete.

### **What are the risks and discomforts of participating?**

The risks of taking this new drug combination is not yet known, thus there may or may not be risks associated with this treatment. You may experience side effects of the drugs

used in this study You will be closely monitored during the trial period and you will be asked to report any side effects to your provider as soon as they occur. Individually, all components of the treatment regimen have been studied and found to have side effects that range from mild and manageable to very serious, including a small risk of death. All efforts will be made to make you comfortable during the treatment period and treat side effects. If the side effects become serious or life threatening, you will receive immune suppressant medication that reverses the effect of the study drugs. If you are not able to tolerate the drugs, your participation in the study will be stopped. You should seek emergency evaluation by calling 911 or going to the Emergency Department in case you experience severe side effects to the medications. Most side effects go away once study medications are stopped, but there is a chance that side effects become permanent. Nivolumab and ipilimumab are FDA-approved biological agents for a group of colorectal cancer patients who have the marker called microsatellite instability high (MSI-H) or deficient mismatch repair (dMMR) to help the body's immune respond better in eliminating the cancer. This group that does not include the type of cancer that you have (Microsatellite stable, MSS), thus the safety information comes mostly from studying the other group. Ipilimumab has the highest potential to cause discomforts and risks among the study drugs used, but in this study, it is used in what is considered a low dose. Patients in previous studies that used this drug combination most commonly experienced diarrhea, tiredness and itchiness. About one third of the patients had more serious side effects such as elevated liver enzymes, elevated lipase (a pancreatic enzyme), low blood counts, and bowel inflammation. About 13 out of 100 patients had to discontinue treatment because of serious side effects such as kidney injury or autoimmune liver inflammation. Thus, while taking the study medications, there is a chance that you will experience some of these side effects. There is a chance that you will have to discontinue this study because of the side effects and hospitalization to treat the side effects might be necessary.

Celecoxib and aspirin are non-steroidal anti-inflammatory drugs (NSAIDs) that are commonly used to treat inflammatory diseases such as arthritis at the dose that is used in the study (Aspirin 325mg daily or Celecoxib 400mg daily). Aspirin at a lower dose (100mg daily) is also used for colon cancer prevention. Aspirin at the 81mg daily dose is also used as an anti-platelet agent to prevent blood clots in the prevention of stroke or heart attack.

The most common side effects of aspirin are heartburn, nausea, vomiting and stomach pain. Elevated liver enzymes were also reported as well as very rare cases of kidney impairment and failure. There is also an increased risk of bleeding. The most serious side effects are rare but include bleeding from a stomach ulcer, kidney failure or aspirin-exacerbated respiratory disease. We take precautions that patients at risk for these serious side effects are not participating in this study. The most common side effects of celecoxib are dyspepsia, nausea, diarrhea and stomach pain. Side effects will be managed by your clinician; however, you might need to discontinue treatment if you have a serious bleeding from an ulcer or if your kidney function significantly worsens.

To receive nivolumab and ipilimumab infusions, you will have to have an IV-line placed. IV-line placements are common procedures but they can be uncomfortable. Mild pain around the IV site is common but goes away quickly when the IV is removed. To monitor the progress of your tumors, you will need to have CT or MRI scans periodically. CT scans will expose you to a high dose of radiation. Repeated doses of radiation can be harmful on the long run. MRI studies do not expose you to harmful

radiation but take longer and can be uncomfortable as you must stay still laying on your back for long periods of time. If you suffer from claustrophobia, you might not be able to tolerate MRI scans.

**How will I know about new risks or important information about the study?**

We will tell you if we learn any new information that could change your mind about taking part in this study.

**How can the study possibly benefit me?**

You may or may not benefit from participating in this trial that will study the benefits and potential harms of this new combination therapy. While patients have been taking this medication combination previously, the potential benefits and safety of this combination have not been previously investigated. Researchers and medical providers think that this new combination will be more effective in treating cancer than receiving the two immune checkpoint therapy drugs (nivolumab and ipilimumab) alone but it is not known in advance who will personally benefit or experience adverse events while taking this medication combination.

**How can the study possibly benefit other people?**

The benefits to science and other people may include a better understanding of how we can make the type of colon cancer patients that you belong to (microsatellite stable, MSS) respond to treatment as well as the group these medications are currently FDA approved for (MSI-H) and which patients in your group will be good candidates for this treatment in the future. We are hoping that the results of this study will lead to new treatments for patients with your type of cancer.

**Are there any costs to participation?**

If you take part in this study, you will not have to pay for any services, supplies, study procedures, or care that are provided for this research only (they are NOT part of your routine medical care). The study medications ipilimumab, nivolumab, aspirin or celecoxib will be provided to you at no cost. However, there may be additional costs to you. These can include costs of transportation and your time to come to the study visits. You or your health insurance must pay for services, supplies, procedures, and care that are part of your routine medical care. You will be responsible for any co-payments required by your insurance.

**Will I be paid for participation?**

You will not be compensated for participating in this study. There is no reimbursement for traveling expenses to the study site.

**What are my choices if I decide not to take part in this study?**

Instead of participating in this study, you have some other choices.

You could:

- The same treatment combination is not available outside of this study for the type of colorectal cancer you have (microsatellite stable (MSS) or proficient mismatch repair pMMR). You might receive the standard of care treatment decided by you and your oncologist. The main treatment options for colorectal cancer that spread to distant sites are traditional chemotherapy, anti-VEGF or EGFR therapy. The first line chemotherapeutic agents are FOLFOX (folinic acid (leucovorin), fluorouracil (5FU) and oxaliplatin), FOLFIRI (folinic acid, 5FU and irinotecan), Capecitabine and 5FU/leucovorin have been used with or without the anti-

angiogenic agent bevacizumab. For high tumor burden or rapidly progressing disease the quadruple chemotherapy FOLFOXIRI (folinic acid, fluorouracil, oxaliplatin, irinotecan) can be considered. For refractory colon cancer drugs regorafenib or trifluridine/tipiracil are recommended. Some patients in this study have already tried these therapies and they didn't work for them. If you have not tried these therapies, you might benefit from trying them. The benefit of chemotherapeutic agents is that they are well studied and have known side effects. The risk associated with chemotherapy is the uncomfortable and sometimes toxic side effects. The side effects of chemotherapy vary based on the combination but the most commonly reported are diarrhea, neuropathy (numbness and tingling sensation in the arms and legs), fatigue, hair loss and cold sensitivity. Some side effects, such as neuropathy can be treatment limiting and irreversible. You should further discuss your chemotherapy options with your oncologist.

- Take part in another study. You might also seek to participate in another study listed at [www.clinicaltrials.gov](http://www.clinicaltrials.gov)
- Receive comfort care only, without any treatment for your disease. This option is called palliative care. You and your oncologist may decide what options are best for you.

#### **How will you keep my data safe and private?**

We will keep information we collect about you confidential. We will share it with others if you agree to it or when we have to do it because U.S. or State law requires it. For example, we will tell somebody if you we learn that you are hurting a child or an older person.

Only research staff will have full access to the data we collect about you. Beyond registration, the information pertaining to you will only include a registration number and will not include any other information that can identify you, such as your name, birthday or address. We will store research documents in a locked file cabinet. All research data will be stored on a password protected computer.

When we publish the results of the research or talk about it in conferences, we will not use your name. If we want to use your name, we would ask you for your permission. We will also share information about you with other researchers for future research but we will not use your name or other identifiers.

Identifiers might be removed from the identifiable private information or identifiable biospecimens and that, after such removal, the information or biospecimens (eg. biopsy or blood samples) could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from the subject or the legally authorized representative, if this might be a possibility. We will not ask you for any additional permission.

#### **What Information Will You Collect About Me in this Study?**

The information we are asking to use and share is called "Protected Health Information." It is protected by a federal law called the Privacy Rule of the Health Insurance Portability and Accountability Act (HIPAA). In general, we cannot use or share your health information for research without your permission. If you want, we can give you more information about the Privacy Rule. Also, if you have any questions about the Privacy Rule and your rights, you can speak to Yale Privacy Officer at 203-432-5919.

The specific information about you and your health that we will collect, use, and share includes:

- Research study records
- Medical and laboratory records of only those services provided in connection with this Study.
- The entire research record and any medical records held by Yale New Haven Hospital created from: 12/01/2019 to: 12/01/2021
- Records about phone calls made as part of this research
- Records about your study visits
- Information obtained during this research regarding
  - HIV / AIDS test results
  - Hepatitis infection
  - Sexually transmitted diseases
  - Other reportable infectious diseases
  - Physical exams
  - Laboratory, x-ray, biopsy and other test results
  - Diaries and questionnaires
  - The diagnosis and treatment of a mental health condition
  - Use of illegal drugs or the study of illegal behavior
  - Records about any study drug you received

**How will you use and share my information?**

We will use your information to conduct the study described in this consent form.

We may share your information with:

- The U.S. Department of Health and Human Services (DHHS) agencies
- Representatives from Yale University, the Yale Human Research Protection Program and the Institutional Review Board (the committee that reviews, approves, and monitors research on human participants), who are responsible for ensuring research compliance. These individuals are required to keep all information confidential.
- The U.S. Food and Drug Administration (FDA) This is done so that the FDA can review information about [the new drug product or device] involved in this research. The information may also be used to meet the reporting requirements of drug regulatory agencies.
- The study sponsor or manufacturer of study drug/device
- Drug regulatory agencies in other countries
- Governmental agencies to whom certain diseases (reportable diseases) must be reported
- Health care providers who provide services to you in connection with this study.
- Laboratories and other individuals and organizations that analyze your health information in connection with this study, according to the study plan.
- Co-Investigators and other investigators
- Study Coordinator and Members of the Research Team
- Data and Safety Monitoring Boards and others authorized to monitor the conduct of the Study

We will do our best to make sure your information stays private. But, if we share information with people who do not have to follow the Privacy Rule, your information will no longer be protected by the Privacy Rule. Let us know if you have questions about this.

However, to better protect your health information, agreements are in place with these individuals and/or companies that require that they keep your information confidential.

**Why must I sign this document?**

By signing this form, you will allow researchers to use and disclose your information described above for this research study. This is to ensure that the information related to this research is available to all parties who may need it for research purposes. You always have the right to review and copy your health information in your medical record.

**What if I change my mind?**

The authorization to use and disclose your health information collected during your participation in this study will never expire. However, you may withdraw or take away your permission at any time. You may withdraw your permission by telling the study staff or by writing to Dr. Michael Hurwitz, 20 York Street, New Haven, CT, 06510 at the Yale University, New Haven, CT 06520.

If you withdraw your permission, you will not be able to stay in this study but the care you get from your doctor outside this study will not change. No new health information identifying you will be gathered after the date you withdraw. Information that has already been collected may still be used and given to others until the end of the research study to insure the integrity of the study and/or study oversight.

**Who will pay for treatment if I am injured or become ill due to participation in the study?**

If you are hurt or injured during this research, you will be given the medical care you may need, but you or your insurance company will be billed for the cost of this treatment. No financial compensation is available for injury or lost wages. You do not give up any of your legal rights by signing this consent form.

**What if I want to refuse or end participation before the study is over?**

Taking part in this study is your choice. You can choose to take part, or you can choose not to take part in this study. You also can change your mind at any time. Whatever choice you make, you will not lose access to your medical care or give up any legal rights or benefits.

We would still treat you with standard therapy or, at your request, refer you to a clinic or doctor who can offer this treatment. Not participating or withdrawing later will not harm your relationship with your own doctors or with this institution.

To withdraw from the study, you can call a member of the research team at any time and tell them that you no longer want to take part.

The researchers may withdraw you from participating in the research if necessary, e.g. because of development of serious side effects.

**What will happen with my data if I stop participating?**

When you withdraw your permission, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to insure the integrity of the study.

**Who should I contact if I have questions?**

Please feel free to ask about anything you don't understand.

If you have questions later or if you have a research-related problem, you can call the Principal Investigator Dr. Michael Hurwitz at (203) 400-4822.

If you have questions about your rights as a research participant, or you have complaints about this research, you call the Yale Institutional Review Boards at (203) 785-4688 or email [hrrp@yale.edu](mailto:hrrp@yale.edu).

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

**Authorization and Permission**

Your signature below indicates that you have read this consent document and that you agree to be in this study.

We will give you a copy of this form.

Participant Printed Name	Participant Signature	Date
Person Obtaining Consent Printed Name	Person Obtaining Consent Signature	Date

Complete if the participant is not fluent in English and an interpreter was used to obtain consent. Participants who do not read or understand English must not sign this full consent form, but instead sign the short form translated into their native language. This form should be signed by the investigator and interpreter only. If the interpreter is affiliated with the study team, the signature of an impartial witness is also required.

Print name of interpreter: \_\_\_\_\_

Signature of interpreter: \_\_\_\_\_ Date: \_\_\_\_\_

**An oral translation of this document was administered to the participant in \_\_\_\_\_ (state language) by an individual proficient in English and \_\_\_\_\_ (state language).**

Print name of impartial witness: \_\_\_\_\_

Signature of impartial witness: \_\_\_\_\_ Date: \_\_\_\_\_

**See the attached short form for documentation.**

Consent Form Template (Biomedical)  
Version 01/21/2019

## **APPENDIX H IPILIMUMAB AND NIVOLUMAB DOSE CALCULATIONS**

### A. Ipilimumab 1ml/kg dose, supplied as 5mg/ml (40ml or 10ml single use vials)

To calculate the total dose:

Patient's weight in kg x 1mg dose = total dose in mg

To calculate the total infusion volume:

Total dose in mg / 5mg/ml (as supplied) = total infusion volume in ml

To calculate the infusion rate:

Total infusion volume in ml / 60minutes = rate of infusion in ml/min

### B. Nivolumab 3ml/kg dose supplied as 10mg/ml (4ml, 10ml or 24ml single use vials)

To calculate the total dose:

Patient's weight in kg x 3mg dose = total dose in mg

To calculate the total infusion volume:

Total dose in mg / 10mg/ml (as supplied) = total infusion volume in ml

To calculate the infusion rate:

Total infusion volume in ml / 90minutes = rate of infusion in ml/min

**Reference:**

Yervoy package insert In: Company Bristol-Myers Squibb, ed.  
Opdivo (nivolumab). In: Bristol-Myers Squibb, ed. [package insert]. www.opdivo.com.

**APPENDIX I PATIENT REGISTRATION FORM**

Yale Cancer Center  
Smilow Cancer Hospital  
Yale School of Medicine  
Department of Medical Oncology  
Principal Investigator: Michael Hurwitz, MD, PhD.

Patient's Name: (Last, First, Middle initial) \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_  
Patient Registration Number: \_\_\_\_\_  
DOB (MM/DD/YYYY): \_\_/\_\_/\_\_\_\_  
Social Security Number: \_\_\_\_-\_\_-\_\_\_\_

Demographics:  
Sex assigned at birth:  Male  Female  
Age:  18-24  25-34  35-44  45-54  55-64  65-74  75-84  85-94  
Race:  White  Hispanic or Latino  Black or African American  Native American or American Indian  Pacific Islander or Asian  Other

Diagnosis of primary tumor: (MM/DD/YYYY) \_\_/\_\_/\_\_\_\_  
Diagnosis of metastasis: (MM/DD/YYYY) \_\_/\_\_/\_\_\_\_  
Sites of metastasis: \_\_\_\_\_  
Histologic characterization of tumor: \_\_\_\_\_  
Confirmed MSS or pMMR  Histochemistry or  PCR or  MSI sensor  
Date performed (MM/DD/YYYY) \_\_/\_\_/\_\_\_\_

History of Lynch syndrome:  Yes  No  
Immunoscore:  1  2  3  4  
Treatment dates: \_\_\_\_\_ Treatment regimen/dose: \_\_\_\_\_  
Treatment dates: \_\_\_\_\_ Treatment regimen/dose: \_\_\_\_\_  
Treatment dates: \_\_\_\_\_ Treatment regimen/dose: \_\_\_\_\_  
Medical History/dates: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Surgical History/dates: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

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Medications:

Name: \_\_\_\_\_ Dose: \_\_\_\_\_  
Name: \_\_\_\_\_ Dose: \_\_\_\_\_

Allergies:

Name: \_\_\_\_\_ Reaction: \_\_\_\_\_  
Name: \_\_\_\_\_ Reaction: \_\_\_\_\_

ECOG performance status

- 0 Fully active, able to carry on all pre-disease performance without restriction  
 1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work

Smoking history:

Never smoker  Former smoker  Current smoker Pack per year: \_\_\_\_\_

### APPENDIX J SAMPLE SIZE CALCULATION

<b>n</b>	<b><math>n_1</math></b>	<b><math>r_1</math></b>	<b><math>r_2</math></b>	<b>Type 1 Error</b>	<b>Power</b>	<b><math>EN_0</math></b>	<b>Probability of early stopping</b>	<b>Interval for <math>w</math></b>	<b>Comment</b>
37	20	1	6	0.0373	0.8018	29.0	0.4706	[0.7304,1]	Minimax
38	17	1	6	0.0404	0.8077	26.3	0.5576	[0.6853,0.7303]	
39	15	1	6	0.0427	0.8025	24.1	0.6202	[0.2693,0.6852]	
41	14	1	6	0.0500	0.8105	23.4	0.6527	[0,0.2692]	Optimal

$n$  is the total number of subjects

$n_1$  is the number of subjects accrued during stage 1

$r_1$ , if  $r_1$  or fewer responses are observed during stage 1, the trial is stopped early for futility

$r_2$ , if  $r_2$  or fewer responses are observed by the end of stage two, then no further investigation of the drug is warranted

$EN_0$  is the expected sample size for the trial when response rate is  $p_0$

Interval for  $w$  is the set of values  $w$  such that the design minimizes  $w * n + (1 - w) * EN_0$

The null hypothesis that the true objective response rate is 8.7%, as observed in the CheckMate 142 trial will be tested against two one-sided alternatives. A 15% effect size will be tested for clinical significance using a Type I (one sided) error rate of 0.5 and type II error of 0.2, yielding an 80% power.  $p_1$ , which is the response probability of the good drug is 23.7%, calculated as  $p_0 + 15\%$  effect size. The 15% is an arbitrarily chosen effect size that is widely used in cancer drug trials. The Simon's two stage design calculator uses this statistical input to calculate  $n$  which is the total number of subjects,  $n_1$ , the number of subjects accrued during stage 1,  $r_1$ , the value for which if  $r_1$  or fewer responses are observed during stage 1, the trial is stopped early for futility,  $r_2$ , the value for which if  $r_2$  or fewer responses are observed by the end of stage two, then no further investigation of the drug is warranted,  $EN_0$  which is the expected sample size for the trial when response rate is  $p_0$  (response probability of poor drug).

#### References:

1. Simon R. Optimal two-stage designs for phase II clinical trials. *Controlled clinical trials*. 1989;10(1):1-10.
2. Jung SH, Lee TY, Kim KM, George S (2004). Admissible two-stage designs for phase II cancer clinical trials, *Statistics in Medicine* 23: 561-569.
3. Simon's Two stage design calculator  
<http://cancer.unc.edu/biostatistics/program/ivanova/SimonsTwoStageDesign.aspx>

## APPENDIX K RESPONSE EVALUATION CRITERIA IN SOLID TUMORS (RECIST) V.1.1

**ORR:** Investigator-assessed objective response rate calculated as patients with best response or complete response (CR) or partial response (PR) divided by the number of patients treated.

**OS:** Time from the first dose to death.

**PFS:** Progression free survival defined as time from first dose to first documented progression or death resulting from any cause, whichever occurred first.

**DCR:** Patients with CR, PR or stable disease (SD) for  $\geq 12$  weeks divided by the number of patients treated.

**PRO:** Patient-reported outcomes, such as functioning, symptoms and quality of life (QOL).

## APPENDIX L PATIENT STUDY CHECKLIST

Patient's Name (Last, First, Middle initial): \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_

DOB (DD/MM/YYYY): \_\_/\_\_/\_\_\_\_

Registration number: \_\_\_\_\_

Proposed treatment start date: \_\_/\_\_/\_\_\_\_

Actual treatment start date: \_\_/\_\_/\_\_\_\_

- Passed Eligibility Checklist
- Signed Consent to Participate in Study
- Completed Patient Registration Form
- Randomized to Treatment Arm A or Treatment Arm B

## APPENDIX M CRITERIA FOR TREATMENT DELAY AND RESUMPTION

Adopted from Supplemental Tables for: Safety of Nivolumab Plus Low-Dose Ipilimumab in Previously Treated Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer Michael Morse et al. Table S1. For grading of adverse events please see Appendix D (CTCAE) Version 5.0

### Treatment Delay Criteria:

- Any grade  $\geq 2$  non-skin-specific TRAE, with the following exceptions:
  - Grade 2 treatment-related fatigue or laboratory abnormalities do not require a treatment delay
- Any grade 3 skin TRAE
- Any grade 3 treatment-related laboratory abnormality, with the following exceptions for asymptomatic amylase or lipase, AST, ALT, or total bilirubin:
  - Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis do not require a dose delay (consultation recommended for grade 3 amylase or lipase abnormalities)
  - In patients with baseline AST, ALT, or total bilirubin within normal limits, treatment would be delayed in case of treatment-related grade  $\geq 2$  toxicity
  - In patients with baseline AST, ALT, or total bilirubin within the grade 1 toxicity range, treatment would be delayed in case of treatment-related grade  $\geq 3$  toxicity

### Treatment resumption criteria:

Patients can resume treatment when the TRAE resolved to grade  $\leq 1$  or to baseline values, with the following exceptions:

- Patients may resume treatment in the presence of grade 2 fatigue

- Patients who have not experienced a grade 3 skin TRAE may resume treatment in the presence of grade 2 skin toxicity
- Patients with baseline grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of grade 2 AST/ALT or total bilirubin
- Treatment-related pulmonary toxicity, diarrhea, or colitis must have resolved to baseline before treatment is resumed
- Treatment-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

Abbreviations: TRAE – treatment-related adverse events, ALT- alanine aminotransferase, AST- aspartate aminotransferase

**Reference:**

Morse MA, Overman MJ, Hartman L, et al. Safety of Nivolumab plus Low-Dose Ipilimumab in Previously Treated Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer. *The oncologist*. 2019.

**APPENDIX N DEFINITIONS OF ADVERSE EVENTS WITH IMMUNE CHECKPOINT INHIBITOR THERAPY**

Treatment Related Adverse Events (TRAEs) are defined as adverse events (AEs) of special clinical interest meeting defined criteria that were grouped by specific categories such as endocrine, GI, hepatic, pulmonary, renal and skin events and had a potential immunologic etiology.

Immune mediated adverse events (IMAEs) are defined as specific events that includes diarrhea and colitis, hepatitis, pneumonitis, nephritis and renal dysfunction, rash, and endocrine events such as adrenal insufficiency, hypophysitis, hypothyroidism/thyroiditis, hyperthyroidism and diabetes mellitus.

**Reference:**

Morse MA, Overman MJ, Hartman L, et al. Safety of Nivolumab plus Low-Dose Ipilimumab in Previously Treated Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer. *The oncologist*. 2019.

**APPENDIX O ADEQUATE ORGAN FUNCTION LABORATORY VALUES**

Hematological

Absolute leukocyte count	≥2500 /mcL
Absolute lymphocyte count (ALC)	≥500 /mcL
Absolute neutrophil count (ANC)	≥1500 /mcL
Platelets	≥100,000 / mcL

Hemoglobin	$\geq 9$ g/dL or $\geq 5.6$ mmol/L without transfusion or EPO dependency (within 7 days of assessment)
<u>Hepatic</u>	
Serum total bilirubin	$\leq 1.5$ X ULN
or	or
Direct bilirubin	$\leq$ ULN for subjects with total bilirubin levels $> 1.5$ ULN
AST and ALT	$\leq 2.5$ X ULN OR $\leq 5$ X ULN for subjects with liver metastases
Albumin	$> 2.5$ mg/dL
<u>Coagulation</u>	
International Normalized Ratio (INR) or Prothrombin Time (PT)	$\leq 1.5$ X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	$\leq 1.5$ X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
<u>Renal</u>	
Serum creatinine	$\leq 1.5$ X upper limit of normal (ULN)
or	or
Measured or calculated creatinine clearance (GFR can also be used in place of creatinine or CrCl)	$\geq 60$ mL/min for subject with creatinine levels $> 1.5$ X institutional ULN

**Reference:**

Eisenhauer EA, Twelves C, Buyse M, Phase I Cancer Trials, a Practical Guide, *Oxford University Press*, 2015

**APPENDIX P SUMMARY OF CURRENT PHASE I/II TRIALS**

Title	Study type	Enrollment	Indication	Intervention	Primary outcome measures	Secondary outcome measures
Nivolumab, Ipilimumab and COX2-inhibition in Early Stage Colon Cancer: an Unbiased Approach for Signals of Sensitivity (NICHE) [NCT03026140]	Phase II, Interventional, open label, single center, randomized allocation, parallel assignment.	60	Early stage (stage 1-3) CRC (both MSS and MSI-H)	Neoadjuvant <u>Control arm:</u> single dose of ipilimumab 1mg/kg on day 1 and two cycles of nivolumab 3mg/kg on day 1 and 15, respectively. <u>Experimental group:</u> single dose of ipilimumab 1mg/kg on day 1, two cycles of nivolumab 3mg/kg one day 1 and 15 and 200mg celecoxib daily until the day before surgery	Incidence of adverse events during the treatment and follow-up (safety) Per CTCAE v 4.0	1. Immune activating capacity of short-term pre-operative immunotherapy [Time Frame: within 2 years after study completion] identify underlying potential escape mechanisms by comparing pre-treatment and post-treatment biopsies 2. Relapse free survival [Time Frame: 3-5 years after last patient inclusion]
RACIN, A Phase I Study of the Combination of Nivolumab Plus Ipilimumab Associated With Low-dose Radiation, Aspirin, and Low-dose Cyclophosphamide, Followed by Nivolumab Maintenance, in Patients With Advanced, TIL-negative Solid Tumors [NCT03728179]	Phase Ia/Ib Interventional, non-randomized, sequential assignment, dose escalation of radiation, open label	50	Patients with advanced, TIL-negative Solid Tumors	<u>Cyclophosphamide:</u> 200mg/m <sup>2</sup> (IV) Q2W from cycle C0 to C4. Nivolumab: 240 mg IV Q2W from cycle C1 to C4. <u>Ipilimumab:</u> 1mg/kg will be administered as IV every 6 weeks (Q6W) from cycle C1 to C4 Aspirin: 300mg orally daily from cycle C1 to C4. Dose escalation of radiation: 0.5Gy, 1Gy, 2Gy, 3Gy	Phases Ia and Ib: Incidence of Treatment-Emergent Adverse Events (Safety and Tolerability) [Time Frame: 3.5 years] Phase Ia and IB: Toxicity and tolerability per CTCAEv.4.03 2. Maximum Tolerated dose (MTD)	1. Objective response rate (ORR)[Time Frame: 3.5 years] Per (RECIST) v.1.1. 2. Disease Control Rate (DCR) [Time Frame: 6,12 and 24 months] Per RECIST v.1.1 3. Progression free survival (PFS) rate [Time Frame: 6,12 and 24 months] Per RECIST v.1.1. 4. Time to Progression (TTP): [Time Frame: 3.5 years] Per RECIST v.1.1

					[Time Frame: 3.5 years]	5. Overall survival (OS) [Time Frame: 12 and 24 months]
PD-1 Antibody Combined With COX Inhibitor in MSI-H/dMMR or High TMB Colorectal Cancer [NCT03638297]	Phase II, Interventional, single group, open label	54	MSI-H/dMMR or High TMB Colorectal Cancer	PD-1 antibody + cox inhibitor BAT1306 + aspirin BAT1306 100mg on day 1 + aspirin 200mg oral (celebrex 400mg oral when there is contraindication to aspirin) on day 1-21 every three weeks	CR (complete response) + PR (partial response) rate according to the RECIST version 1.1 guidelines.	1. Progression free survival [Time Frame: 2 years] 2. Overall survival time [Time Frame: 5 years] 3. Disease control rate [Time Frame: 6 months] Per RECIST version 1.1 4. Toxicity assessed using the NCI common toxicity criteria, version 4.0. [Time Frame: 2 years] 5. Duration of response [Time Frame: 2 years]
An Open Label Phase II Study Combining Nivolumab and Celecoxib in Patients With Advanced " Cold " Solid Tumors (NICE-COMBO) [NCT03864575]	Phase II, Interventional, Single group assignment, Simon's two-stage Minimax design, open label	68	Cancer types with an indication of treatment with anti-PD1 antibodies, metastatic, IDO1 positive ( $\geq 5\%$ expression of tumor cells) and non T-cell infiltrated tumors (<1% T cells infiltrating the tumor bed)	Celecoxib 400 mg/d nivolumab 240 mg every two weeks	ORR Objective response rate [Time Frame: at week 12 from onset of treatment]	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Number of participants with treatment-related adverse events as assessed by CTCAE v4.0 [Time Frame: from first dose to day 28 post last dose] 2. Efficacy - Duration of response (DOR) [Time Frame: From date of randomization until the date of first documented progression or date of death from any cause, whichever came first, assessed up to 60 months] per RECIST v1.1 3. Efficacy - Time to response (TTR) [Time Frame: From onset of

						<p>treatment to response of cancer through study completion, an average of 12 months is expected] Per RECIST v1.1.</p> <p>4. Disease control rate (DCR) [Time Frame: at week 12 from onset of treatment] Per RECIST v1.1.</p> <p>5. Progression-free survival (PFS) [Time Frame: From date of randomization until the date of first documented progression or date of death, whichever comes first, assessed up to 60 months ] Per RECIST v1.1), or death due to any cause, if occurring sooner than progression.</p> <p>6. Overall survival (OS) [Time Frame: From date of randomization until the date of death, assessed up to 60 months]</p>
<p>PRIMMO: a phase II study combining PD-1 blockade, radiation and immunomodulation to tackle cervical and uterine cancer [NCT03192059]</p>	<p>Phase II, multi-center, open-label, non-randomized, 3-cohort study with a safety run-in in</p>	<p>43+</p>	<p>Recurrent/refractory cervical carcinoma, endometrial carcinoma or uterine sarcoma</p>	<p>Pembrolizumab 200mg q3w IV, Radiation 3 fractions of 8Gy 48 hours apart, Vitamin D 2000IU daily, Lansoprazole 180mg uneven weeks, 30mg even weeks, aspirin 325mg daily, cyclophosphamide 50mg daily, curcumin 2g daily</p>	<p>Objective response rate (ORR) at week 26 per immune-related response criteria (irRC)</p>	<p>Safety per (CTCAE4.0), the ORR at week 26 per RECIST criteria, the best overall response (BOR), progression-free survival (PFS), overall survival (OS) and quality of life (QoL)</p>

## APPENDIX Q DEFINITION OF ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

Adverse events (AE) are defined as any untoward or unfavorable occurrence in a human research subject (physical or psychological harm) temporally associated with the individual's participation in the research (whether or not considered related to participation in the research).

Serious adverse events (SAE) are defined as any adverse event that results in any of the following outcomes: death, a life-threatening experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect, or any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

### Reference:

Administration OoR. IRB Policy 710 Reporting Unanticipated Problems Involving Risks to Subjects or Others, including Adverse Events. In: University Y, ed April 15 2014

## APPENDIX R BRIEF OUTLINE OF AMERICAN SOCIETY OF CLINICAL ONCOLOGY (ASCO) GUIDELINES BY CTCAE GRADE

There will be a high level of suspicion that any new adverse symptom might be immunotherapy related. In general, **Grade 1 toxicities** will be closely followed without treatment interruption. Exceptions are some neurologic, cardiac and hematologic toxicities. Treatment will be held for most **Grade 2 toxicities** and will be resumed when symptoms and/or laboratory abnormalities revert to Grade 1 or less. A 0.5-1.0mg/kg/day prednisone equivalent corticosteroid dose may be administered.

Treatment will be held for **Grade 3 toxicities** and high dose corticosteroids will be initiated (prednisone equivalent of 1-2mg/kg/day, (alternatively methylprednisolone IV 1-2mg/kg/day). Infliximab may be given if symptoms and/or laboratory values don't improve within 48-72 hours with high dose corticosteroids. In case of liver toxicity, we will use non-TNF- $\alpha$  agents instead to avoid the hepatotoxic properties of Infliximab. Treatment might be reinitiated when symptoms and/or laboratory values return to Grade 1 or less. There will be no dose adjustments made. Treatment will be permanently discontinued in case of Grade 4 toxicity, except for endocrinopathies that can be generally managed by hormone replacement.

### Reference:

Brahmer JR, Lacchetti C, Schneider BJ, et al. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline. *Journal of Clinical Oncology*. 2018;36(17):1714-1768.

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