TITLE: Novel Exploratory Study to Test Combination of Botensilimab and Balstilimab Immunotherapy in Patients with Resectable Colorectal Cancer (NEST-1)

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Weill Cornell Medical College-NewYork Presbyterian Hospital (WCMC/NYPH)
NewYork-Presbyterian Brooklyn Methodist Hospital (BMH)
NewYork-Presbyterian Queens (NYPQ)
Hereafter, all above mentioned locations are referred to as WCMC/NYPH.

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Statement of Compliance

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from WCM, unless disclosure on ClinicalTrials.gov is federally required.

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Institution Name

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Principal Investigator's Name	**Principal Investigator's Signature**	**Date**

Table 1: List of Abbreviations

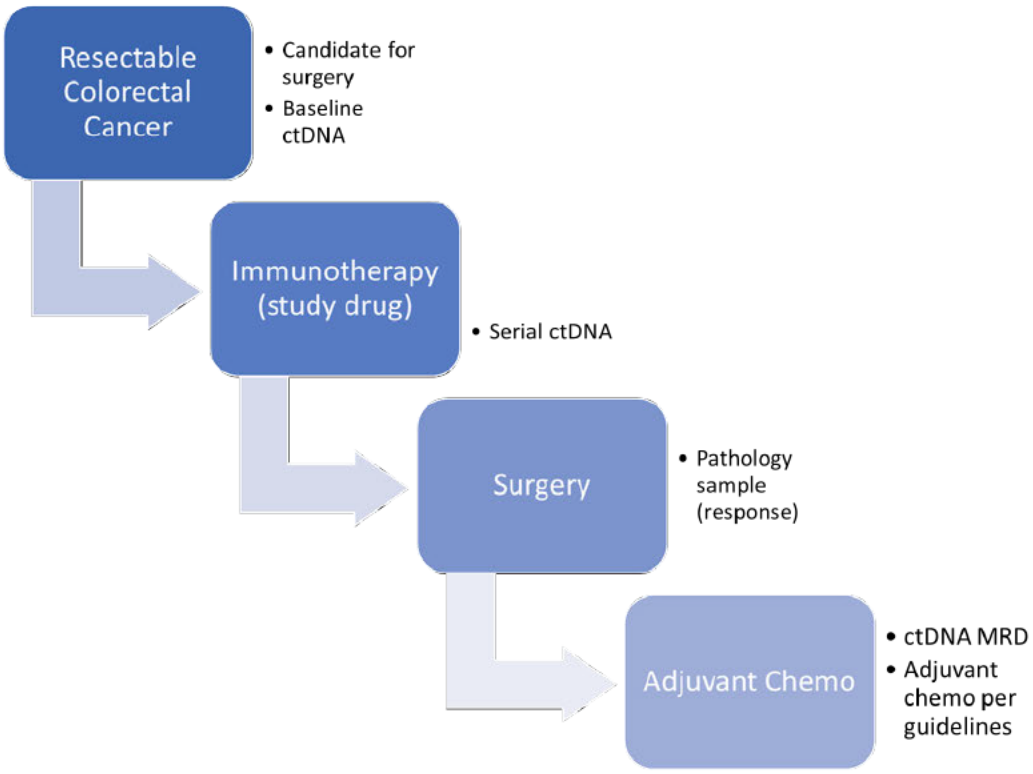
ADCC	Antibody-dependent cellular cytotoxicity
AE	Adverse Event
bal	Programmed cell death protein 1 (PD-1) inhibitor balstilimab
bot	Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) inhibitor botensilimab
bot/bal	Combination of CTLA-4/PD-1 with 1 dose of the CTLA-4 inhibitor bot and 2 doses of the PD-1 inhibitor bal is referred to as bot/bal regimen in the protocol
CFR	Code of Federal Regulations
CRF	Case Report Form
CTLA4	Cytotoxic T lymphocyte-associated protein 4
CTSC	Clinical Translational Science Center
dMMR/MSI-H	Mismatch repair deficient/microsatellite-high
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	Informed Consent Form
Ig	Immunoglobulin
IND	Investigational New Drug
IPI/NIVO	Combination regimen of CTLA-4/PD-1 inhibitors ipilimumab and nivolumab
IRB	Institutional Review Board
mAb	Monoclonal antibody
MRD	Minimal Residual Disease
NOAEL	No-observed- adverse-effect level
PD-1	Programmed cell death protein 1
PHI	Protected Health Information
PI	Principal Investigator
pMMR/MSS	Mismatch repair proficient/microsatellite stable
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
Treg	Regulatory T cell
UIRTSO	Unanticipated Problem Involving Risks to Subjects or Others
WCM	Weill Cornell Medicine

1. Protocol Summary

Full Title:	Novel Exploratory Study to Test combination of Botensilimab and Balstilimab Immunotherapy in Patients with Resectable Colorectal Cancer (NEST-1)
Short Title:	NEST-1
Clinical Phase:	II
Principal Investigator:	Manish A. Shah, MD
Study Description:	This study aims to assess the feasibility, safety, and efficacy of using a combination of a programmed cell death protein 1 (PD-1) inhibitor (balstilimab – bal) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) inhibitor (botensilimab - bot) in the neoadjuvant setting in patients with colorectal cancer, prior to resection.
Sample Size:	This study will initially enroll 12 participants (Cohort A). Additional participants may be added. *Additional cohorts as protocol version 3.0:* Cohort B - 12 participants Cohort C - 12 participants
Study Population:	Patients with resectable colorectal cancer prior to surgery
Enrollment Period:	18 months
Study Design:	This is a multi-cohort, single-center, open-label, phase 2 study in which patients will receive a combination of PD-1 inhibitor, balstilimab, and CTLA-4 inhibitor, botensilimab, prior to resection in patients with colorectal cancer. This study enrolled an initial cohort (Cohort A) who received 1 dose of the CTLA-4 inhibitor, botensilimab, and 2 doses of the PD-1 inhibitor, balstilimab. Based on the safety and feasibility profile of the initial cohort, additional cohorts were added. Cohort B and C will receive 1 dose of the CTLA-4 inhibitor, botensilimab, and 4 doses of the PD-1 inhibitor, balstilimab. Cohort C will only enroll patients with dMMR/MSI-High colorectal cancer. Safety and feasibility of will be assessed by the number of potentially treatment-related SAEs/AEs that lead to delays in planned surgical resection.
Description of Sites/ Facilities Enrolling Participants:	Weill Cornell Medical College-NewYork Presbyterian Hospital (WCMC/NYPH) NewYork-Presbyterian Brooklyn Methodist Hospital (BMH)

	NewYork-Presbyterian Queens (NYPQ)
Study Duration:	18 months
Participant Duration:	6 months
Study Agent/Device Name Intervention Description:	Balstilimab (AGEN2034 - bal) is a human monoclonal antibody (mAb) that targets PD-1. Botensilimab (AGEN1181 - bot) is a fragment crystallizable (Fc)-engineered human immunoglobulin (Ig) G1 (IgG1) mAb that targets CTLA-4. Balstilimab and botensilimab will be given intravenously at a dose of 240 mg and 75 mg respectively. Number of doses will vary based on cohort.
Primary Objective:	- To assess the anti-tumor effects of using a combination of balstilimab, a PD-1 inhibitor, and botensilimab, a CTLA-4 inhibitor, (bot/bal regimen) in a neoadjuvant setting among patients with resectable colorectal cancer prior to surgery -To assess feasibility and safety of using a combination of balstilimab, a PD-1 inhibitor, and botensilimab, a CTLA-4 inhibitor (bot/bal regimen), in a neoadjuvant setting among patients with resectable colorectal cancer prior to surgery
Secondary Objectives:	- To assess changes in circulating tumor DNA (ctDNA) in patients receiving a combination of balstilimab, a PD-1 inhibitor, and botensilimab, a CTLA-4 inhibitor, in a neoadjuvant setting among patients with resectable colorectal cancer prior to surgery
Exploratory Objectives:	- To assess changes in liquid biopsy, and panel-based circulatory/tissue immune cells and immune biomarkers, in patients with resectable colorectal cancer receiving combination immunotherapy prior to surgery; as well as to assess changes in ctDNA beyond 30 days post-resection as assessed by standard of care practices in this patient population (blood/tissue), and to correlate molecular reoccurrence (via ctDNA) with clinical and radiographic data

1.1 Schema



ctDNA – circulating tumor DNA; MRD – minimal residual disease

1.2 Study Objectives and End Points

1.2.1 Primary Objectives and Endpoints

Primary Objective	Primary Endpoints (reported for Cohort)
To assess feasibility and safety of using a combination of balstilimab, a PD-1 inhibitor, and botensilimab, a CTLA-4 inhibitor, (bot/bal regimen) in a neoadjuvant setting among patients with resectable colorectal cancer prior to surgery	Safety Cohort A: Number of participants who experience potentially treatment-related SAEs according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 at 90 days following the last treatment with balstilimab or botensilimab
	Feasibility: Cohort A: Number of participants who experience treatment-related complications leading to delays of 12 weeks or more in surgery after treatment initiation (Day 0)
To assess the anti-tumor effects of using a combination of balstilimab, a PD-1 inhibitor, and botensilimab, a CTLA-4 inhibitor, (bot/bal regimen) in a neoadjuvant setting among patients with resectable colorectal cancer prior to surgery	Cohort A and B: Pathological overall response (pOR) rate of each cohort determined by analysis of tissue resected during surgery
	Cohort C: Composite rate of clinical complete response or major pathological response at 6 months

1.2.2 Secondary Objectives

Secondary Objectives	Secondary Endpoints (reported per cohort)
To assess changes in circulating tumor DNA (ctDNA) in patients receiving a combination of balstilimab, a PD-1 inhibitor, and botensilimab, a CTLA-4 inhibitor, (bot/bal regimen) in a neoadjuvant setting among patients with resectable colorectal cancer prior to surgery	All Cohorts: Changes in Minimal Residual Disease assessed using ctDNA pre- and 30 days post-surgical resection

1.2.3 Exploratory Objectives

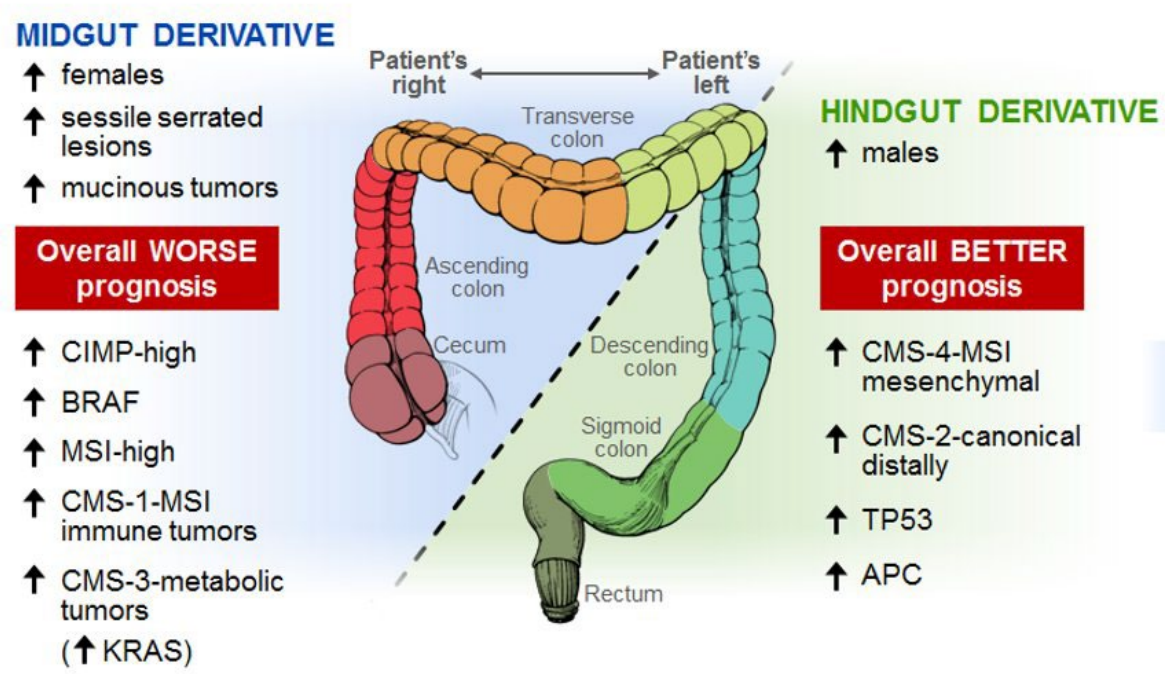
- To assess changes in liquid biopsy, and panel-based circulatory/tissue immune cells and immune biomarkers, in patients with resectable colorectal cancer receiving combination immunotherapy prior to surgery
- To assess changes in ctDNA beyond 30 days post-resection as assessed by standard of care practices in this patient population (blood/tissue), and to correlate molecular reoccurrence (via ctDNA) with clinical and radiographic data

2. Background

2.1 Disease

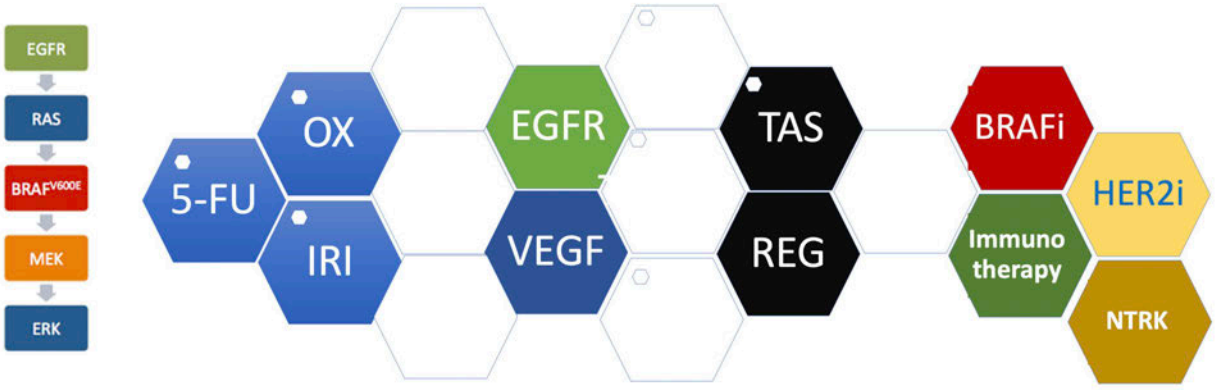
Colorectal Cancer is one the leading cause of cancer diagnoses as well as death from cancer worldwide. Colorectal cancer accounts for over 135,000 new cancers diagnosed annually in the United States. Despite recent advances in treatment, it remains the third most common cause of cancer mortality, causing at least 50,000 deaths per year. (Siegel, et al, 2016; Ryerson, et al, 2016). While the term colorectal cancer includes colon and rectal cancer together, it is a combination of different diseases and biological entities. Even sidedness matters. As shown in Figure 1, the mutational profiles, therapy options, as well as outcomes are very different for patients with LEFT-sided versus RIGHT-sided colon cancers. This is secondary to potentially different embryological origins (Midgut derivative versus Hindgut derivative).

Figure 1: Simplistic diagram illustrating the differences in biology and outcomes in patients with colon and rectal cancers.



Survival for patients with colorectal cancer has improved from a median of 6 months in the advanced/metastatic setting to upwards of a median of 3-4 years. The reason for that is not just one drug but a combination of multiple drugs and advances over the years. These include advances in the surgical and other allied specialties. Figure 2 illustrates these various 'tools in our toolbox' that are now available for patients with advanced/metastatic colorectal cancer.

Figure 2: Overview of treatment options in patients with advanced/metastatic colon and rectal cancers. Illustrated on the left-hand side of the panel are the traditional chemotherapy agents. [1]



Amongst various treatment options, one of the biggest advances for patients with colorectal cancer and for the field of cancer has been the advent of immunotherapy. Immunotherapy in the context of this trial and protocol refers to the traditional programmed cell death protein 1 (PD-1) inhibitors and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) inhibitors. In patients with cancer, these PD-1 or PD-L1 inhibitors are often used alone (e.g., pembrolizumab, nivolumab (NIVO), durvalumab, dostarlimab), or in combination with CTLA-4 inhibitors (e.g., ipilimumab (IPI), tremelimumab).

However, in the context of colorectal cancer, immunotherapy is only an option for advanced/metastatic colorectal cancers that are mismatch repair deficient/microsatellite-high (dMMR/MSI-H). While in the early-stage setting, the proportion of dMMR/MSI-H colorectal cancers are estimated to be about 15-20% of the cases, in the metastatic setting it is only around 4%. For most colorectal cancers that are mismatch repair proficient/microsatellite stable (pMMR/MSS) immunotherapy whether it is PD-1 inhibitor alone or in combination with a CTLA-4 inhibitor does not work. There is a plethora of negative studies in this space unfortunately.

In recent years, immune checkpoint inhibitors (ICIs) have revolutionized the treatment of a number of cancers. They target the programmed cell death ligand-1 (PD-L1), Programmed cell death protein 1 (PD-1) or cytotoxic T-lymphocyte antigen-4 (CTLA-4) pathways. These immune escape mechanisms allow tumors to progress by evading the body's immune response. ICIs inhibit these pathways and activate lymphocytes to recognize tumor antigens, thereby enhancing tumor killing (Pardoll, et al, 2012). They have shown activity and efficacy in a number of malignancies including melanoma, lung cancer,

[1] Traditional chemotherapy agents are often coupled with biologics/targeted therapies (EGFR or VEGF inhibitors). In the salvage setting pills like TAS-102 (TAS) and Regorafenib (REG) are options. Finally for specific subsets, BRAF inhibitors for BRAFV600E mutant colorectal cancer, immunotherapy for mismatch repair deficient/microsatellite instability-high (dMMR/MSI-H) cancers, NTRK-inhibitors for NTRK-fusion positive cancers, and HER2-directed therapies for HER2-poisitive cancers are also now in guidelines.

renal cell cancer, bladder cancer, head and neck cancer, and Hodgkin lymphoma. (Topalian, et al, 2012; Brahmer, et al, 2012; Rosenberg, et al, 2016; Robert, et al, 2015; Ferris, et al, 2016; Gettinger, et al, 2016).

Immunotherapy alone in patients with metastatic colorectal cancer (mCRC) had been of limited value until recently. The factors identified behind immunotherapies working for some cancers while not others include tumor related factors (mutational burden) and host related factors (immune response). In colorectal cancers, these tumors can be classified as microsatellite instability-high (dMMR/MSI-H) with a high mutational burden (Lynch-syndrome like) and microsatellite stable (pMMR/MSS), with a low mutational burden. The results of a landmark immunotherapy trial published in 2015 for the first time showed great promise for immunotherapies in mCRC. Of note, while seven out nine patients who were MSI-H had an objective response, 0 out 18 patients who were MSS had a response to anti-PD-1 blockade. (Le, et al, 2015) Unfortunately, dMMR/MSI-H tumors constitute a very small proportion (~4%) of mCRC. Therefore, novel approaches are needed to make immunotherapy a viable option for MSS tumors, which constitute more than 96% of the tumors.

What is new however, is the increasing awareness that while immunotherapy may not work for patients with cancers that have escaped or metastasized to different organs, it has the potential to work in early-stage settings. Of particular interest is the neoadjuvant setting (before surgery). This lends several advantages and has a lot of rationale to back this approach. From a scientific study perspective, it brings the opportunity of assessing both the blood and tumor tissue for response to any novel therapy, as well as an early readout (pathologic overall response - pOR). It also allows for studying the cancer and the tumor microenvironment for assessment of changes secondary to any novel therapy including immunotherapy.

Building upon these observations, the NICHE clinical trial was launched in Europe and the results published in Nature Medicine. Updated results were just unveiled at the American Society of Clinical Oncology (ASCO) meeting in June 2022 this year which has sparked a lot of interest in this space.

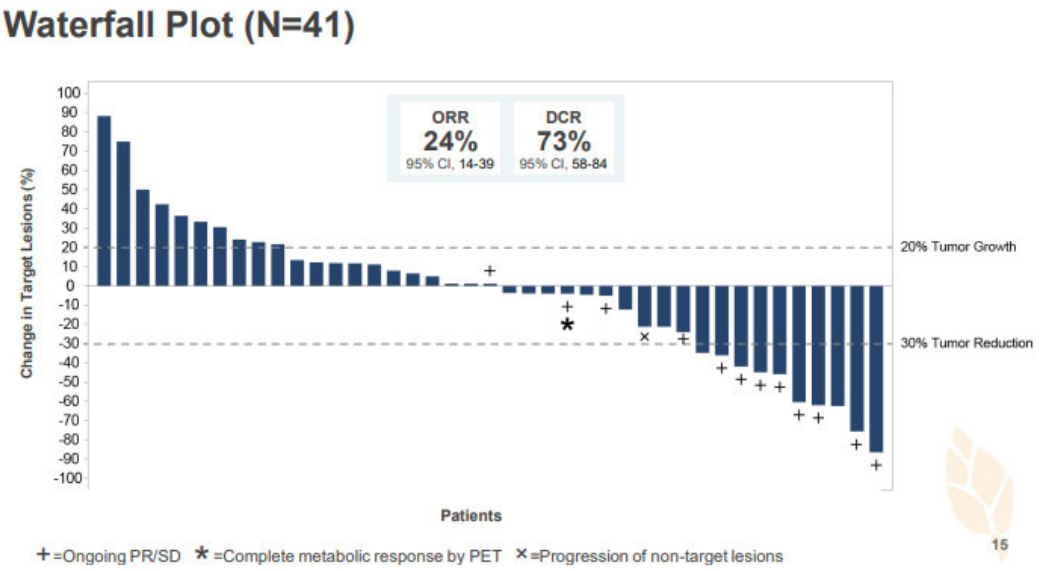
“The NICHE study was the first neoadjuvant immunotherapy study in colon cancer to show impressive responses in 100% of dMMR (n= 20) and 27% of pMMR (n= 15).” This was the initial data that showed feasibility and safety of the approach that is being proposed in our trial as well. Updated analysis and data at ASCO 2022 was on thirty patients with pMMR and 32 with dMMR tumors were evaluable for the efficacy analyses. “In the pMMR cohort, pathologic response was observed in 9/30 (30%, 95% CI 14-46%) patients, consisting of 7 MPR (including 3 pathologic complete responses {pCR}) and 2 partial responses. The conclusions by the authors and team were These data confirm our previously published results of the NICHE study, with responses to neoadjuvant nivolumab plus ipilimumab in 30% of pMMR and 100% of dMMR CC in the completed original cohorts. Validation of the dMMR responses in a large group of dMMR patients is ongoing and has the potential to change current practice (Clinical trial information: NCT03026140 - https://clinicaltrials.gov/ct2/show/NCT03026140).”

The goal of our study is to build upon the NICHE study. What is novel and different about our approach is more robust assessment of circulatory tumor markers that would be done real-time, as well as the use of novel agents. The NICHE study used the CTLA-4/PD-1

regimen of IPI/NIVO. Our study will be assessing the anti-tumor effects of the combination of balstilimab, a PD-1 inhibitor, and botensilimab, a CTLA-4 inhibitor, (bot/bal regimen) in patients with colorectal cancer in the neoadjuvant setting. This regimen's safety is already well-established. What is remarkable is activity in the metastatic settings in patients with pMMR/MSS colorectal cancers that had not been previously demonstrated with other agents in this space. As noted in more detail later in the protocol, a key distinction of botensilimab (bot) from ipilimumab (IPI) is the Fc-optimized IgG1 backbone that enhances its binding to human FcγRIIIA and other Fcγ receptors and exploits a novel mechanism of action (Waight et al. 2018). In vitro and in vivo data suggest that FcγR co-engagement is important for reaching optimal CTLA-4 antagonism (Investigator's Brochure for AGEN1181). Results showing activity in pMMR/MSS colorectal cancers were initially presented at the American Association for Cancer Research (AACR) annual meeting in 2019, with detailed results unveiled in July 2022 at the World GI Congress in Barcelona, Spain at the annual meeting by the European Society of Medical Oncology (ESMO) (Bullock et al. 2022).

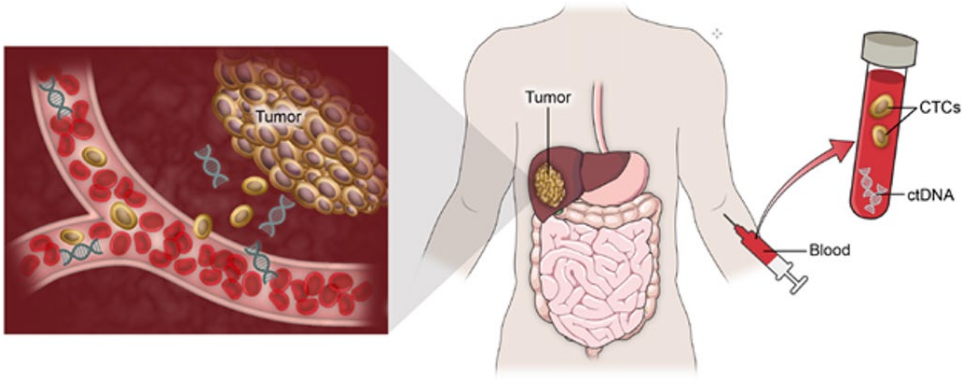
At the 2022 ESMO annual meeting, results were presented as a late breaking abstract from an expanded phase 1A/B trial (NCT03860272) of a combination of botensilimab and balstilimab given to patients with metastatic microsatellite stable colorectal cancer (MSS CRC). In this study (N=41) of heavily pre-treated patients (average number of prior therapies was 4), the measured ORR was 24% (10/41), disease control rate (DCR) was 73% (30/41), and DOR ranged from 0.0 ± 17.0+ months. Of further interest, patients without liver metastases (N=24) had even higher response rates with an ORR of 42% (10/24) and DCR of 96% (23/24). Eight out of the 10 responses were ongoing at the time of presentation. Additionally, the combination of botensilimab and balstilimab had an overall favorable safety profile. Most AEs were grade 1 or 2. Twenty-four percent (24%) of participants experienced grade 3 TRAEs and no grade 4 or 5 TRAEs were reported. Diarrhea/colitis was the only grade 3 TRAE occurring in more than one patient (10%) (Bullock et al. 2022). These recent results provide further rationale for utilizing a combination of botensilimab and balstilimab in patients with colorectal cancer from both from an efficacy and safety perspective.

Figure 3. Efficacy of a combination of botensilimab and balstilimab in patients with metastatic MSS CRC (Bullock et al. 2022).



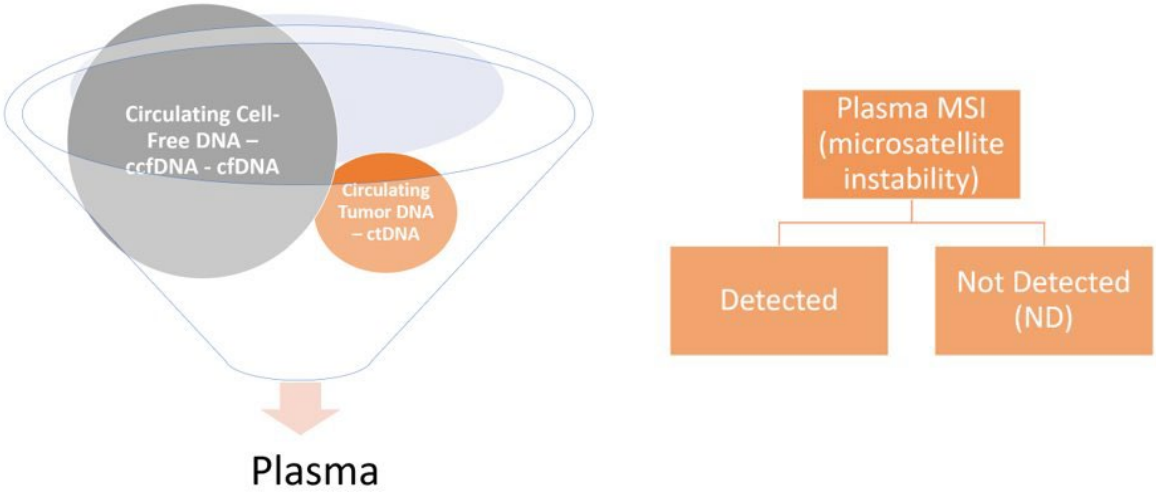
In addition to all the advances in drugs/therapeutics, what is also new is the development of the field of liquid biopsies, which is revolutionizing cancer care. The term liquid biopsy refers to circulating tumor DNA (ctDNA) or circulating tumor cells (CTC) assessment in the blood of the patient, rather than conventional biopsy sample collection.

Figure 4: Simplistic overview of the 2 kinds of liquid biopsies (circulating tumor DNA – ctDNA and circulating tumor cells (CTCs)



In patients with colorectal and other cancers, liquid biopsies not only allow for assessment of mutational status from the circulating tumor DNA or cells, but also assessment of other methylated markers, as well as assessment of microsatellite instability to help inform who may be a candidate for immunotherapy. These are derived from the plasma component of the blood.

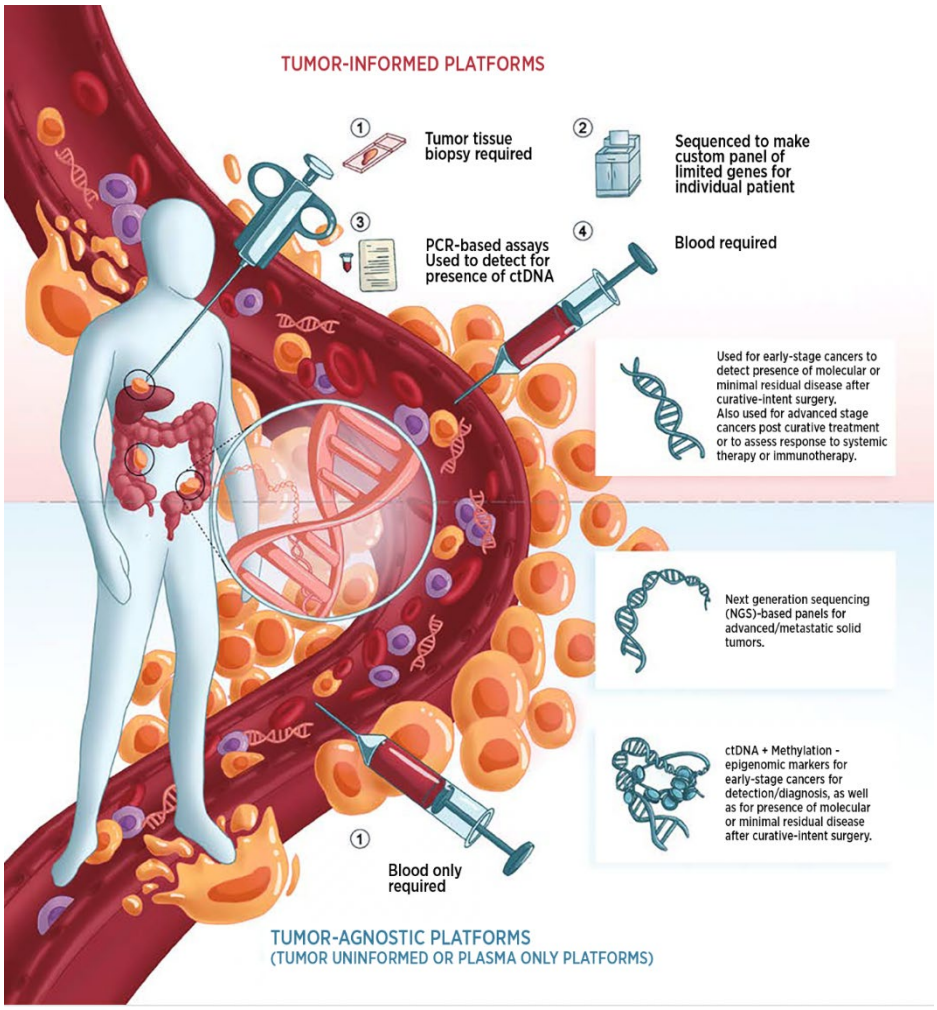
Figure 5: Circulating tumor DNA (ctDNA) is derived from the plasma component of blood. [2]



It is of value to understand that there are different kinds of liquid biopsies. Figure 4 from one of our recent reviews/editorials illustrates how they differ in terms of what they assess, how they assess it, and current indications/applications. We will be using these novel assessments of circulating markers in the current proposed trial as markers of early readout and response/recurrence.

[2] The predominant component of plasma is actually normal circulating cell free DNA (normal DNA) abbreviated as cfDNA or ccfDNA. Additionally, it allows for assessment of other variables e.g., the microsatellite instability status.

Figure 6: Comparing Tumor-Informed Versus Tumor-Uninformed ctDNA Assays. [3]

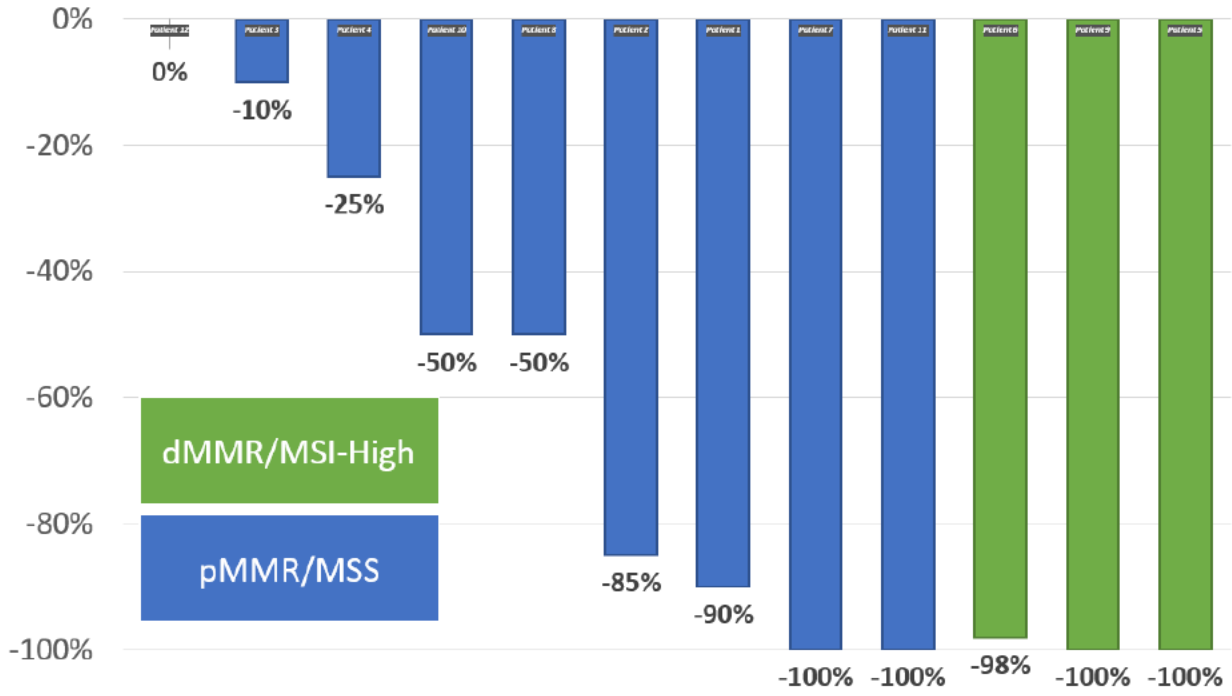


Updates as of September 5, 2023 (Protocol Version 3.0)

This trial opened earlier in 2023 and is ongoing. Enrollment from a safety as well as efficacy perspective has shown very promising results prompting an expansion to cohort B and cohort C. No patients in cohort A had surgery delayed to a treatment related adverse event.

Figure 7. Efficacy of a combination of botensilimab and balstilimab in patients with advanced colorectal cancer (Cohort A)

[3] There are 2 broad categories of ctDNA assays: tumor-informed versus tumor-uninformed (plasma-only/tumor-agnostic assays). Some are cancer-specific, whereas others are custom-built for each individual's cancer (patient-specific).
Abbreviations: ctDNA, circulating tumor DNA; PCR, polymerase chain reaction (From Kasi PM. ASCO Daily News. Jan 10, 2022)
https://dailynews.ascopubs.org/do/10.1200/ADN.22.200792/full/



Specifically, the goal for cohort B would be to focus on patients with pMMR/MSS patients but give the immunotherapy more time to 'brew' and act on the cancer to result in potentially more killing. Therefore, instead of 1 dose of the BOT/BAL and 1 additional dose of the BAL immunotherapy, patients will end up getting the same 1 dose of the BOT/BAL but 3 additional doses of the BAL immunotherapy. This would result in more exposure/time for the immunotherapy to act on tumor cells. Instead of surgery happening at 4 weeks as in Cohort A, surgery will happen at a minimum of 8 weeks from C1D1. As noted in the waterfall plot Figure 4, as well as the following table, the longer someone waited to get their surgery, the greater the likelihood of deeper responses including the findings of 100% responses or no residual tumor. Also of note, no one's surgery was delayed due to a treatment related adverse event. The primary safety endpoint was met.

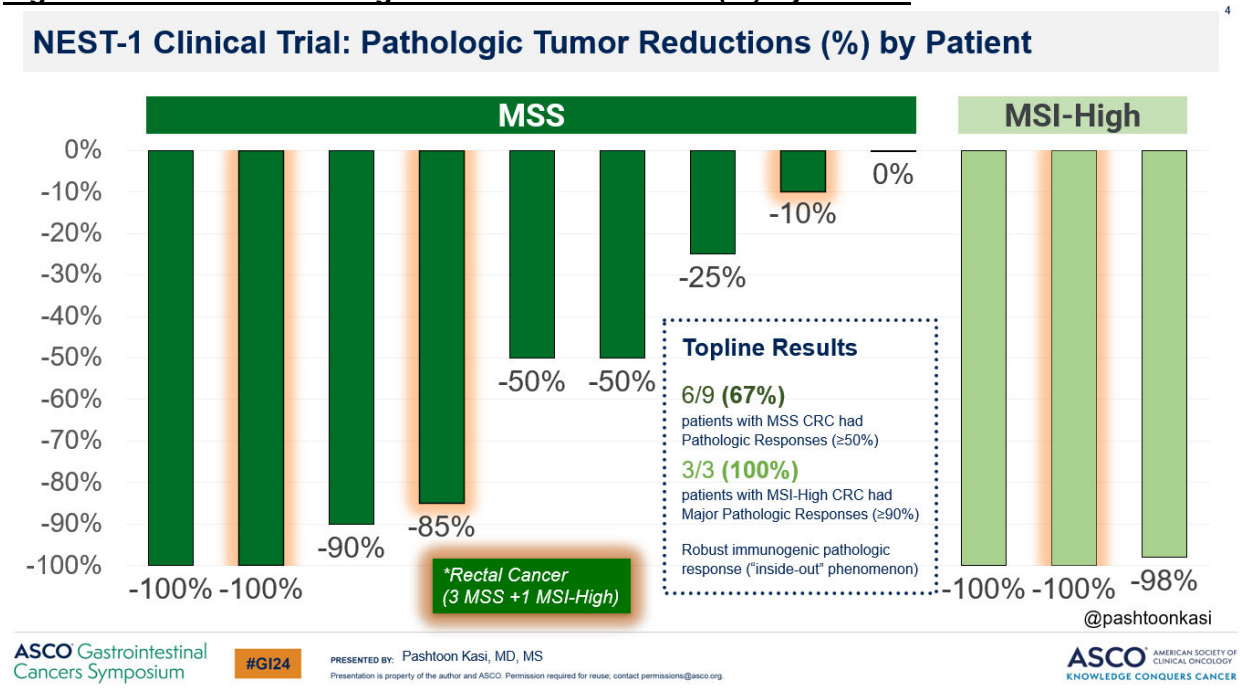
Table 2. NEST-1 Initial 9 Participants Tumor Response (%) and Time to Surgery in MSS vs MSI-H

	MSS									MSI-H		
ID	11	7*	1	2*	8	10	4	3*	12	5	6	9*
Response	**100%**	**100%**	**90%**	**85%**	**50%**	**50%**	**25%**	**10%**	**0%**	**100%**	**98%**	**100%**
Stage (initial clinical)	T3N1a IIIB	T2N0 I	T2N1a IIIA	T3bN2a IIIB	T3bN2b IIIC	T3dN2b IIIC	T3N2a IIIB	T3aN1b IIIB	TXN0	T3N2b IIIC	T3N2a IIIB	T3dN2b IIIC
Stage (final pathology)	T0N0 *No tumor*	T0NX *No tumor*	T1N0 I	T1N0 I	T3N0 IIA	T3N0 IIA	T3N1b IIIB	T4aN2b IIIC	T2N1a IIIA	T0N0 *No tumor*	T2N0 I	T0N0 *No tumor*
Days to surgery	38	64	30	24	36	27	21	29	29	34	42	57

Cohort C focuses solely on the dMMR/MSI-High subset of immune responsive patients. In Cohort A, we saw virtually no tumor each time a dMMR/MSI-High patient got

immunotherapy. This has brought into question the need for any surgery in these patients. Based on these preliminary responses, patients in cohort C will get immunotherapy but surgery will be optional. This is also in alignment with ongoing trials globally that have reported similar excellent responses. Participants in Cohort C will instead have a “Watch-&-Wait” approach so that the patients/providers can essentially monitor the patient to see if immunotherapy is able to eradicate/cure all the cancer and therefore, obviate the need for any surgery. From an efficacy analysis, the 3 patients already treated in the cohort A that had dMMR/MSI-High tumors, would be included in the analyses for this cohort pre-planned analysis.

Figure 8. Cohort A Pathological Tumor Reductions (%) by Patient



Based on this, a pilot to assess the preliminary efficacy with short term follow up after 1 dose of the BOT/BAL and 3 additional doses of BAL (6 weeks of immunotherapy) and reassessment in the clinic every 4-6 weeks and imaging between 8-16 weeks as standard of care. This treatment is to be followed by standard treatment as determined by the treating physician, such as chemoradiotherapy and surgery or transanal/endoscopic resection. Patients who had a clinical/pathologic response after completion of immunotherapy would have the option of watch-and wait and close interval follow up without chemoradiotherapy and surgery as per standard NCCN guidelines. However, this decision would be made by the multi-disciplinary tumor board and the treating physician. Plans to proceed with standard chemoradiation, chemotherapy and/or surgery or watch-and-wait will be as per the standard NCCN guidelines.

Following immunotherapy, treating physicians and patients may decide to treat with standard therapies, such as radiation, chemotherapy, and/or surgical resection, or opt for a watch-and-wait approach, depending on the patients’ clinical response to immunotherapy. While information regarding participant next treatment will be collected,

decisions regarding treatment following immunotherapy will not be dictated by this protocol.

Future Directions

With outcomes of these large neoadjuvant chemotherapy-based studies in public domains, we plan to generate data that would be pivotal in changing the standard of care and increasing the proportion of patients who are cured with the addition of immunotherapy to the treatment plan.

Recently two meta-analyses which have included hundreds of patients clearly show the significant number of patients who are not cured by the current standard of care with the number/proportion of individuals cancer-free dropping with each year unfortunately shown below (Aliseda et al 2024 and Davey et al 2023 respectively).

With propensity-based analyses, we plan to compare the outcomes of these cohorts in our NEST trial treated with immunotherapy with these existing datasets treated with treated with chemotherapy.

2.2 Investigational Agent/Device, or Surgical Treatment/Method

2.2.1 Balstilimab

Balstilimab (bal) is a human monoclonal antibody that targets programmed cell death protein 1 (PD-1). Engagement of PD-1 by its ligands, programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2), leads to signal transduction that inhibits important aspects of T-cell function, including proliferation, cytokine production, and cytolytic activity. Balstilimab potently inhibits PD-1 binding to PD-L1 and PD-L2 and is intended to reverse the immunosuppressive effects of this signaling pathway in the context of tumor immuno-surveillance by T cells.

2.2.1.1 Balstilimab Non-clinical Data

The utility of PD-1 as a therapeutic antibody target in human cancer has been well characterized. A human IgG4 antibody with Ig kappa light chains (IgG4κ) mAb directed against PD-1 (nivolumab, Opdivo®) has been evaluated in thousands of subjects covering a range of IV doses, from 0.1 mg/kg to 20 mg/kg (Topalian et al. 2012). Balstilimab is physicochemically comparable to nivolumab (anti-PD-1 specific IgG4 antibodies with Igκ light chains), and a side-by-side in vitro pharmacological characterization of balstilimab and nivolumab demonstrated that these 2 antibodies were functionally comparable.

Balstilimab toxicity was assessed in a 4-week GLP repeat dose study in cynomolgus monkeys and in a human tissue cross reactivity study. No clinically relevant findings were identified. Details of these studies can be found in the Investigator's Brochure for balstilimab.

The no-observed- adverse-effect level (NOAEL) of balstilimab from the 4-week cynomolgus monkey toxicity study was 40 mg/kg. Histopathology findings at 300 mg/kg included vascular/perivascular inflammation in a variety of tissues. The collective histology, IHC, PK and anti-drug antibody (ADA) data were consistent with an immune-mediated vasculitis caused by ADA formation in a proportion of monkeys given 300 mg/kg balstilimab. ADA development is a recognized phenomenon in non-human primate studies as it relates to the antigenicity of an administered humanized antibody and these types of events are typically not correlated with the formation of ADA in human subjects (Frazier et al. 2015; Ponce et al. 2009; Rojko et al. 2014). Other treatment-related findings in the 4-week study were consistent with pharmacodynamic effects including changes in lymphoid cellularity in the spleen (increased at ≥ 40 mg/kg) and thymus (decreased at 300 mg/kg) and an increase in mononuclear cell infiltration in the brain (≥ 40 mg/kg). These findings related to some changes in organ weights that showed a trend towards recovery following the 4-week non-dosing phase. Cytokine measurements from the 4-week toxicity study in cynomolgus monkeys and from an in vitro cytokine release assay in human whole blood revealed no findings that are considered related to cytokine release syndrome.

For detailed information on preclinical characterization, manufacturing, and

administration of balstilimab, please refer to the Investigator's Brochure for balstilimab.

2.2.1.2 Balstilimab Clinical Data

Currently, there are multiple ongoing clinical studies of balstilimab in patients with metastatic or locally advanced solid tumors and in advanced cervical cancer. Balstilimab is being evaluated both as a single agent and in combination with botensilimab (AGEN1181 - bot), as in this study. For detailed information on each study, please refer to the current Balstilimab Investigator's Brochure.

As of 20 December 2022, 942 participants across the Agenus Inc. balstilimab clinical program have been exposed to balstilimab as monotherapy or in combination with other drugs. The following are the most commonly occurring AEs in the balstilimab clinical program, irrespective of causality: anemia, nausea, diarrhea, fatigue, decreased appetite, vomiting, urinary tract infection, pyrexia, abdominal pain, constipation, cough, headache, back pain, aspartate aminotransferase increased, asthenia, dyspnea, pruritus, arthralgia, alanine aminotransferase increased, hypokalemia, hyponatremia, peripheral edema, blood alkaline phosphatase increased, hypothyroidism, blood creatinine increased, and hypomagnesemia.

Immune-mediated AEs (imAEs) are anticipated AEs in the immune-checkpoint inhibitor drug space. As of the 20 December 2022, the following Grade 3+ imAEs have been reported in the balstilimab clinical program: immune-mediated enterocolitis, immune-mediated hypothyroidism, immune-mediated dermatitis, immune-mediated hepatitis, immune-mediated nephritis, immune-mediated thyroiditis, immune-mediated pancreatitis, immune-mediated pneumonitis, immune-mediated myocarditis, immune-mediated arthritis, and immune-mediated lung disease.

Since earlier versions of this protocol were developed, updated Investigator's Brochures for balstilimab (Editions 9.0 and 10.0) have included revised and additional safety information. The informed consent documents have been updated to reflect the most current risk information for balstilimab. For additional information regarding safety, pharmacology, and clinical experience, please refer to the most current Investigator's Brochure

2.2.2 Botensilimab

Botensilimab is a novel, human, fragment crystallizable (Fc)–engineered immunoglobulin (Ig) G1 (IgG1) anti–cytotoxic T-lymphocyte antigen 4 (CTL-4) antibody designed to exploit a novel mechanism by which increased Fc engagement enhances antigen-specific effector T-cell responses. In addition, this Fc-modified antibody improves the immunological synapse by binding with very high affinity to Fc gamma receptors (FcγR fragment-crystallizable gamma receptors (γRs) located on immune cells. In addition to enhancing T-effector cell (T_{eff}) responses, this receptor-ligand interaction may target regulatory T-cells (T_{reg}) via antibody-dependent cellular cytotoxicity (ADCC) or antibody-

dependent cellular phagocytosis (ADCP). Botensilimab is being developed to treat subjects with advanced or refractory malignancies.

2.2.2.1 Botensilimab Non-clinical Data

Botensilimab selectively binds with high affinity to human CTLA-4 (bivalent: equilibrium dissociation constant [KD] 0.43 nM; monovalent: KD 0.45 nM), as determined by surface plasmon resonance. Botensilimab does not bind to other members of the CD28 superfamily. Botensilimab cross-reacts with cynomolgus monkey (*Macaca fascicularis*) CTLA-4, with an affinity within 3-fold of that for human CTLA-4. However, botensilimab does not cross-react with rodent CTLA 4 (Investigator's Brochure for AGEN1181, Section 4).

Flow cytometric analysis data demonstrated that consistent with its engineered Fc region, botensilimab bound with higher affinity than the parental IgG1 to FcγRIIA R131, FcγRIIB, FcγRIIIA V158, and FcγRIIIA F158. Fc-FcγR interactions have been shown to play an important role for mAb–directed effector cell activities, as well as mAb-dependent forward signaling into target cells via receptor clustering. In the case of mAbs targeting CTLA-4, the interaction with FcγR on antigen-presenting cells (APCs) enhances antigen-specific T-cell responses and tumoricidal activity (Waight et al. 2018). CTLA-4 is also expressed by Tregs, which can impair tumor immunity by multiple suppressive mechanisms (Wing et al. 2008; Zou 2006). One such mechanism involves CTLA-4-mediated removal of CD80 and CD86; CD80 or CD86 are hijacked by CTLA-4 from the surface of activated APCs by a process termed trans-endocytosis, then degraded within CTLA-4-expressing cells. This attenuates the immune stimulatory potential of APCs (Qureshi, et al. 2011).

Recent preclinical and clinical data have indicated that some immunomodulatory monoclonal antibodies, including anti–CTLA-4, have additional capacity to preferentially deplete T_{regs} in tumor microenvironment by ADCC, resulting in an increase in the intra-tumoral ratio of T_{effs} to T_{regs}, and tumor rejection (Bulliard et al. 2013; Marabelle et al. 2013; Romano et al. 2015; Selby et al. 2013; Simpson et al. 2013). This mechanism involves the co binding of anti-CTLA 4 antibodies to T_{regs} located within the tumor and FcγR-expressing effector cells (e.g., natural killer [NK] or myeloid cells), resulting in ADCC or ADCP. The *in vitro* preclinical data with botensilimab are aligned with this report. An *in vitro* study has been performed with isolated human T_{regs} and T_{effs} from whole blood and expanded *in vitro* that maintain their lineage properties. ADCC activity was measured by activation of apoptosis (Caspase 3/7 activation) in activated T_{reg} or T_{eff} cells in co-culture with an NK cell line expressing FcγRIIIA and a fixed concentration of botensilimab or botensilimab IgG1. Botensilimab increased apoptosis relative to botensilimab IgG1, and the induction of apoptosis was stronger in T_{regs} compared to T_{effs}.

A 4-week Good Laboratory Practice (GLP) study was conducted in cynomolgus monkeys: 15 males and 15 females in study groups, and 4 males and 4 females in the recovery group. Botensilimab was administered by a slow IV bolus once weekly at dosages of 0, 5, 30, or 100 mg/kg. Administration at 5, 30, and 100 mg/kg once weekly for 29 days resulted in widespread inflammatory changes in several organ systems, such as change in size and weight of the organs, discoloration (kidney cortex, adrenal gland, etc.), hepatocellular hypertrophy, and inflammation in stomach, colon, etc. One male in the 30 mg/kg dose group was euthanized on Day

14 due to declining clinical condition. Morbidity was attributed primarily to pulmonary findings, including edema and hemorrhage. All other animals in the study group survived to their scheduled terminal euthanasia on Day 29. Most botensilimab-related inflammatory changes were not fully reversible after a 1-month recovery phase.

A GLP tissue cross reactivity study with human tissues indicated the epithelial staining was cytoplasmic in nature. In addition, mAb binding to cytoplasmic sites in tissue cross-reactivity studies generally is considered of little to no toxicologic significance due to the inability of antibody drugs to access the cytoplasmic compartment *in vivo*. For further details, refer to the Investigator's Brochure for AGEN1181.

2.2.2.2 Botensilimab Clinical Data

Botensilimab is being developed as monotherapy and in combination with other anticancer agents across a range of advanced solid tumors, including tumor types unresponsive to immunotherapy. The clinical program includes several Phase 1, 2, and 3 clinical studies in the United States, Europe, and other countries. As of 13 December 2022, 315 participants have been exposed to botensilimab as a part of the Agenus botensilimab clinical program. The most frequently reported TEAEs (≥ 10%) in the botensilimab clinical program, regardless of causality include fatigue, diarrhea, nausea, decreased appetite, anemia, pyrexia, vomiting, colitis, cough, abdominal pain, chills, hypokalemia, AST increased, hyponatremia, ALT increased, pruritus, arthralgia, headache, dyspnea, weight decreased, constipation, rash maculo-papular, dehydration, blood creatinine increased, blood alkaline phosphatase increase, peripheral edema, hypotension, and hypoalbuminemia.

As of 13 December 2022, the following serious adverse reactions had been reported: immune-mediated enterocolitis (0.32%); colitis (13.65%); and diarrhea (5.39%).Although the risks of botensilimab in terms of diarrhea, colitis, and rash require proactive mitigation measures, other risks appear less prominent than anticipated, such as hepatitis, pneumonitis, and hypophysitis. These proactive mitigation measures include close follow up (every 2 weeks) and early use of guideline endorsed supportive care measures to treat the relevant immune mediated events. These risks are to be evaluated against the promising numbers of durable responses seen in the Phase 1 Study C-800-01. Taken together, these data suggest that botensilimab is indeed differentiated from earlier generation anti–CTLA-4 antibodies and support the continued investigation of botensilimab in the treatment of a number of solid tumors. For additional information, please refer to the current Investigator's Brochure for Botensilimab.

Updated Investigator's Brochures for botensilimab (Editions 6.0 and 7.0) have also provided revised safety information for this agent. The informed consent documents have been updated accordingly to ensure the most current risk information is reflected. For additional information regarding safety, pharmacology, and clinical experience, please refer to the most current Investigator's Brochure

2.3 Rationale

2.3.1 CTLA-4 Inhibition

The discovery of ICIs has dramatically altered the landscape of cancer therapy. CTLA-4, an inhibitory checkpoint protein, is a down-regulator of the immune response. Enhanced immune responsiveness has been demonstrated by inhibiting downregulation of T-cell activation via CTLA-4 receptor antagonism. Based on its robust clinical efficacy in metastatic melanoma subjects, ipilimumab (Bristol Myers Squibb), an IgG1 anti CTLA-4 antibody, has been approved in the United States of America (USA) for the treatment of metastatic melanoma and in Europe for previously treated metastatic melanoma (Lipson and Drake 2011; Page et al. 2013).

A key distinction of botensilimab relative to ipilimumab, though, is the incorporation of an Fc-optimized IgG1 backbone that enhances antibody binding to human FcγRIIIA and other Fcγ receptors and exploits a novel mechanism of action (Waight et al. 2018). *In vitro* and *in vivo* data suggest that FcγR co-engagement is important for reaching optimal CTLA-4 antagonism (Investigator's Brochure for AGEN1181).

Vargas and colleagues demonstrated a striking correlation between FcγR biology and improved clinical response to ipilimumab in melanoma subjects with high tumor mutational burden and inflamed tumors. They noted that subjects with the germline high-affinity FcγRIIIA polymorphism (V158) exhibited a significant advantage in survival over those that harbored only the low-affinity FcγRIIIA allele (F158) (Arce Vargas et al. 2018). Based on our *in vitro* data, botensilimab binds strongly to both FcγRIIIA-F158 and FcγRIIIA-V158 (Investigator's Brochure for AGEN1181). Consequently, this approach to the development of the anti–CTLA-4 antibody botensilimab may offer a significant clinical benefit to cancer subjects for whom current immunotherapy may be ineffective, as well as in subjects with other tumor types in whom CTLA-4 blockade is not included as a part of their treatment.

2.3.2 PD-1 Inhibition

It is increasingly understood that cancers are recognized by the immune system, and under some circumstances, the immune system may control or even eliminate tumors (Dunn et al 2004). PD-L1 is part of a complex system of receptors and ligands that are involved in controlling T-cell activation. The PD-1 receptor (CD279) is expressed on the surface of activated T cells (Keir et al. 2007). It has 2 known ligands: PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273) (Okazaki & Honjo, 2007). PD-1 and PD-L1/PD-L2 belong to a family of immune checkpoint proteins that act as co-inhibitory factors, which can halt or limit the development of T-cell response. PD-L1 is constitutively expressed by B-cells, dendritic cells (DCs), and macrophages (Sun et al. 2018). When PD-L1 binds to PD-1, an inhibitory signal is transmitted into the T cell, which reduces cytokine production and suppresses T-cell proliferation. Tumor cells exploit this immune checkpoint pathway as a mechanism to evade detection and inhibit immune responses. Based on these findings, an anti-PD-1/PD-L1 antibody could be used therapeutically to enhance anti-tumor immune responses in participants with cancer. Results of pre-clinical and clinical studies of mAbs targeting the PD-L1/PD-1 pathway have shown evidence of clinical activity and a manageable safety profile, supporting the hypothesis that an anti-PD-1/PD-L1 antibody could be used to therapeutically enhance antitumor immune response in cancer participants (Brahmer et al., 2010; Topalian et al. 2014; Topalian et al. 2019).

2.4 Risk/Benefit Assessment

2.4.1 Known Potential Risks

mAbs directed against immune checkpoint proteins, such as those directed against PD-1 and CTLA-4, aim to boost endogenous immune responses directed against tumor cells. By stimulating the immune system, however, there is the potential for adverse effects on normal tissues.

Most adverse drug reactions seen with the ICIs are thought to be due to the effects of inflammatory cells on specific tissues. These risks are generally events with a potential inflammatory or immune-mediated mechanism, and which may require more frequent monitoring and/or unique interventions such as immunosuppressants and/or endocrine therapy. These immune-mediated effects can occur in nearly any organ system and are most commonly seen as gastrointestinal AEs such as colitis and diarrhea; pneumonitis/interstitial lung disease; hepatic AEs such as hepatitis and liver enzyme elevations; skin events such as rash and dermatitis; and endocrinopathies including hypo- and hyperthyroidism.

Additionally, both drugs are intravenously administered immunoglobulins, and thus have the potential to cause infusion-related reactions (IRRs), or hypersensitivity (anaphylaxis and serious allergic reactions. Allergic reactions, including severe reactions such as anaphylaxis have been observed after administration of mAbs and other foreign proteins usually after repeated exposure. Severe reactions are rare. IRRs occur during dosing and may present with manifestations similar to hypersensitivity, such as pruritus, rash, headache, flushing, sweating, tachycardia, dyspnea, bronchospasm, hypotension, dizziness or lightheadedness, fever, chills, myalgia, nausea, vomiting, and hemodynamic instability. These reactions are more common with higher doses, higher rates of infusion, and in subjects with a history of allergies. Although IRRs are similar to hypersensitivity reactions in their clinical presentation, they predominantly occur at the first exposure to drug, and are uncommon at subsequent exposures.

In a study of combination therapy with balstilimab and botensilimab (Study C-800-01), 79.6% patients experienced at least 1 TRAE, 51.9% patients experienced at least 1 SAE, and 19.4% patients experienced at least 1 treatment-related SAE. As of the data cutoff, 27 (25.0%) patients died, and 17 deaths were due to disease progression (and assessed as unrelated to the study drugs). Fatal TEAEs were reported in 12.0% patients, including multiple organ dysfunction syndrome (4 patients), respiratory failure (3 patients), and sepsis, acute respiratory distress syndrome, acute respiratory failure, intracranial hemorrhage, intestinal obstruction, and small intestinal perforation (1 patient each).

2.4.1.1 Balstilimab Risks

The safety profile of balstilimab is consistent with that observed for the PD-1 inhibitors on the market. Immune-mediated AEs are the most relevant events for this type of molecule. Gastrointestinal, respiratory, endocrine, hepatic, renal, and skin disorders have been observed across the studies, with balstilimab as monotherapy and in combination with zalifrelimab or botensilimab. Colitis, pneumonitis, nephritis, and effects on the hypophyseal-adrenal axis are more frequently reported as serious

and related thus far; these effects are considered “identified risks” for the molecule. As balstilimab is administered intravenously, infusion-related reactions are possible.

Potential risks associated with balstilimab treatment include, but are not limited to:

- anemia
- nausea
- diarrhea
- fatigue
- vomiting
- colitis
- immune-mediated enterocolitis
- pneumonitis
- adrenal insufficiency
- immune-mediated nephritis
- hypothyroidism and hyperthyroidism
- diabetes mellitus (with ketoacidosis and diabetic coma)
- hypophysitis
- rash
- hepatitis
- joint pain (arthralgia)
- decreased appetite
- urinary tract infection
- fever
- abdominal pain
- constipation
- cough
- headache
- back pain
- weakness (asthenia)
- shortness of breath
- itchy skin (pruritis)
- joint
- low potassium in blood (hypokalemia)
- low sodium in blood (hyponatremia)
- swelling (peripheraledema)

For additional information, please see the investigational brochure for balstilimab.

2.4.1.2 Botensilimab Risks

The overall safety data of botensilimab from the ongoing study were broadly compatible with the anticipated safety profile of this product with similar rates of colitis/diarrhea, rash, and thyroid disorders to other anti–CTLA-4 antibodies, whereas the rates of observed hepatitis, hypophysitis, and pneumonitis appear less prominent than anticipated. As botensilimab is administered intravenously, infusion-related reactions are possible.

Potential risks associated with botensilimab treatment include, but are not limited to:

- Anemia
- Nausea
- Diarrhea
- Fatigue
- Colitis
- Immune-Mediated Enterocolitis
- Decreased appetite
- Fever
- Vomiting
- Cough
- Abdominal pain
- Chills
- Low Potassium in blood (Hypokalemia)
- Low Sodium in blood (Hyponatremia)
- Aspartate aminotransferase increased.
- Alanine aminotransferase increased.
- Itchy skin (Pruritis)
- Joint pain (Arthralgia)
- Headache
- Shortness of breath (Dyspnea)
- Weight decreased
- Constipation
- Rash maculo-papular
- Dehydration
- Blood Creatinine increased
- Swelling (peripheral edema)
- Hypotension
- Low levels of blood protein called albumin (Hypoalbuminemia)

For additional information, please see the investigational brochure for botensilimab.

2.4.2 Known Potential Benefits

Studies of balstilimab and botensilimab's efficacy in treating various tumor types as monotherapy or in combination with each other are still ongoing. Accordingly, participants in this study cannot be guaranteed any benefit. Preliminary results from a study of combined balstilimab and botensilimab therapy (Study C-800-01) have demonstrated treatment responses across an array of "cold" tumors, including MSS colorectal cancer, and post-PD-1/PD-L1 treated tumors (e.g. non-small cell lung cancer). Tumor types responding to botensilimab monotherapy include several diseases not expected to respond to immunotherapy, including ampullary cancer (pancreaticobiliary phenotype), and post-PD-1/PD-L1 treated tumors including cervical cancer, MSS endometrial cancer, and melanoma with the FcγR genotype predicted not to respond to first-generation CTLA-4 inhibitors.

In a study of balstilimab monotherapy, an ORR of 15.0% with a median DOR of 11.0 months. In a study of balstilimab in combination with CTLA-4 inhibitor, zalifrelimab, (Study C-550-01) treated patients had an ORR of 25.6%. The median DOR was not

estimable, as the response was still ongoing. Comparing the 2 mature datasets, an absolute increase of 10% in ORR and 2.3% in CR rate was noted for combination therapy vs. monotherapy. After adjustment for differences in distribution of age, PD-L1 expression status, histology, and prior use of bevacizumab, the difference in ORR between both studies increased to 12.1% ($p = 0.014$). This analysis supports that the differences observed in ORR and CR rate were due to treatment, as opposed to differences in the enrolled populations related to known prognostic factors. As noted above, in Study C-800-01, multiple tumor types responded to balstilimab in combination with botensilimab, with many of those responses currently ongoing.

A complete list of responses across tumor types is provided in the investigational brochures for balstilimab and botensilimab.

2.4.3 Assessment of Potential Risks and Benefits

While both botensilimab and balstilimab have risks, their safety profiles are similar to other CTLA-4 and PD-1 inhibitors, respectively. Additionally, although rates of diarrhea/colitis and rash appear like those seen with first-generation anti-CTLA-4 antibodies, the Phase 1 data from Study C-800-01 suggest a lack of hypophysitis and pneumonitis, as well as potentially less autoimmune hepatitis, which might be due to the structural and functional innovations in the botensilimab molecule. Furthermore, preliminary data from Study C-800-01 have demonstrated treatment responses among patients with colorectal cancer treated with combination botensilimab and balstilimab therapy.

From a safety perspective, this protocol has built in multiple checks to monitor patient safety, including regular AE assessments; review by the Data Safety and Monitoring after enrollment of the first 6 patients; and a stopping rule defined in Section 14.1, if patients experience an unacceptable number of treatment-related surgical delays. Furthermore, patients with any immune-related toxicity will be managed as per the tables outlined in Appendix A and B.

2.5 Correlative Studies Background

The correlatives for this study can be broadly divided into:

- Immune Correlates (Pre- and Post- immunotherapy)
- Tumor Correlates (Pre- and Post- immunotherapy including surgical specimens)

The specifics and timing of sample collection are detailed in the schedule of testing/companion laboratory manual for this clinical trial. Both in-house research/clinical as well as commercially available platforms would be utilized to accomplish the goals listed.

Broadly speaking as noted, the main goal would be study 2 main correlates (tissue/blood pre- and post-immunotherapy) from a) an immune perspective and b) tumor perspective. As noted in detail in the schedule of testing and companion laboratory manual, there are additional serial collections from a blood testing perspective to allow for assessment of blood-based immune markers as well as tumor based liquid biopsies (e.g., circulating tumor DNA testing – ctDNA testing).

Analysis of immune cells by flow cytometry: Analysis of immune cell populations via flow cytometry will allow for more quantitative and in-depth profiling of immune cells. In addition, these experiments will be done at the Cornell at the time of sample collection.

Immune cell analysis of peripheral blood and tumor tissue: Frequencies of innate (monocytes, macrophages, neutrophils, dendritic cells, NK cells) and adaptive (CD4+ T cells, CD8+ T cells, NKT cells, B cells) immune cells present in the peripheral blood or tumors of patients will be determined via flow cytometry. In addition, we will examine expression of inhibitory markers on T cells including, but not limited to, PD-1, CTLA-4, TIM3 and LAG3. For peripheral blood samples we will compare frequencies of immune cell populations at the several serial time points as denoted in the table. Tumor infiltrating cells in tumor tissue samples will be compared at two time points for the tissue as denoted in the schedule of testing/companion laboratory manual.

Serum cytokine and chemokine analysis: Use of a Multiplex assay will allow us to examine multiple cytokine and chemokine targets at multiple time points with use of minimal serum sample. These again would be done at Cornell at the time of sample collection. Serum will be collected from peripheral blood of patients at the different time points as denoted in the table. A bead based multiplex assay will be used to quantitatively measure levels of different cytokines and chemokines at various time points. This analysis will be done at the Cornell.

From a tumor microenvironment perspective, would evaluate for immune related changes including but not limited to PD-L1 and PD-1 expression and tumor-infiltrating lymphocytes.

Tumor correlates:
We will have 2 timepoints of tumor evaluation in terms of tissue i.e. pre- and post-immunotherapy (on the standard of care biopsy sample at diagnosis if possible, and on the surgical specimen post therapy).

Additionally, as noted, a unique and strong aspect of this study is the use of liquid biopsies (ctDNA/circulating tumor DNA testing) at multiple serial timepoints.

- We will measure the variant allele fraction (%) and the spectrum of mutations through next generation sequencing based platforms.
- Tumor mutation burden would also be estimated and studied.
- On tissue sequencing would be to assess mutational status and guideline recommended markers.

Of note, if enough tissue is not available from the biopsy sample, analysis would then be reserved primarily for the surgical specimen.

3. Study Design

3.1 Overall Design

This is a non-randomized, open-label, phase II study to assess the feasibility, safety, and efficacy of using a combination of PD-1 inhibitor, balstilimab, and CTLA-4 inhibitor, botensilimab, in the neoadjuvant setting in patients with colorectal cancer, prior to resection. This study includes an initial safety and feasibility cohort (Cohort A) and will have the ability to add additional cohorts based on preliminary results.

Cohort A: In Cohort A, participants will receive an initial dose of botensilimab (75 mg IV) and balstilimab (240mg IV), followed by a second dose of balstilimab approximately 2 weeks after the initial dose, prior to receiving surgical resection of their colorectal cancer.

Cohort B: In Cohort B, a combination of balstilimab (240mg IV Q2W) and botensilimab (75 mg IV) will be given to patients with colorectal cancer, followed by three additional doses of balstilimab given approximately every 2 weeks prior to surgical resection.

Cohort C: In Cohort C, a combination of balstilimab (240mg IV Q2W) and botensilimab (75 mg IV) will be given to patients with d-MMR or MSI-high colorectal cancer, followed by three additional doses of balstilimab given approximately every 2 weeks prior to scheduled surgical resection. Given the evidence of increased efficacy in this population, treating surgeon and patients may decide to complete surgical resection or opt for a watch and wait approach, depending on the patients' clinical response to immunotherapy.

3.2 Scientific Rationale for Study Design

Rationale for Initial Cohort (Cohort A)
This is a proof-of-principle, exploratory, phase-2 study looking at anti-tumor effects of this novel combination bot/bal immunotherapy regimen. The neoadjuvant platform is the perfect setting both from an immunological, efficacy, as well as correlative standpoint.

Rationale for Cohort B and C (based on data as of September 2023)
As of September 2023, this protocol enrolled 12 participants in the initial feasibility cohort (Cohort A). Each participant received an initial dose of balstilimab (240mg IV) and botensilimab (75mg IV), followed by a second dose of balstilimab 240mg IV two weeks after the initial dose. At this time, all (12 out of 12 – 100%) participants have completed surgery with no delays due to treatment-related adverse events. These preliminary results support that the combination of bot/bal at this dose is both safe and feasible in the neoadjuvant setting. Survival follow-up is ongoing.

Of the participants who have been assessed for pathological response to date, tumor regression has ranged from 10-100%. Evidence suggests that allowing increased time for bot/bal to exert an affect in the neoadjuvant setting may increase tumor regression. Thus, the investigators believe that by adding additional time and 2 more doses of the PD-1 treatment, may increase therapeutic effect without compromising safety.

As noted by the landmark paper in the New England Journal of Medicine and plenary presentation at ASCO 2022, "Owing to the complications of surgery and the high frequency of pathological complete response, interest in organ-sparing nonoperative management is increasing. The use of clinical complete response that is achieved with neoadjuvant treatment as a surrogate for pathological complete response provides patients with a nonoperative option that results in a survival benefit that is similar to that in patients undergoing surgical resection." (Cercek et. al., 2022) Our preliminary data (Figure 4) alongside these landmark publications and clinical rationale supporting this warrants the formal investigation of the "Watch-&-Wait – W&W" approach for the subset of patients who have dMMR/MSI-High tumors. This forms the basis of the Cohort C in this protocol.

3.3 Justification for Dose

3.3.1 Dose Rationale for Balstilimab

Balstilimab has been evaluated in the Phase 1 setting at doses up to 10 mg/kg Q2W with no maximum tolerated dose identified. Although balstilimab can be dosed Q2W or Q3W, Q2W was selected for Study C 800 25 to best match potential concomitant therapies in this patient population (e.g., future combinations with FOLFOX and bevacizumab). The 240 mg fixed dose was selected for this study as it approximates the RP2D of 3 mg/kg but confers several potential advantages over body weight-based dosing, described below. This study will allow for further characterization of the PK profile of 240 mg fixed dose balstilimab vs prior PK data with weight-based dosing, including allowing for assessment of PK parameters across a range of body weights.

Preliminary serum balstilimab concentrations, serum balstilimab anti-drug antibody (ADA), dosing, demographic, covariate, and response data from balstilimab as monotherapy (ongoing Study C-700-01) and in combination with zalifrelimab (ongoing Study C-550-01) were analyzed via NCAs and pooled for population pharmacokinetics and exposure-response (PopPK-ER) analyses.

A 2-compartment model with linear clearance described balstilimab concentration-time data following IV administration. Analyses noted no time- or concentration-dependency in balstilimab clearance. Geometric mean (geometric CV%) serum balstilimab estimates were 0.907 (40.4%) L/d for clearance, 6.82 (31.8%) L for steady-state volume of distribution, and 9.42 (49.3%) days for terminal elimination half-life. A separate preliminary analysis of ongoing Study C-800-01 found no clinically significant effect of botensilimab coadministration on balstilimab PK and vice-versa. Covariate effects on serum balstilimab pharmacokinetics were minor and do not suggest that balstilimab dose individualization is required.

PopPK results suggest patient body weight is not a critical factor in normalizing systemic balstilimab exposure among adult patients. The similarity of balstilimab exposure between body weight-dosing (mg/kg) and fixed-dosing (mg) supports the use of either dosing regimen. Balstilimab delivered as 240 mg Q2W (fixed dose) is expected to deliver a similar balstilimab dose and systemic exposure as the 3 mg/kg Q2W (RP2D based on body weight) evaluated in Study C 700 01, Study C-550-01, and Study C-800-01.

3.3.2 Dose Rationale for Botensilimab

Preliminary botensilimab PK analyses support a shift from body weight-dosing to fixed-dosing. In general, mAbs targeting immune checkpoints have a volume of distribution (V_d) that is similar to blood plasma and extracellular fluids with body composition and organ function having little impact on overall systemic drug exposure.

Based on preliminary data from 124 patients treated in the ongoing Phase 1 Study C-800-01: the geometric mean (GeoCV) serum botensilimab clearance is 0.378 L/d (46.5%), steady-state V_d is 5.53 L (34.9%), and terminal elimination half-life ($t_{1/2}$) is 12.6 d (41.4%). There is no observed effect of body weight on clearance, which is the main determinant of drug exposure (AUC). The selected dose of 75 mg of botensilimab is expected to produce serum drug concentrations and systemic drug exposure comparable to a 1.07 mg/kg botensilimab dose for a 70 kg patient. The potential benefits

of transitioning to a fixed dosing regimen include decreased dosing errors, drug administration burden, waste, and cost.

The similarity of botensilimab exposure between body weight-dosing (mg/kg) and fixed-dosing (mg), including AUC steady state (AUC_{ss}), maximum observed drug concentration at steady-state (C_{max-ss}), and minimum observed drug concentration at steady-state (C_{min-ss}) for dose levels 50 mg and 150 mg, supports the use of either dosing regimen. The dose range, 50–150 mg, best captures the therapeutic dose range observed in the current Phase 1 Study C-800-01, thus the proposed dose of 75 mg, which is within this range, will provide data necessary to further optimize the balance between safety and anti-tumor efficacy.

The overall clinical, PK, and pharmacodynamic data from Study C-800-01 to date indicate that the observed therapeutic dose range for botensilimab monotherapy is between approximately 1 and 3 mg/kg. There were no monotherapy objective responses seen at doses below 1 mg/kg (although instances of prolonged SD were observed at low dose levels) and safety signals were not favorable at 3 mg/kg. It is important to note that no objective responses were observed with botensilimab monotherapy doses < 1 mg/kg in the Phase 1 Study C-800-01. Accordingly, a 75 mg dose of botensilimab represents a lower dose level within the previously studied range with a reasonable chance of achieving a clinical objective response. In addition the lower dose of 75 mg is expected to yield meaningfully different non-overlapping exposure based on a 3-fold difference from the higher dose, in accordance with guidance from the Friends of Cancer white paper on Drug Development (Friends of Cancer Research 2021).While a dose/response or dose/toxicity relationship for botensilimab is not yet proven, it is expected that these relationships exist and will become clear with further study based on precedent with CTLA-4 monotherapy (Ascierto 2017).

3.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the **Schedule of Assessments (SoA), Section 6.1**. The end of the study is defined as completion of the last visit or procedure shown in the SoA in the trial globally.

4. Subject Selection

4.1 Study Population

Subjects with a diagnosis of resectable, localized/advanced adenocarcinoma of the colon and/or rectum who meet the inclusion and exclusion criteria will be eligible for participation in this study.

4.2 Inclusion Criteria (All Cohorts)

1. Capable of and willing to provide informed, written consent
2. Age greater than or equal to 18 years
3. Histologically, cytologically, or clinically confirmed adenocarcinoma of the colon or rectal cancer

4. Plans for surgical resection as determined by the treating physician and principal investigator
5. Eastern Cooperative Oncology Group (ECOG) performance status of less than or equal to 2
6. Adequate organ and bone marrow reserve function, as indicated by the following laboratory values:
    a. Adequate hematological function, defined as absolute neutrophil count (ANC) ≥ 1.5 × 10^9/L, platelet count ≥ 100 × 10^9/L, and hemoglobin levels of ≥ 8 g/dL without recent transfusion (defined as a transfusion that has occurred within 2 weeks of the hemoglobin measurement).
    b. Adequate liver function, defined as total bilirubin level ≤ 1.5 × upper limit of normal (ULN), aspartate aminotransferase (AST) ≤ 2.5 × ULN, and alanine aminotransferase (ALT) ≤ 2.5 × ULN.
    c. Adequate renal function defined as calculated creatinine clearance ≥ 40 mL/min as determined by the Cockcroft-Gault equation
7. If capable of becoming pregnant, or getting someone else pregnant, must be willing to use highly effective contraception from Screening period through 90 days following the last dose of study drug

4.2.1 Cohort B Specific Inclusion Criteria

1. Classification of tumor as Microsatellite stability (MSS) based on mismatch repair testing

    a. Patients can enroll in cohort B while awaiting mismatch repair testing results. If noted to be dMMR/MSI-High, they would be still considered evaluable and moved to cohort C.

4.2.2 Cohort C Specific Inclusion Criteria

1. Presence of deficient (d) DNA mismatch repair (dMMR) and/or Microsatellite instability high (MSI-High) report.
    a. MMR status must be assessed by immunohistochemistry (IHC) for MMR protein expression (MLH1, MSH2, MSH6, PMS2) where loss of one or more proteins indicates dMMR. dMMR may be determined by any CLIA-certified lab
    b. Patients who are known to have Lynch syndrome, and have been found to carry a specific germline mutation in an MMR gene (MLH1, MSH2, MSH6, PMS2) are also eligible for Cohort C

4.3 Exclusion Criteria (All Cohorts)

1. Presence of metastases on routine, standard of care radiographic imaging (no stage 4 allowed)
2. Previous treatment with immune checkpoint inhibitors targeting CTLA-4, PD-1 or PD-L1

3. Currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigation device within 3 weeks of first dose of current study drug
4. Patients with rectal cancer must not have plans to receive neoadjuvant radiation prior to completion of immunotherapy
5. Persistent toxicity of National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 Grade > 1 severity that is related to prior therapy.
   Note: Sensory neuropathy or alopecia of Grade ≤ 2 are acceptable.
6. Known severe (Grade ≥ 3) hypersensitivity reactions to fully human monoclonal antibodies, antibody, or severe reaction to immuno-oncology agents, such as colitis or pneumonitis requiring treatment with steroids; or has a history of interstitial lung disease, any history of anaphylaxis, or uncontrolled asthma
7. Pregnant or breastfeeding – individuals capable of becoming pregnant must have a negative serum β-HCG test within 72 hours prior to receiving the first dose of study drug. Women are considered of non-childbearing potential if they meet any of the following criteria:
   a. ≥ 45 years of age and has not had menses for >1 year.
   b. Amenorrheic for > 2 years without a hysterectomy and/or oophorectomy and follicle stimulating hormone value in the postmenopausal range upon pretrial (Screening) evaluation.
   c. Status is post-hysterectomy, -oophorectomy, or -tubal ligation.
8. Active infection requiring treatment
9. Active HIV, Hepatitis B or C with uncontrolled disease, as determined by the treating investigator or PI.
   EXCEPTION: Patient with non-active, controlled disease will be allowed to participate in study.
10. Active or history of autoimmune disease that requires systemic treatment within 2 years of the start of study drug (i.e., with use of disease-modifying agents, corticosteroids, or immunosuppressive drugs). Subjects with autoimmune conditions requiring hormone replacement therapy or topical treatments are eligible.
11. Live vaccination within 28 days prior to receiving the first dose of immunotherapy
12. Receiving systemic corticosteroid therapy 1 week prior to the first dose of study drug or receiving any other form of systemic immunosuppressive medication. Corticosteroid use as a premedication for IV contrast allergies/reactions is allowed. Subjects who are receiving daily corticosteroid replacement therapy are also an exception to this rule. Daily prednisone at doses of ≤ 7.5 mg or equivalent hydrocortisone dose are examples of permitted replacement therapy. Use of inhaled or topical corticosteroids is permitted.
13. Clinically significant (i.e., active) cardiovascular disease: cerebral vascular accident/stroke or myocardial infarction within 6 months of enrollment, unstable angina, congestive heart failure (New York Heart Association class ≥ II), or

serious uncontrolled cardiac arrhythmia requiring medication that may prevent surgery

14. History or current evidence of any condition, therapy, any active infections, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating Investigator or the PI.

4.4 Lifestyle Considerations

During this study, participants of childbearing potential are required to use highly effective methods of contraception for 120 days following the last dose of study drug. Please see Table 3 for a list of highly effective methods of contraception.

Table 3. Highly Effective Methods of Contraception (<1% Failure Rate)

Non hormonal Methods	Hormonal Methods
• Total sexual abstinence (evaluate in relation to the duration of the clinical study and the preferred and usual lifestyle choice of the participant) • Vasectomised sexual partner (with participant assurance that partner received post- vasectomy confirmation of azoospermia) • Tubal occlusion • Intrauterine device (provided coils are copper-banded)	• Implants: Etonogestrel-releasing implants (e.g. Implanon® or Norplant®) • Intravaginal devices: Ethinylestradiol/etonogestrel-releasing intravaginal devices (e.g. NuvaRing®) • Injection: Medroxyprogesterone injection (e.g. Depo-Provera®)[a] • Combined Pill: Normal and low dose combined oral contraceptive pill • Patch: Norelgestromin/ethinylestradiol-releasing transdermal system (e.g. Ortho Evra®) • Mini pill: Progesterone based oral contraceptive pill using desogestrel: Cerazette® is currently the only highly effective progesterone-based • Levonorgestrel-releasing intrauterine system (e.g., Mirena®)[a]

Additionally, participants may not receive live vaccines during the study, as well as for 180 days after the last dose of balstilimab or botensilimab. Vaccination with an inactivated vaccine is permitted at any time as per investigator discretion and COVID-19 vaccination is permitted.

Participants may not donate blood starting from receiving the first dose of balstilimab or botensilimab, until 30 days following the last dose of balstilimab or botensilimab.

Participants should agree to not take herbal remedies with immune-stimulating properties (e.g., mistletoe extract) or that are known to potentially interfere with major organ function (e.g., hypericin).

4.5 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an abnormal lab value may be rescreened. Rescreened participants will be assigned a different participant number than was assigned during the initial screening.

4.6 Strategies for Recruitment and Retention

Subjects will be referred by treating oncologists in WCM outpatient clinics. We anticipate accruing approximately 1 patient/month from WCM clinic sites.

Pre-screening of potential subjects may be performed by investigators or delegates under their direct supervision.

The trial will be listed on the Joint Clinical Trials Office (JCTO) public website in order to help generate referrals.

Subjects will not be compensated for taking part in this trial.

5. Registration Procedures

5.1 Subject Registration (WCM only)

Subjects will be registered within the WRG-CT as per the standard operating procedure for Subject Registration.

5.2 Subject Registration (Sub-sites)

Not applicable.

6. Study Procedures

6.1 Schedule of Assessments

	Table 4: Cohort A Schedule of Assessments									
	Screening[i]	Treatment Visit 1	Treatment Visit 2	Toxicity Check pre-op 1[e]	Toxicity Check pre-op 2[e]		Short-Term Follow-Up			Long-Term Follow-Up[g]
							Post-op Follow up Visit-1[k]	Post-op Follow-up Visit-2[k]	Final AE Review[k]	
	-28 to -1 Days	Day 0	Day 14 (-2 to + 5 days)	Day 28 (+/- 7 days)	Day 42 (+/- 7 days)	Surgical Resection[j] 1-6 weeks post Treatment 2	1 – 3 weeks post-op (+/- 7 days)	4 – 6 weeks post-op (+/- 7 days)	Day 104 (+/- 14 days)	Approx. Q3M – Q6M
Balstilimab administration		X	X							
Botensilimab administration		X								
Informed consent	X									
Demographics	X									
Medical history	X									
Concurrent meds	X	X	X				X	X		
Complete Physical exam	X	X	X				X	X		
Vital signs	X	X	X				X	X		
Height	X									
Weight	X	X	X				X	X		
β-HCG	X[d]									
CBC w/diff, plts[h]	X	X	X				X	X		
Serum chemistry [a,h]	X	X	X				X	X		
EKG (as indicated)	X									

Adverse event evaluation		X	X				X	X	X	
Tumor tissue collection [b]	X						X[f]			
Biomarker/Research blood draw [c]		X	X	X	X		X	X		
Recurrence/ Cancer Treatment Data								X		X
Mortality								X		X

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT[AST], SGPT[ALT], sodium
b: Please note tumor tissue if not present is not an exclusion. But if archival is present, we would do correlative studies on both the biopsy/surgery sample. Similarly, collection of tumor tissue is not required in the LTFU period, but if obtained as part of standard of care, it may be used for correlative studies.
D: Serum pregnancy test (people capable of becoming pregnant)
c: Please see lab manual. Biomarker blood draw should be performed prior to receiving investigational product at Treatment Visit 1
e: Toxicity bloodwork is recommended, but not required, every 2-4 weeks as necessary, per physician's discretion, until surgery; if surgery is done prior to Day 28 or 46, blood draws will not be performed
f: Tumor tissue collection listed in follow-up visit 1 is referring to tumor tissue that will be made available to the research team from surgical resection
g: Long-term follow up evaluations will be conducted per standard clinical practice; medical history, current medical notes, current treatment and hospitalization data, current medications, and any laboratory data may be abstracted from the patient's medical records; long-term follow up will continue until death or for a maximum of 2 years from time of surgery, whichever occurs first. It is not required that any tumor tissue be collected during the follow-up phase, and no tissue will be collected for research-only purposes, but if tissue is collected as part of standard of care, it will be made available for correlative studies
h: If screening bloodwork (CBC and serum chemistry) is done within 3 days prior to Treatment Visit 1, then it does not need to be repeated. CBCs and serum chemistries drawn on Treatment Visits 1 and 2 must be completed prior to administering investigational product
i: Standard of care, pre-operative imaging should be available to confirm that the patient does not have metastases, but this imaging is not required to be from within the 28-day screening period, as long as it is acceptable per standard clinical practice as determined by the treating physician or study principal investigator
j: Surgery will be performed per standard clinical practice by the treating physicians. No research specific procedures will be performed. The research team will have access to any laboratory testing performed as a part of standard pre-operative and intra-operative care via the participant's medical record. The research team will receive samples of the resected tumor tissue for research purposes, as well as standard pathology reports.
K: May be performed as a clinic visit or phone call. Visits at the other sites/oncology offices would be acceptable. Data will be collected from those visits.

Table 5: Cohort B Schedule of Assessments

	Screening[i]	Treatment Visit 1	Treatment Visit 2	Treatment Visit 3	Treatment Visit 4	Pre-Op Toxicity Checks[e]	Surgical Resection[k] 1-6 weeks post Treatment 4	Short-term Follow-Up		Long-term Follow Up [g]
								Post-Op Follow Up Visit-1[l]	Final AE Check[l]	
	-28 to -1 Days	Day 0	Day 14 (-2 to + 5 days)	Day 28 (-2 to +5 days)	Day 42 (-2 to +5 days)	Day 56 (-7 to +14 days)		1-4 weeks post-op (-2 to +5 days)	Day 132 (± 7 days)	Q3-6 months
Balstilimab administration		X	X	X	X					
Botensilimab administration		X								
Informed consent	X									
Demographic Information	X									
Medical history	X									
Concurrent meds	X	X	X	X	X			X		
Complete Physical exam	X	X	X	X	X			X		
Vital signs	X	X	X	X	X			X		
Height	X									
Weight	X	X	X	X	X			X		
β-HCG	X[d]									
CBC w/diff, plts[h]	X	X	X	X	X	X		X		
Serum chemistry [a,h]	X	X	X	X	X	X		X		
EKG (as indicated)	X									
Tumor Markers (as indicated)[j]	X	X	X	X	X					
Adverse event evaluation		X	X	X	X			X	X	
Tumor tissue collection [b]	X		X					X[f]		
Biomarker/Research blood draw [c]		X	X	X	X	X		X		
Recurrence/ Cancer Treatment Data										X
Mortality										X

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT[AST], SGPT[ALT], sodium
b: Please note if archival tumor tissue if not present, patients are still eligible. But if archival tumor tissue is available, it will be used for correlative studies on both the

biopsy/surgery sample. Collection of tumor tissue is not required in the long-term follow-up period, but if obtained as part of standard of care, it may be used for correlative studies.
c: Please see lab manual. Biomarker blood draw should be performed prior to receiving investigational product at Treatment Visit 1
d: Serum pregnancy test (people capable of becoming pregnant)
e: Toxicity bloodwork is recommended, but not required, every 2-4 weeks as necessary, per physician's discretion, until surgery; if surgery is done prior to Day 56, blood draws will not be performed
f: Tumor tissue collection listed in the follow-up visit is referring to tumor tissue that will be made available to the research team from surgical resection
g: Long-term follow up evaluations will be conducted per standard clinical practice; medical history, current medical notes, current treatment and hospitalization data, current medications, and any laboratory data may be abstracted from the patient's medical records; long-term follow up will continue until death or for a maximum of 2 years from time of surgery, whichever occurs first. It is not required that any tumor tissue be collected during the follow-up phase, and no tissue will be collected for research-only purposes, but if tissue is collected as part of standard of care, it will be made available for correlative studies
h: If screening bloodwork (CBC and serum chemistry) is done within 3 days prior to Treatment Visit 1, then it does not need to be repeated. CBCs and serum chemistries drawn on Treatment Visits must be completed prior to administering investigational product (or have been performed and resulted within 3 days prior)
i: Standard of care, pre-operative imaging should be available to confirm that the patient does not have metastases, but this imaging is not required to be from within the 28-day screening period, as long as it is acceptable per standard clinical practice as determined by the treating physician or study principal investigator
j: CEA; CA19-9; ctDNA
k: Surgery will be performed per standard clinical practice by the treating physicians. No research specific procedures will be performed. The research team will have access to any laboratory testing performed as a part of standard pre-operative and intra-operative care via the participant's medical record. The research team will receive samples of the resected tumor tissue for research purposes, as well as standard pathology reports.
L: May be performed as a clinic visit or phone call. Visits at the other sites/oncology offices would be acceptable. Data will be collected from those visits.

Table 6: Cohort C Schedule of Assessments									
	Screening[i]	**Treatment**	**Treatment**	**Treatment**	**Treatment**	**Pre-Op**	[illegible]	**Short-term Follow-Up**	**Long-term**

		Visit 1	Visit 2	Visit 3	Visit 4	Toxicity Check[e]		Post-Op Follow Up Visit-1[m]	Final AE Check[m]	Follow Up [g]
	-28 to -1 Days	Day 0	Day 14 (-2 to + 5 days)	Day 28 (-2 to +5 days)	Day 42 (-2 to +5 days)	Day 60 (±7 days)		1-4 weeks post-op (-2 to +5 days)	Day 132 (± 7 days)	Q3-6 months
Balstilimab administration		X	X	X	X					
Botensilimab administration		X								
Informed consent	X									
Demographic Information	X									
Medical history	X									
Concurrent meds	X	X	X	X	X			X		
Complete Physical exam	X	X	X	X	X			X		
Vital signs	X	X	X	X	X			X		
Height	X									
Weight	X	X	X	X	X			X		
β-HCG	X[d]									
CBC w/diff, plts[h]	X	X	X	X	X	X		X		
Serum chemistry [a,h]	X	X	X	X	X	X		X		
EKG (as indicated)	X									
Tumor Markers (as indicated)[j]	X	X	X	X	X					
Adverse event evaluation		X	X	X	X			X	X	
Tumor tissue collection [b]	X		X					X[f]		
Biomarker/Research blood draw [c]		X	X	X	X	X		X		
Recurrence/ Cancer Treatment Data										X
Mortality										X

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT[AST], SGPT[ALT], sodium
b: Please note tumor tissue if not present is not an exclusion. But if archival is present, we would do correlative studies on both the biopsy/surgery sample. Similarly, collection of tumor tissue is not required in the LTFU period, but if obtained as part of standard of care, it may be used for correlative studies.

c: Please see lab manual. Biomarker blood draw should be performed prior to receiving investigational product at Treatment Visit 1
d: Serum pregnancy test (people capable of becoming pregnant)
e: Toxicity bloodwork is recommended, but not required, every 2-4 weeks as necessary, per physician's discretion, until surgery; if surgery is done prior to Day 56, blood draws will not be performed
f: Tumor tissue collection listed in follow-up visit 1 is referring to tumor tissue that will be made available to the research team from surgical resection
g: Long-term follow up evaluations will be conducted per standard clinical practice; medical history, current medical notes, current treatment and hospitalization data, current medications, and any laboratory data may be abstracted from the patient's medical records; long-term follow up will continue until death or for a maximum of 2 years from time of surgery, whichever occurs first. It is not required that any tumor tissue be collected during the follow-up phase, and no tissue will be collected for research-only purposes, but if tissue is collected as part of standard of care, it will be made available for correlative studies
h: If screening bloodwork (CBC and serum chemistry) is done within 3 days prior to Treatment Visit 1, then it does not need to be repeated. CBCs and serum chemistries drawn on Treatment Visits must be completed prior to administering investigational product (or have been performed and resulted within 3 days prior)
i: Standard of care, pre-operative imaging should be available to confirm that the patient does not have metastases, but this imaging is not required to be from within the 28-day screening period, as long as it is acceptable per standard clinical practice as determined by the treating physician or study principal investigator
j: CEA; CA19-9; ctDNA
k: Surgery will be performed per standard clinical practice by the treating physicians. No research specific procedures will be performed. The research team will have access to any laboratory testing performed as a part of standard pre-operative and intra-operative care via the participant's medical record. The research team will receive samples of the resected tumor tissue for research purposes, as well as standard pathology reports.
l: The joint patient-surgeon decision to defer surgery for a watch and wait (Watch-&-Wait W&W) approach is allowed as long as the patient is demonstrating sustained response as determined by clinical, radiographic, endoscopic, and/or blood-based biomarkers. If the plan is for W&W approach, patients will be seen once a month (+/- 1 week window) for a total of 6 months from the time of initiation of therapy after which they will have standard of care follow ups every 3-6 months as per treating physician for a maximum of 2 years from the last dose of investigational product.
m: May consist of a clinic visit or telephone call. Visits at the other sites/oncology offices would be acceptable. Data will be collected from those visits.

7. Study Intervention

7.1 Study Intervention/Device Description

Botensilimab is an Fc-engineered IgG1 mAb. Botensilimab drug product is a clear-to-opalescent, colorless-to-yellow liquid that is free of visible particulates. Botensilimab drug product is supplied as a sterile, single-use solution for IV administration in a United States Pharmacopeia (USP)-compliant Type 1 borosilicate glass vial. Each drug product vial contains a deliverable amount of 50 mg of Botensilimab in a formulation buffer containing L-histidine, L arginine, and polysorbate 80 at pH 6.5 (Investigator's Brochure for Botensilimab).

Balstilimab is a fully human monoclonal IgG4 antibody designed to block PD-1 binding to PD L1 and PD-L2. Balstilimab drug product is supplied as a sterile, single-use solution for IV injection in a 2 mL or 10 mL glass vial.

Each drug product 2 mL vial contains a withdrawable volume of 1 mL with 50 mg balstilimab at a nominal concentration of 50 mg/mL formulated in 20 mM histidine, 250 mM sorbitol, and 0.01% polysorbate 80 pH 6.0. Each drug product 10 mL vial contains a withdrawable volume of 5 mL, with 50 mg balstilimab at a nominal concentration of 10 mg/mL formulated in 20 mM histidine, 250 mM sorbitol, and 0.02% polysorbate 80 pH 6.0 (Investigator's Brochure for Balstilimab).

Cohort A:

Intervention	Dose	Timing
Botensilimab (bot)	75 mg	Treatment Visit 1
Balstilimab (bal)	240 mg	Treatment Visits 1 and 2

Cohorts B and C:

Intervention	Dose	Timing
Botensilimab (bot)	75 mg	Treatment Visit 1
Balstilimab (bal)	240 mg	Treatment Visits 1-4

7.2 Availability

Botensilimab and balstilimab are investigational agents supplied to investigators by Agenus Biosciences Inc.

7.3 Acquisition and Accountability

Botensilimab and balstilimab will be shipped in transport cool containers (2°C to 8°C) that are monitored with temperature control devices.

Botensilimab and balstilimab drug product must be stored at 2°C to 8°C until use, with a temperature log maintained daily. All medication boxes supplied to each study center must be stored carefully, safely, and separately from other drugs.

Botensilimab and Balstilimab Inventory Records– The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and

disposition of all agents/device received from Agenus Inc. on a Drug Accountability Record Form (DARF).

7.4 Formulation, Appearance, Packaging, and Labeling

Packaging and labeling will be in accordance with applicable local and national regulatory requirements and applicable Good Manufacturing Practice (GMP) guidelines. Please see Section 7.1 for additional information.

7.5 Product Storage and Stability

Botensilimab and balstilimab drug product vials are single-use and stored at 2-8 °C and protected from light. The clinical site will maintain a record of drug product storage conditions, including regular confirmation of continuous storage at 2-8 °C.

Please refer to the current version of the Investigator's Brochures and/or Pharmacy Manuals for complete storage, handling, dispensing, and infusion information for botensilimab and balstilimab (Investigator's Brochure for Botensilimab and Investigator's Brochure for Balstilimab).

7.6 Preparation

Please refer to Botensilimab and Balstilimab Pharmacy Manual for instructions on preparation and administration.

Any unused portion of solution should be discarded in biohazard waste disposal, with final disposal by accepted local and national standards of incineration.

Study drug documentation must be maintained including all processes required to ensure drug is accurately administered. This includes documentation of drug storage, administration and, as applicable, storage temperatures, reconstitution, and use of required processes (e.g., required diluents, administration sets).

7.7 Dosing and Administration

Botensilimab and balstilimab are to be administered via continuous IV infusion over 30 (± 5) minutes.

7.7.1 Dosing Delays/Dose Modifications

No dose modifications will be allowed for either drug. Participants experiencing DLTs will be permanently discontinued from study drug. The period of the DLT assessment begins on Day 1 (first dose) for each subject and continues for 28 days following the last dose of investigational product. A DLT will be defined as the occurrence of any drug-related toxicity as detailed below that is clearly not associated with the underlying disease, a concomitant medication, or comorbidity. Toxicity will be graded according to NCI CTCAE version 5.0.

General DLTs

- Any death not due to disease under investigation

Hematologic DLTs

- Grade 3 thrombocytopenia with clinically significant bleeding (i.e., requires hospitalization, transfusion of blood products, or other urgent medical intervention), or Grade 4 thrombocytopenia regardless of bleeding
- Grade ≥3 febrile neutropenia (ANC < 1.0×10^9/L and fever > 101°F/38.3°C)
- Grade 4 neutropenia (lasting more than 5 days)
- Grade 4 anemia, unless explained by underlying disease

Non-Hematologic DLTs

- Any Grade ≥3 non-hematologic AEs, whether immune-related or not, will be considered a DLT **with the exception of**:
  - Any Grade 3 endocrinopathy that is controlled with systemic corticosteroid therapy and/or hormone replacement therapy
  - Grade 3 AE of tumor flare (defined as local pain, irritation, or rash localized at sites of known or suspected tumor)
  - Grade 3 fatigue
  - Transient (< 72 hours) nausea, vomiting, and/or diarrhea in the absence of maximal medical therapy
  - Grade ≥ 3 electrolyte abnormality that lasts < 72 hours, unless the patient has clinical symptoms.
  - Asymptomatic amylase and lipase elevation
- Any Grade ≥ 2 drug-related uveitis or eye pain or reduction of visual acuity will be adjudicated as a DLT if it does not respond to topical therapy and does not improve to Grade 1 severity within 2 weeks of initiation of topical therapy OR if it requires systemic treatment.
- Toxicities indicating a high risk of a fatal drug-induced liver injury causing hepatocellular injury (not cholestatic injury)
  - Hy's Law: > 3-fold elevations above the patient's baseline of alanine aminotransferase (ALT) or aspartate aminotransferase (AST), with elevation of serum total bilirubin to > 2X patient's baseline, and without initial findings of cholestasis (elevated serum alkaline phosphatase [AP])

Dosing/Procedure-Related Toxicities

- Any Grade ≥ 3 infusion reaction AE

7.8 General Concomitant Medication and Supportive Care Guidelines

All concomitant medications will be recorded and/or updated on subject medication log throughout the course of the study and saved in subject binder, if applicable. Guidelines

for treatment of IRRs and irAEs can be found in Appendix A and Appendix B, respectively.

7.9 Duration of Therapy and Criteria for Treatment Discontinuation

In the absence of treatment delays due to adverse event(s), treatment may continue per the schedule of events for the participant's assigned cohort or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Subject decides to withdraw from the study, or
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator.

7.10 Duration of Follow Up

Participants will be in short term (active) follow-up until completion of both the in clinic follow-up visit and completion of the AE/SAE collection period. After this, participants will move to long term follow up, where they will be followed per clinical practice. Study personnel will continue to obtain mortality, recurrence, and cancer treatment data until death or for a maximum of 2 years following surgery. Participants who discontinue treatment for unacceptable adverse events will be followed, at minimum, until resolution or stabilization of the adverse event.

7.11 Measures to Minimize Bias: Randomization and Blinding

This is an open label, non-randomized study.

7.12 Study Intervention/Follow-up Compliance

Both botensilimab and balstilimab will be given intravenously in the clinic, under supervision. All infusion information (e.g. time, dose, administrator, vitals, any reactions, etc.) will be recorded via eCRF.

Up to 3 attempts to contact participants who miss scheduled visits will be made in order to reschedule.

8. Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

8.1 Discontinuation of Study Intervention

Discontinuation from botensilimab and balstilimab does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following:

- Evaluation of AEs
- Blood draw to assess:
    - CBC
    - CMP
    - ctDNA
    - Other biomarkers pertaining to immunological and tumor DNA in the tissue tumor/blood

8.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive required dose of botensilimab or balstilimab within the timeframe defined in the SoA
- Participant lost to follow-up after several attempts to contact subject to schedule study visit.

The reason for participant discontinuation or withdrawal from the study will be recorded on the appropriate electronic Case Report Form (eCRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

8.3 Lost to Follow Up

A participant will be considered lost to follow-up if he or she fails to return for any of the scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within the timeframe designated in the SoA and counsel the participant on the importance

of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

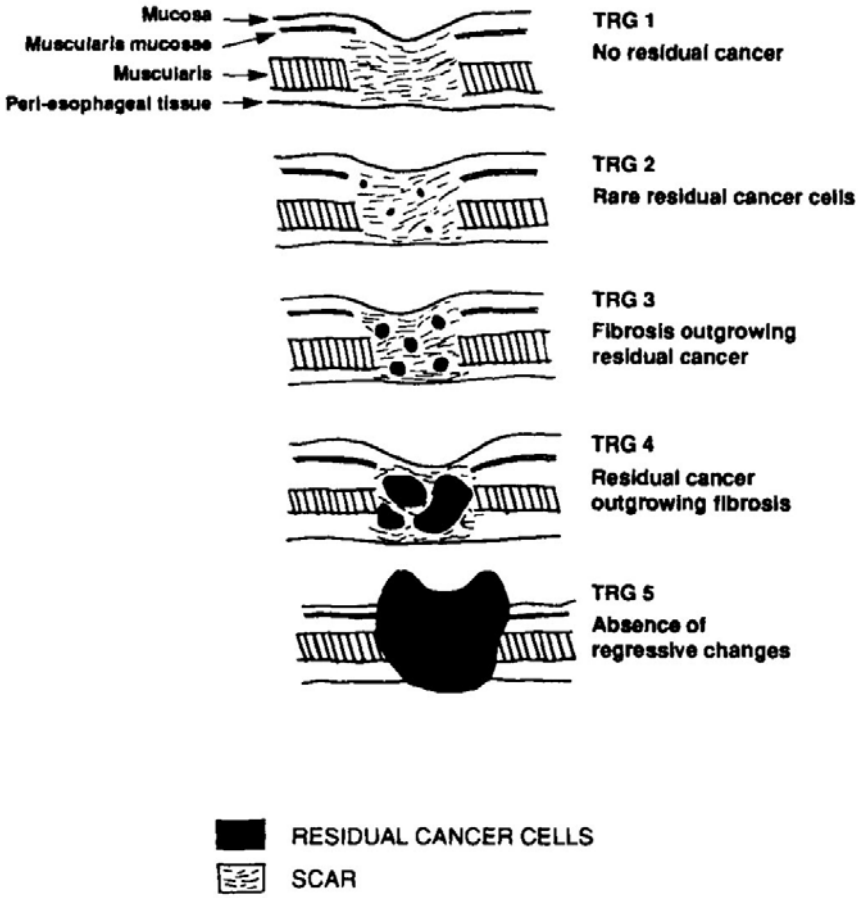
9. Measurement of Effect

9.1 Pathological Response Criteria

Resected tumors will be examined in their entirety, and regression of resected tumors was assessed by estimating the percentage of residual viable tumor of the macroscopically identifiable tumor bed, as identified on routine hematoxylin and eosin (H&E) staining. In addition, regression will be classified using the Mandard tumor regression grading system. Major pathologic response (MPR) will be defined as ≤10% of residual viable tumor cells (or ≥ 90% response), corresponding to Mandard tumor regression grade 1 (CR) or 2 (near-CR). PR will be defined as at least 50% tumor regression. However, considering the lack of consensus on the definition of PR after immunotherapy, tumors with >50% and <90% residual viable tumor will be labeled accordingly as '10–50% tumor regression', as per the NICHE study (Chalabi *et. al*., 2020).

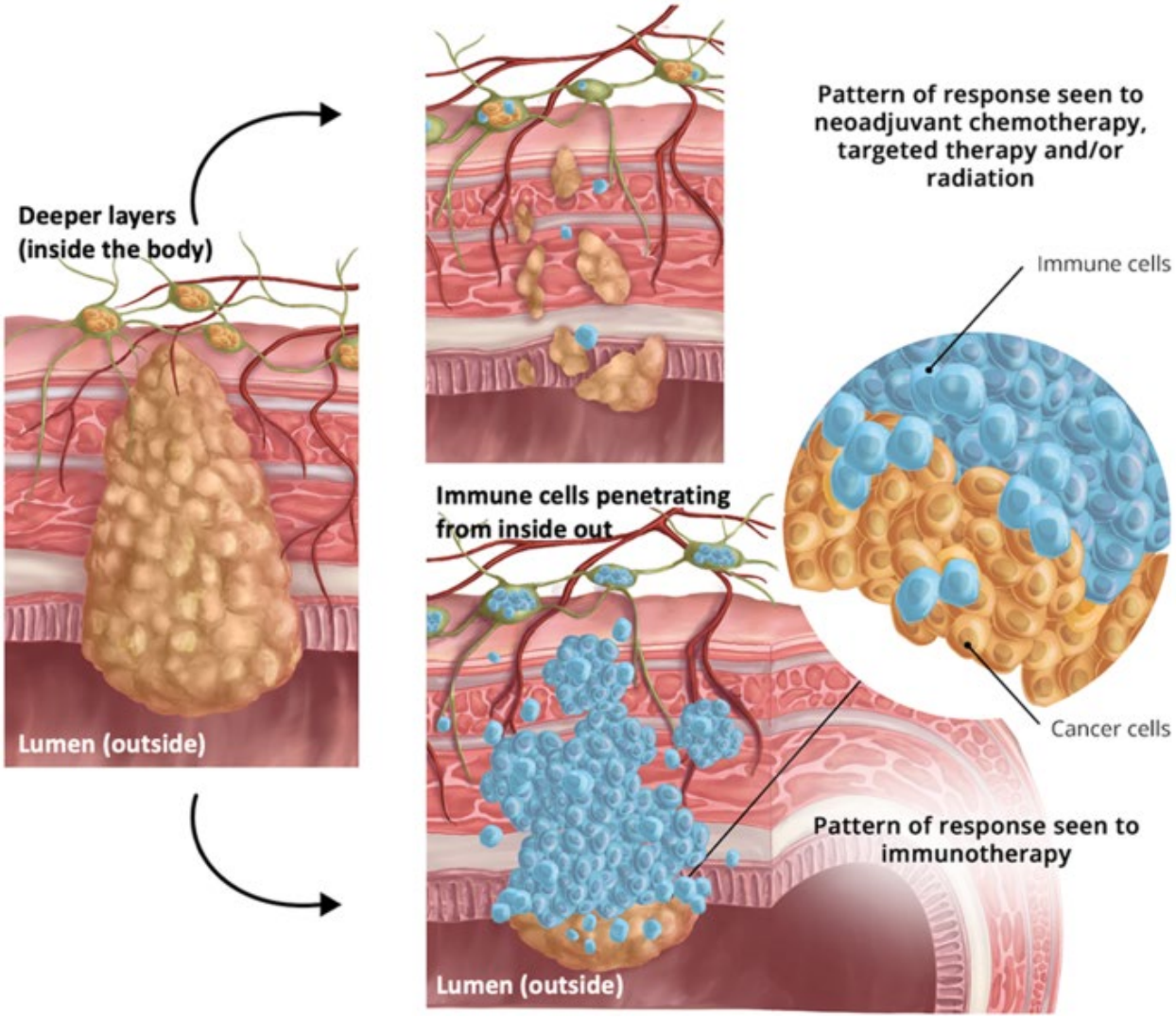
Pathological Response Category	**Definition**
Major pathological response (MPR; CR + near-CR)	≤10% of residual viable tumor cells (or ≥ 90% response)
Partial Response (PR) 50-90% tumor regression	Tumors with more than 10%, but less than 50% residual viable tumor
10–50% tumor regression	Tumors with >50% and <90% residual viable tumor
No response (≤10% tumor regression)	Greater than or equal to 90% residual viable tumor cells

Figure 9: Illustration showing assessment of pathological overall response on the surgical specimen



Additionally based on our own findings from Cohort A, we will also describe the pattern of immune response and location of residual cells, since we noted a novel and unique pattern of how the immune cells were wiping out the cancer as noted in Figure 10 below:

Figure 10: 'Inside-out' (serosa-to-mucosa) regression pattern of response seen in patients with colon and rectal cancer receiving botensilimab plus balstilimab in the neoadjuvant setting.



9.2 Cohort C Composite Clinical Complete or Major Pathological Response

Cohort C consists of only MSI-high/dMMR patients. There is reason to believe that as a whole, this patient population may have overall better responses than the MSS population. In this cohort we propose an organ-sparing strategy. If the patient is maintaining a sustained clinical response to bot/bal as determined by clinical, radiographic, endoscopic, and/or blood-based biomarker assessments, the treating surgeon/oncologist and patient may choose to either still elect for surgery or opt for a "watch and wait" (Watch-&-Wait – W&W)

approach. For participants who elect to undergo surgery, pathological response will be determined as described in Section 9.1. The key endpoint here will be "Major pathological response (MPR; CR + near-CR) as noted in Section 9.1. Patients who opt for non-operative management based on clinical response will be considered to have CR. Unfortunately, as opposed to rectal cancer where there are clear guidelines on what complete response means as far as endoscopic and radiographic assessment, there are not clear guidelines in colon cancer. Radiographic presence of disease also overestimates the presence of any residual tumor. Therefore, complete response will be determined by the treating physician and surgeon based on composite subjective and objective assessments of clinical, radiographic, endoscopic, blood-based biomarker assessments, and/or the absence of clinical and radiographic progression. Not all aforementioned criteria must be met to determine CR due to discrepancies between these findings and pathological response. If the plan is for W&W approach, patients will be seen once a month (+/- 1 week window) for a total of 6 months from the time of initiation of therapy after which they will have standard of care follow ups every 3-6 months as per treating physician for a maximum of 2 years from the last dose of investigational product.

9.3 ctDNA assessment

ctDNA assessment would be done using inhouse/available commercial platforms as outlined in the lab manual.

10. Data Reporting / Regulatory Considerations

10.1 Data Collection

The data collection plan for this study is to utilize REDCap to capture all treatment, toxicity, efficacy, and adverse event data for all enrolled subjects.

10.1.1 REDCap Cloud

REDCap Cloud supported by Weill Cornell CTSC, is a secure web-based commercial grade electronic data capture (EDC) and management system that is 21 CFR Part 11, HIPAA, and FISMA compliant. It is also HITRUST and ISO27017 27018 certified. The REDCap Cloud platform extends the features of academic REDCap that are suitable for building and managing projects ranging from online surveys and databases through Phase III clinical trials.

10.2 Regulatory Considerations

10.2.1 Institutional Review Board/Ethics Committee Approval

As required by local regulations, the Investigator will ensure all legal aspects are covered, and approval of the appropriate regulatory bodies obtained before study initiation.

Before initiation of the study at each study center, the protocol, the ICF, other written material given to the patients, and any other relevant study documentation will be submitted to the appropriate Ethics Committee. Written approval of the study and all relevant study information must be obtained before the study center can be initiated or the IP is released to the Investigator. Any necessary extensions or renewals of IRB approval must be obtained for changes to the study, such as amendments to the protocol, the ICF,

or other study documentation. The written approval of the IRB together with the approved ICF must be filed in the study files.

The Investigator will report promptly to the IRB any new information that may adversely affect the safety of the patients or the conduct of the study. The Investigator will submit written summaries of the study status to the IRB as required. On completion of the study, the IRB will be notified that the study has ended.

All agreed protocol amendments will be clearly recorded on a protocol amendment form and will be signed and dated by the original protocol approving signatories. All protocol amendments will be submitted to the relevant institutional IRB for approval before implementation, as required by local regulations. The only exception will be when the amendment is necessary to eliminate an immediate hazard to the trial participants. In this case, the necessary action will be taken first, with the relevant protocol amendment following shortly thereafter.

Once protocol amendments or consent form modifications are implemented at the lead site, Weill Cornell Medicine, updated documents will be provided to participating sites, as applicable. Weill Cornell Medicine must approve all consent form changes prior to local IRB submission.

Relevant study documentation will be submitted to the regulatory authorities of the participating countries, according to local/national requirements, for review and approval before the beginning of the study. On completion of the study, the regulatory authorities will be notified that the study has ended.

All protocol deviations will be recorded on the Protocol Deviation log. Protocol deviations will be reported to the IRB per the following guidelines:

http://www.brany.com/wp-content/uploads/2018/07/Information-Sheet-for-Researchers-%E2%80%93-Reportable-Events.pdf

10.2.2 Ethical Conduct of the Study

The Investigators and all parties involved should conduct this study in adherence to the ethical principles based on the Declaration of Helsinki, GCP, ICH guidelines and the applicable national and local laws and regulatory requirements.

This study will be conducted under a protocol reviewed and approved by the applicable ethics committees and investigations will be undertaken by scientifically and medically qualified persons, where the benefits of the study are in proportion to the risks.

10.2.3 Informed Consent

The investigator or qualified designee must obtain documented consent in accordance with ICH-GCP and local regulations, as applicable, from each potential subject or each subject's legally authorized representative prior to participating in the research study. Subjects who agree to participate will sign the approved informed consent form and will be provided a copy of the signed document.

The initial ICF, any subsequent revised written ICF and any written information provided to the subject must approved by IRB prior to use. The ICF will adhere to IRB requirements,

applicable laws and regulations.

10.2.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor-Investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.2.5 Record Retention

Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. After completion of the study, all documents and data relating to the study will be kept in an orderly manner by the Investigator in a secure study file. Essential documents should be retained for 2 years after the final marketing approval in an ICH region or for at least 2 years since the discontinuation of clinical development of the investigational product. In addition, all participants medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

11 Statistical Considerations

11.1 Study Design/Endpoints

This is a phase-2 study with an initial planned treatment of n=12 patients on this novel bot/bal immunotherapy regimen to study anti-tumor effects and serial ctDNA assessments alongside feasibility of this approach.

11.2 Sample Size/Accrual Rate

Cohort A
This cohort will enroll up to 12 subjects, with 15 subjects expected to be screened for eligibility.

Based on previous studies, the null hypothesis is that the probability of achieving a pathologic overall response (pOR) to treatment is ≤ 5% versus the alternative, ≥ 35% requires a total sample size of 12 patients (including a minimum of 3 dMMR patients) with 90% power at a one-sided significance level of 0.10. We estimate a high retention rate given that the primary endpoint data collection will occur at approximately one-month post-treatment initiation (see Section 6.1).

No interim analysis is planned from an efficacy perspective, but from a safety perspective, please see Section 14.1.

As noted, cohort A has successfully completed enrollment with very promising results. Based on these, following Cohorts B and C would be enrolled in this protocol.

Cohort B:
This cohort will enroll up to 12 subjects. In contrast with cohort A, cohort B is restricting enrollment to pMMR/MSS patients only. Participants who receive at least 1 dose of BOT/BAL will be considered evaluable.

Given updated data from 2 meta-analyses published of neoadjuvant chemotherapy trials, up to 25% pathologic responses is the benchmark (Aliseda et. al., 2024; Davey et al., 2023). Cohort B will enroll 12 subjects. Cohort A also enrolled 9 MSS participants who will be included in the overall analysis as they received at least 1 dose of bot/bal. Therefore, we expect a total of 77 evaluable patients to assess pathological response. A sample size of 77 achieves 84.1% power to detect a difference (P1-P0) of 0.15 using a one-sided exact test with a target significance level of 0.05. The actual significance level achieved by this test is 0.031. These results assume that the population proportion under the null hypothesis (P0) is 0.25. This would translate into a clinically meaningful improvement of pathologic overall responses from 25% with chemotherapy to at least 40% with the novel immunotherapy combination. Sub-analysis may be performed based on number of doses of bal and time to surgery.

No interim analysis is planned from an efficacy perspective, but from a safety perspective, please see Section 14.1.

Cohort C:
For cohort C, due to the unprecedented activity of immunotherapy for the patients with dMMR/MSI-High colorectal cancers, this cohort will enroll an additional 12 subjects with a similar 15 subjects expected to be screened for eligibility. Note for the efficacy analyses using a composite of clinical complete or major pathologic response as noted in section 9.1, the null hypothesis is that the probability of achieving a pathologic overall response (pOR) to treatment is ≤ 80% versus the alternative = 100% requires a total sample size of 15 patients with 90% power at a one-sided significance level of 0.10. No interim analysis is planned from an efficacy perspective. From a safety perspective, all SAEs would be reported for this cohort. However, there are no DLTs evaluable specifically in terms of delay in the planned surgery since for those undergoing a watch-&-wait (W&W) approach surgery is optional.

11.3 Stratification Factors

Participants will not be stratified, but we will include descriptive analyses for dMMR/MSI-H versus pMMR/MSS patients.

11.4 Analysis of Endpoints

11.4.1 Analysis of Primary Endpoints

The primary endpoints include a) the proportion of patients who achieve a pOR (or in the case of Cohort C composite clinical response rate); 2) the rate of AEs; and 3) the proportion of patients where the treatment is deemed infeasible. These endpoints will be summarized as using counts and proportions. A corresponding two-sided exact 95% confidence interval (CI) by the Clopper-Pearson method will also be calculated.

The efficacy analysis will be carried out following a modified intention-to-treat principle in

all treated patients with pathologic response assessment.

Safety and feasibility will be assessed by any delay in surgery of over 12 weeks from treatment initiation (Day 0) due to any treatment related AE/SAEs. If greater than 2 out of the first 6 patients, or greater than 4 out of the total 12 patients, have treatment related delays in surgery greater than 12 weeks from treatment initiation (Day 0), then this combination/dosages of botensilimab and balstilimab will not be considered safe or feasible in this population. Please note, delays due to the ongoing COVID-19 pandemic will not contribute to this endpoint analysis.

For cohort B, this would be a delay of over 16 weeks since there is a planned 4-week delay to give immunotherapy more time to work for anyone undergoing a planned surgery.

For cohort C, this would be a delay of over 16 weeks again since there is a planned 4-week delay to give immunotherapy more time to work for anyone undergoing a planned surgery. For those undergoing a watch-&-wait (W&W) approach, this would not be applicable since surgery is optional.

11.4.2 Analysis of Secondary Endpoints

For continuous biomarker measurements obtained at various time points and difference in biomarker values between time points, summary statistics including mean, standard deviation, median, and range will be provided. Linear mixed-effects models will be used to model longitudinal biomarker values. Simultaneous testing of general linear hypotheses will be used to evaluate contrasts of interest. Safety and feasibility will be summarized in terms of counts and proportions with the exact 95% CI as appropriate. Descriptive summary statistics will also be generated separately for dMMR and pMMR patients.

11.5 Interim Analysis

Not applicable

11.6 Reporting and Exclusions

11.6.1 Evaluation of Toxicity

All subjects will be evaluable for toxicity from the time of their first treatment with balstilimab or botensilimab.

11.6.2 Evaluation of Response

All subjects included in the study will be assessed for response to treatment if they have received a single dose of botensilimab, both doses of balstilimab, and surgical resection with tissue collection.

12. Adverse Event Reporting Requirements

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The

investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safe use of the drug or device under investigation. Safety will be monitored by evaluation of adverse events reported by subjects or observed by investigators or research staff, as well as by other investigations such as clinical laboratory tests, x-rays, electrocardiographs, etc.

12.1 Adverse Event Definition

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

12.1.1 Investigational Agent Risks (Expected Adverse Events)

Table 8: SARs for Balstilimab Considered Expected for Safety Reporting Purposes

			Number of Patients Exposed (N = 595)		
System Organ Class	**Preferred Term**	**Frequency Category**	**All SARs n (%)**	**Life-Threatening SARs n (%)**	**Fatal SARs n (%)**
Gastrointestinal disorders	Immune-mediated enterocolitis[a]	Common	21 (3.5)	1 (0.2)	0
	Diarrhoea	Common	11 (1.8)	0	0
	Colitis	Uncommon	7 (1.2)	0	0
Respiratory, thoracic and mediastinal disorders	Pneumonitis	Common	5 (0.8)	0	1 (0.2)
	Immune-mediated lung disease[b]	Uncommon	2 (0.3)	0	0

Renal and urinary disorders	Immune-mediated nephritis	Uncommon	3 (0.5)	0	1 (0.2)
	Nephritis	Uncommon	0	0	0
Endocrine disorders	Adrenal insufficiency	Uncommon	3 (0.5)	0	0
	Immune-mediated adrenal insufficiency	Uncommon	0	0	0
	Hypophysitis	Uncommon	1 (0.2)	0	0
	Immune-mediated hypophysitis	Uncommon	1 (0.2)	0	0

Abbreviations: SAR: serious adverse reaction.
Events including diarrhoea, colitis, pneumonitis, nephritis, adrenal insufficiency, and hypophysitis are only considered expected if assessed as related to balstilimab or immune related.
All life-threatening and fatal events will be considered unexpected for safety reporting.
Note: N is the number of patients exposed to balstilimab in Agenus-sponsored studies (593) plus 2 patients from the Investigator-sponsored study who experienced a SAR; n is the number of patients who experienced a SAR.
Data presented are from the safety database as of the data cutoff date of 21 December 2021.
[a] Two events of immune-mediated enterocolitis are from the Investigator-sponsored study. Other data from this study are not included in this report.
[b] Only events with lower-level term of immune-mediated pneumonitis will be considered expected.

Additionally, arthralgia is recognized as a risk of balstilimab. Utilizing a Standardized MedDRA Query (SMQ), 56 TEAEs were identified as having been assessed as related to balstilimab, including 45 events of arthralgia and 11 additional adverse drug reactions (ADRs) from SMQ Arthritis (3 ADRs of immune-mediated arthritis, 2 ADRs of arthritis, and 1 ADR each of polyarthritis, musculoskeletal stiffness, joint swelling, joint stiffness, joint range of motion decreased, and neck pain). All 56 ADRs were nonserious and mild to moderate in severity (42 Grade 1 events and 14 Grade 2 events).

Due to the potential of monoclonal antibodies to cause immune-mediated reactions, and as arthralgia is not a symptom of underlying malignancies, arthralgia has been identified as a non-important, identified risk for balstilimab. A potential strategy for mitigation of this risk includes symptomatic treatment and medical management for patients who develop symptoms following administration of balstilimab.

Table 9: Serious Adverse Reactions for Botensilimab Considered Expected for Safety Reporting Purposes

			Number of patients exposed (N) = 143		
System Organ Class	**Preferred Term[a]**	**Frequency Category**	**All SARs n (%)**	**Fatal SARs n (%)**	**Life-Threatening SARS n (%)**
Metabolism and Nutrition disorders	Dehydration[b]	Common	3 (2.1)	0	0
	Colitis	Common	4 (2.8)	0	0

Gastrointestinal disorders	Diarrhoea	Common	6 (4.2)	0	0
	Immune-mediated enterocolitis	Common	16 (11.2)	1 (0.7)	1 (0.7)

Abbreviations: IMP: investigational medicinal product; SAR: serious adverse reaction.
[a] Lowest Level Terms within the preferred term are considered expected.
[b] Dehydration is considered expected only if it is associated with another SAR. Note: n = number of patients who have experienced the SAR.
Data presented from the safety database as of data cutoff date of 13 December 2021.

12.1.2 Adverse Event Characteristics and Related Attributions

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

12.1.3 Recording of Adverse Events

All adverse events will be captured from signing of the informed consent form through 90 days following the last treatment with balstilimab or botensilimab and recorded on a subject specific AE log. The AE log will be maintained by the research staff and kept in the subject's research chart.

12.1.4 Reporting of AEs to an IRB

All AEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link:

http://www.brany.com/wp-content/uploads/2018/07/Information-Sheet-for-Researchers-%E2%80%93-Reportable-Events.pdf

12.1.5 Reporting Events to Participants

Any events that cause a change in anticipated risks to participants will result in an ICF change, which will be provided to participants.

12.1.6 Events of Special Interest

An AESI is one of scientific and medical interest specific to understanding of the investigational product and may require close monitoring and rapid communication by the investigator to Agenus Inc. An AESI may be serious or nonserious. The rapid reporting of AESIs allows ongoing surveillance of these events in to characterize and

understand them in association with the use of this investigational product.

The following types of AEs are considered AESIs:

- IRRs
- Hypersensitivity/anaphylactic reactions
- irAEs, including but not limited to the following
    - Immune-mediated pneumonitis
    - Immune-mediated nephritis
    - Immune-mediated colitis
    - Immune-mediated hepatitis
    - Immune-mediated adrenal insufficiency
- Abnormal hepatic function meeting Hy's Law Criteria

IRRs and hypersensitivity/anaphylactic reactions with a different underlying pharmacological etiology are considered AESIs. Anaphylaxis and IRRs have some common manifestations and may be difficult to distinguish from each other. IRRs are commonly observed during or shortly after the first time of exposure to therapeutic mAbs delivered through IV infusion. These reactions are less common following subsequent exposures. Unlike IRRs, anaphylaxis is a rare allergic mediated event, usually occurring after subsequent exposure to an antigen, and it is most commonly accompanied by severe systemic, skin and/or mucosal reactions. The investigator is advised to carefully examine adverse reactions observed during or shortly after drug infusion and consider the above- mentioned facts prior to making a final diagnosis.

AESIs for immune checkpoint inhibitors include, but are not limited to, events with a potential inflammatory or immune-mediated mechanism and which may require more frequent monitoring and/or interventions such as steroids, immunosuppressants, and/or hormone replacement therapy. An irAE is defined as an AE that is associated with drug exposure and is consistent with an immune-related mechanism of action and where there is no clear alternate etiology. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an irAE diagnosis. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the irAE. Some potential irAEs include colitis, hepatitis, pneumonitis, nephritis, and adrenal insufficiency. Please refer to Appendix B for further instructions on managing irAEs.

Cases where a patient shows elevations in liver biochemistry may require further evaluation and may need to be reported as SAEs. The criteria for a potential Hy's Law case are AST or ALT ≥3X ULN together with TBL ≥2X ULN at any point during the study following the start of study medication irrespective of an increase in ALP.

12.1.7 Reporting of Pregnancy

Only pregnancies considered by the Investigator as related to study drug (e.g., resulting

from a drug interaction with a contraceptive medication) are considered as AEs. Investigators must actively follow up, document, and report the outcome of these pregnancies, even if subjects are withdrawn from the trial.

All pregnancies need to be reported to Agenus Inc. within 24 hours of awareness. The Pregnancy Report Form or relevant form should be completed and faxed directly to Agenus Inc. at 1-781-674-4261 or emailed to Adverse.Events@Agenusbio.com.

Any abnormal outcome must be reported as an SAE in an expedited manner, as described in Section 12.2, whereas normal outcomes must be reported within 45 days from delivery. If a participant's partner becomes pregnant during the trial, consent should be obtained from the pregnant partner to follow the outcome of their pregnancy.

In the event of a pregnancy in a subject occurring during the trial, the subject must be discontinued from trial medication immediately.

12.2 Definition of SAEs

SAEs include death, life threatening adverse experiences, hospitalization or prolongation of hospitalization, disability or incapacitation, overdose, congenital anomalies and any other important medical events that may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed in this definition.

12.2.1 Reporting of SAEs to an IRB

All SAEs occurring on this study will be reported to the study-affiliated IRB according to IRB policy accessible via:

https://www.brany.com/wp-content/uploads/2021/05/2022-Feb-Information-Sheet-for-Researchers-Reportable-Events-20211216.pdf

12.2.2 Reporting of SAE to FDA

IND application sponsor must report any suspected adverse reaction to study treatment that is both serious and unexpected. Unexpected fatal or life-threatening suspected adverse reactions represent especially important safety information and must be reported to FDA as soon as possible but no later than **7 calendar days** following the sponsor's initial receipt of the information. All other serious unexpected suspected adverse reactions must be reported to FDA within 15 calendar days following sponsor's initial receipt of the information.

i. death,
ii. a life-threatening adverse event,
iii. in-patient hospitalization or prolongation of existing hospitalization,
iv. a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
v. a congenital anomaly or birth defect, or
vi. important medical events

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or research subject and may require medical or surgical intervention to prevent one of the outcomes listed as serious.

Submit SAE Reports to:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biologic Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

12.2.3 Reporting of SAE to Agenus Inc.

Institution will send Agenus Inc. copies of any and all SAEs either related or unrelated to suspect product and reports filed with the FDA or other applicable regulatory authorities, as well as copies of any correspondence with the FDA or other applicable regulatory authorities, regarding any and all SAEs/AESIs, irrespective of causal association with the Study Drug(s) from signing of informed consent through 90 days following the last treatment with balstilimab or botensilimab, within 1 business days of such report or correspondence being sent to the FDA or other applicable regulatory authorities. Copies should be faxed directly to Agenus Inc. at 1-781-674-4261 or emailed to Adverse.Events@Agenusbio.com.

12.3 AE/SAE Follow Up

All SAEs and AEs reported during this study will be followed until resolution or until the investigator confirms that the AE/SAE has stabilized, and no more follow-up is required. This requirement indicates that follow-up may be required for some events after the subject discontinues participation from the study. Any SAEs which are suspected to be related to product after the end of reporting period should be sent to Agenus Inc.

12.4 Time Period and Frequency for Event Assessment and Follow Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, via review of medical records, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate eCRF. Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Research staff will record all reportable events with start dates occurring any time after informed consent is obtained until 90 days following the last treatment with balstilimab or botensilimab. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization. If at any point the investigator becomes aware of any AEs/SAEs that they would consider possibly, probably, or definitely related to the investigational product, Agenus Inc should be notified.

13. Unanticipated Problems Involving Risks to Subjects or Others

13.1 Definition of Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSO)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

13.1.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPIRTSOs) to the reviewing Institutional Review Board (IRB). The UPIRTSO report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UPIRTSO;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UPIRTSO.

To satisfy the requirement for prompt reporting, UPIRTSOs will be reported using the following timeline:

- UPIRTSOs will be reported to the IRB within 5 days of the investigator becoming aware of the event.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), Food and Drug Administration (FDA), and the Office for Human Research Protections (OHRP) within 1 month of the IRB's receipt of the report of the problem from the investigator.

14. Data and Safety Monitoring Plan (DSMP)

This study will utilize the WCM Institutional Data Safety Monitoring Committee (DSMC) for safety monitoring and follow its policies and procedures. All patients who receive at least one dose of balstilimab and botensilimab will be considered evaluable for safety parameters. AEs will be collected and documented at every visit. SAEs will be documented and immediately reported to all concerned authorities as per institutional and sponsor guidelines. This study will be conducted in accordance with the guidelines of the 2001 NCI approved DSMP for the WCM Cancer Institute. Reports to the DSMC will include the following information: accruals, targets, responses, AEs and evidence of reporting to appropriate review committees.

At the time of protocol initiation, the WCM DSMC will review the IRB approved protocol, the DSMP and any stopping guidelines. A WCM DSMC periodic report will be submitted after enrollment of the first 6 participants and then every six months following the first DSMC Periodic Report submission. The WCM DSMC may also convene as needed if stopping criteria are met or other safety issues arise from communications of the PI and/or IRB.

14.1 Stopping Rules

- If there are ≥ 2 patients out of the first 6 patients, or ≥ 4 patients out of the full 12 participants (≥33%), with AEs/SAEs that lead to a delay in surgery beyond 12 weeks from treatment initiation, enrollment will be stopped and this combination and dosages of botensilimab and balstilimab will not be considered safe or feasible in this population (primary endpoints)
    - Please note, any COVID-related procedural delays (i.e. if a patient tests positive for COVID during pre-operative testing causing surgery to be delayed) will not contribute to this stopping rule.
- Similarly, if there are ≥4 of the full 12 participants' experience Grade 3+ AEs that are classified as possibly, potentially, or definitely related to either investigational product, then enrollment will be stopped.
- Similar rules apply to Cohort B except for the cutoff for delay in surgery is beyond 16 weeks from treatment initiation since there is a planned 4-week delay to give immunotherapy more time to work. The enrollment would be stopped to reassess the treatment plan and supportive care strategies to mitigate the side effects of immunotherapeutic agents.
- The stopping rules in terms of delay for surgery do not apply for Cohort C since there is no planned surgery. However, if there are ≥5 of the full 15 participants' experience Grade 3+ TRAEs, the enrollment would be stopped to reassess the treatment plan and supportive care strategies to mitigate the side effects of immunotherapeutic agents.

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Appendix A: Management Guidelines for Botensilimab and Balstilimab Infusion–Related Reactions

CTCAE Grade	Treatment	Premedication At Subsequent Dose Administration
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the Investigator	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hours	Stop infusion and monitor symptoms Additional appropriate medical therapy may include but is not limited to the following: – IV fluids – Antihistamines – NSAIDS – Acetaminophen – Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the Investigator If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate. Otherwise, dose administration will be held until symptoms resolve, and the subject should be premedicated for the next scheduled dose Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug administration	Subject may be premedicated 1.5 h (± 30 minutes) prior to infusion with the following: Diphenhydramine 50 mg PO (or equivalent dose of antihistamine) Acetaminophen 500-1000 mg PO (or equivalent dose of antipyretic)
Grade 3-4 *Grade 3* Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) *Grade 4* Life-threatening; pressor or ventilatory support indicated	Stop infusion Additional appropriate medical therapy may include but is not limited to: – IV fluids – Antihistamines – NSAIDs – Acetaminophen – Narcotics – Oxygen – Pressor – Corticosteroids – Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the Investigator Hospitalization may be indicated Subject is permanently discontinued from further study drug administration	No subsequent dosing

Appendix B: Toxicity Management Guidelines for irAEs

General instructions:

1. Corticosteroid tapers should be initiated upon irAE improving to Grade 1. A typical taper duration is approximately 4 to 6 weeks, but the taper should be completed more quickly when clinically feasible. If additional immunosuppressive therapy (e.g., infliximab, vedolizumab) has been used, the taper duration should be shorter (< 2 to 4 weeks).
2. For situations where AGEN1181/balstilimab have been held, the agent(s) may only be resumed if 1) criteria for permanent discontinuation are not met; 2) irAE has reduced to Grade 1 or resolved; and, if applicable, 3) corticosteroids have been tapered to ≤ 10 mg prednisone or equivalent per day.
3. AGEN1181/balstilimab should be permanently discontinued if irAE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks. Note additional criteria in the table below for permanent discontinuation.
4. For severe and life-threatening irAEs, IV corticosteroids are preferred to oral.
5. For categories of irAE not listed below, follow standard of care society guidelines (e.g., ASCO, SITC, NCCN).
6. For any Grade 3 to 4 irAE where permanent discontinuation is not mandated below, discussion with Sponsor must occur prior to resuming study treatment.
7. When infliximab is used in patients who may resume AGEN1181 consider administering three doses according to the prescribing information at 0, 2, and 6 weeks (Remicade (infliximab) Package Insert 2021) and continuing with maintenance infliximab.

Immune-related AEs	Toxicity Grade or Conditions (NCI CTCAEv5.0)	Action Taken with AGEN1181	irAE Management with Corticosteroid and/or Other Therapies	Monitor and Follow-up
Diarrhea/colitis	Grade 1-2	Withhold (only resume following discussion with Sponsor).	Consider treatment with infliximab (5-10 mg/kg) as initial therapy once non-immune causes of diarrhea/colitis have been reasonably excluded. If infliximab is not given initially or symptoms do not improve within 24-48 hours post-infliximab treatment, administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper. If corticosteroids alone are given initially (dose of 1-2 mg/kg prednisone or equivalent), then treatment with infliximab (5-10 mg/kg) is recommended if symptom improvement is not noted after 3 days on corticosteroids. If symptoms persist following treatment with infliximab and steroids,	Notify Sponsor (Medical Monitor) within 24 hours. Monitor subjects for signs and symptoms of enterocolitis (i.e., diarrhea, abdominal pain, blood, or mucus in stool with or without fever) and of bowel perforation (i.e., peritoneal signs and ileus). Evaluate for infectious etiologies (e.g., *C difficile*). Subjects with Grade ≥ 2 diarrhea suspicious for colitis should consider GI consultation for discussion of endoscopy if the diagnosis is uncertain. Subjects with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid, electrolytes and corticosteroids should be substituted via IV infusion. Consider evaluation/trending

			recommend GI consultation and consider treatment with vedolizumab (may require dosing by level if hypoalbuminemia present) or additional immunosuppressive therapy.	with fecal calprotectin or lactoferrin.
	Grade 3-4	Permanently discontinue	Consider treatment with infliximab (5-10 mg/kg) in addition to corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent, and consider IV) as initial therapy. If corticosteroids alone are given initially (dose of 1-2 mg/kg prednisone or equivalent), then treatment with infliximab (5-10 mg/kg) is recommended if symptom improvement is not noted after 3 days on corticosteroids. If symptoms persist following treatment with infliximab and steroids, recommend GI consultation and consider treatment with vedolizumab (may require dosing by level if hypoalbuminemia present) or additional immunosuppressive therapy.	
Maculopapular rash	Grade 1-2	Continue	Treat with topical corticosteroids for Grade 1-2 per guidelines.	Ensure adequate evaluation to confirm etiology or exclude other causes. Perform TBSE. Consider dermatologic consultation/biopsy.
	Grade 3-4	Withhold	For Grade 3-4, administer corticosteroids and obtain dermatology consult.	
Hyperthyroidism	Grade 2	Continue	Treat with nonselective beta-blockers (e.g., propranolol) or thionamides as appropriate.	Monitor for signs and symptoms of thyroid disorders.
	Grade 3-4	Withhold		
Hypothyroidism	Grade 2-4	Continue	Initiate thyroid replacement hormones (e.g., levothyroxine or liothyronine) per SoC.	Monitor for signs and symptoms of thyroid disorders.
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of prednisone 1-2 mg/kg or equivalent) followed by taper. Consider infliximab (5 mg/kg) if symptom improvement is not noted after 3 days on high-dose IV corticosteroids.	Monitor subjects for signs and symptoms of pneumonitis. Evaluate for potential pulmonary infectious etiologies. Evaluate subjects with suspected pneumonitis with CT imaging. Add prophylactic antibiotics for opportunistic infections.

	Grade 3-4	Permanently discontinue		
AST/ALT elevation or increased bilirubin	Grade 2	Withhold	Administer corticosteroids (initial dose of prednisone 0.5-1 mg/kg or equivalent) followed by taper.	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value has returned to baseline or is stable). Evaluate for other causes, including medications, worsening liver metastases, viral infection (e.g., hepatitis panels), biliary obstruction, etc.
	Grade 3-4	Permanently discontinue	Administer corticosteroids (initial dose of prednisone 1-2 mg/kg or equivalent) followed by taper. In the case of Grade 3- 4 AE refractory to steroids, infliximab should not be given (due to potential for hepatotoxicity). MMF may be considered.	
T1DM or hyperglycemia	New onset T1DM or Grade 3-4 hyperglycemia associated with evidence of β-cell failure	Withhold	Initiate insulin replacement therapy for subjects with T1DM. Administer appropriate therapy for diabetic ketoacidosis as indicated.	Monitor subjects for hyperglycemia or other signs and symptoms of diabetes. Obtain routine lab panel for autoimmune DM evaluation. Treatment with study therapy may resume once the subject is clinically stable.
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal replacements as clinically indicated.	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency).
	Grade 3-4	Withhold		
Nephritis and renal dysfunction	Grade 2	Withhold	Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	Monitor changes of renal function Evaluate for prerenal and obstructive nephropathy with labs, urine electrolytes, renal US, etc.
	Grade 3-4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	Management per society guidelines and best SoC.	Ensure adequate evaluation to confirm etiology or exclude other causes.
	Grade 3-4	Withhold or permanently discontinue based on the type of event. Discussion with the Sponsor required prior to resuming therapy.		

Abbreviations: AE = Adverse event; ALT = alanine aminotransferase; AST = aspartate aminotransferase; β = beta; irAE = immune-related adverse event; IV = intravenous(ly); ASCO = American Society of Clinical Oncology; CT = computed topography; GI = gastrointestinal; MMF = mycophenolate mofetil; NCI CTCAE v5.0 = National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0; NCCN = National

Comprehensive Cancer Network; SITC = Standard International Trade Classification; SoC = standard of care; T1DM = type 1 diabetes mellitus; TBSE = total body skin exam; US = ultrasound.

Appendix C: Protocol Version History and Summary of Changes

Version	Section	Change	Rationale
7.0*	Full Protocol	Updated protocol version number and date throughout the document.	Administrative update to reflect the current protocol version.
7.0*	Full protocol	Removed references to the expansion of Cohort B and the addition of Cohort D throughout the protocol.	Cohort expansion and the additional cohort described in Protocol Version 4.0 were reviewed internally but were not approved for implementation at the institution and therefore were never implemented.
7.0	Section 2	Added language noting the availability of updated Investigator's Brochures for both study drugs.	To acknowledge the availability of the most recent Investigator's Brochures and ensure alignment with the updated safety information reflected in the informed consent document.
6.0	Full protocol	Version date and PI were updated throughout the protocol.	Previous PI unable to serve as PI so new PI was identified to complete the role
5.0	Full protocol	Version date and PI were updated throughout the protocol.	Previous PI left the institution and this study was transferred to a new PI.
4.0*	Full protocol	Version date was updated to April 11, 2024, throughout the text.	To reflect the updated protocol version and version date
4.0*	Full protocol	Formatting and grammatical changes were made throughout the text	To ensure clarity and readability
4.0*	Table of Contents	The table of contents was updated.	To reflect accurate page numbers in the updated protocol
4.0*	Protocol Summary Full Protocol	This amendment includes the addition of a fourth cohort, Cohort D, which will specifically enroll 30 rectal patients. This amendment also expands Cohort B to accrue an additional 56 patients for a total of 68 in Cohort B.	Addition of Cohort D and the expansion of Cohort B are based on the continued efficacy and responses observed without any safety signals of patients in Cohort A, including four rectal patients.
4.0*	Section 6.1	Clarified that participants in Cohorts C and D that opt for a W&W approach will be followed in long term follow-up for a maximum of 2 years from the last dose of investigational product	To match the follow-up described for participants that receive surgery
4.0*	Section 6.1	Clarified that pre-treatment blood work may be obtained within a 3 day window prior to all treatment visits	To reduce the time burden of patients on treatment visit days
4.0*	Section 7.7.1	Updated the DLT time period from 28 days post-surgery to 28 days following the last dose of study drug	To account for if participants do not receive surgery
3.0	Full protocol	Version date was updated to September 5, 2023, throughout the text.	To reflect the updated protocol version and version date
3.0	Full protocol	Formatting and grammatical changes were made throughout the text	To ensure clarity and readability
3.0	Title Page Protocol Summary	Added NewYork-Presbyterian Brooklyn Methodist Hospital (BMH) and NewYork-Presbyterian Queens (NYPQ) as participating	BMH and NYPQ are network locations within WCM Meyer Cancer Center. By adding these

		locations.	locations, we are able to enroll a greater number of patients within our catchment area, including patients from the boroughs Brooklyn and Queens.
3.0	Table of Contents	The table of contents was updated.	To reflect accurate page numbers in the updated protocol
3.0	Protocol Summary Full Protocol	This amendment includes the addition of two additional cohorts to this protocol, in addition to the original 12 participants (now referred to as Cohort A). Cohort B and C will receive 1 dose of the CTLA-4 inhibitor, botensilimab, and 4 doses of the PD-1 inhibitor, balstilimab. Cohort C will only enroll patients considered dMMR/MSI-High. Cohort B and C will each enroll an additional 12 participants for a total of 36 participants.	At the time of this amendment, the first 12 participants have completed surgery with promising preliminary efficacy results and no major safety concerns. Therefore, enrollment is being expanded. Further rationale is available in Sections 2.1 and Section 3.2.
3.0	Section 1.2 Section 1.2.1 Section 1.2.2	Updated the primary and secondary objectives and endpoints from bullet format to a table format, and included which endpoints correspond to the appropriate Cohort. The primary endpoint for Cohort C was added.	Table format improves clarity and readability. While Cohort B has the same primary efficacy endpoint as Cohort A based on pOR, Cohort C does not require surgery and therefore has a different primary efficacy endpoint that is a composite of pathological complete response and clinical complete response.
3.0	Section 2.1	Updated background with preliminary data from the first 12 participants enrolled	To provide rationale for this amendment to add additional cohorts based on preliminary efficacy and safety data
3.0	Section 2.2	Updated background on balstilimab and botensilimab based on current IBs with data cutoffs of December 2022.	Updated IBs have been released with updated clinical data based on ongoing clinical trials.
3.0	Section 2.4.1.1 Section 2.4.1.2	Updated risks of balstilimab and botensilimab based on current IBs (balstilimab IB version 8.1 and botensilimab IB version 5.0).	Updated IBs have been released with updated risk frequencies.
3.0	Section 3.1	The study design description has been updated to include descriptions of newly added Cohorts B and C.	To reflect the addition of two additional enrollment cohorts.
3.0	Section 3.2	Updated the scientific rationale for study design to include additional rationale for Cohorts B and C	To provide rationale for this amendment to add additional cohorts
	Section 4.2 Section 4.2.1	Updated 3rd inclusion criteria to say "Histologically, cytologically, or clinically confirmed adenocarcinoma of the colon *or rectal cancer as long as there are no plans for neoadjuvant radiation for the patients with rectal cancer. Note: patients can enroll in cohort B while awaiting mismatch repair testing results. If noted to be dMMR/MSI-High, they would be still considered evaluable and moved to cohort C.*" Section 4.2.1 was added to specify additional	In order to clarify that patients with rectal cancer are eligible; that Cohort C must be dMMR/MSI-High; and that availability of mismatch repair testing results are not required prior to enrollment,

		inclusion criteria for Cohort C, which requires participants to be dMMR/MSI-High.	
3.0	Section 4.4	Updated the contraception requirements to specify that "participants of childbearing potential are required to use highly effective methods of contraception *for 120 days following the last dose of study drug*."	Previously the timeline was not clearly specified for how long participants must continue contraception after completing their last dose of study drug.
3.0	Section 6.1	Added Schedule of Assessments (SoA) Table for Cohorts B and C. LDH and phosphorus were removed from screening labs. The subsections outlining visit assessments in bulleted form was removed	The addition of schedule of assessments tables was based on addition of two additional enrollment cohorts, which each include additional doses of balstilimab. LDH and phosphorus were removed to prevent delays in enrollment as they are additional labs that are not always drawn as standard practice. Subsections that reiterate the assessments to be performed at each visit were removed to avoid redundancy and potential for discrepancies with the SoA tables. Any additional information from those sections were added to the table footnotes.
3.0	Section 7.1	Updated to include a description of the dosing and timing of botensilimab and balstilimab in Cohort B and C.	Updated based on addition of two additional enrollment cohorts
3.0	Section 9.1	Added PR row to the table describing classification of pathological response.	The table now matches the description in the text. The previous omission of a row for PR was an error.
3.0	Section 9.1	The following sentence was also added to the description of pathological overall response: "Additionally based on our own findings from Cohort A, we will also describe the pattern of immune response and location of residual cells, since we noted a novel and unique pattern of how the immune cells were wiping out the cancer as noted in Figure 8 below" along with Figure 8.	This was added based on preliminary results from the first 12 participants.
3.0	Section 9.2	Added additional section titled "Cohort C Composite Clinical Complete or Major Pathological Response".	This section describes how the primary efficacy endpoint for Cohort C will be measured.
3.0	Section 10.1 Section 10.1.1	Electronic Data Capture system description updated to reflect the use of REDCap Cloud, as opposed to academic REDCap.	REDCap Cloud is being used as the EDC in this study as it is 21 CFR Part 11 compliant.
3.0	Section 11.2 Section 11.4.1	Statistical plan updated to include Cohort B and C with rationales for the selected sample size and description of endpoint analyses.	Updated based on addition of two additional enrollment cohorts
3.0	Section 14.1	Added the following stopping rules to the DSMP for Cohort B and C: • Similar rules apply to Cohort B except for the cutoff for delay in surgery is	The initial stopping rules only applied to the first 12 participants (Cohort A). Additional stopping rules have been added for Cohort B

		beyond 16 weeks from treatment initiation since there is a planned 4-week delay to give immunotherapy more time to work. The enrollment would be stopped to reassess the treatment plan and supportive care strategies to mitigate the side effects of immunotherapeutic agents. • The stopping rules in terms of delay for surgery do not apply for Cohort C since there is no planned surgery. However, if there are ≥5 of the full 15 participants' experience Grade 3+ TRAEs, the enrollment would be stopped to reassess the treatment plan and supportive care strategies to mitigate the side effects of immunotherapeutic agents.	and C.
3.0	References	Cerceck et. al 2022 was added to the references section of the protocol.	This article was referenced in the rationale for the addition of Cohort C
3.0	Appendix C	Added Appendix C: Protocol Version History and Summary of Changes	For organizational purposes, the protocol amendment summary of changes has been added as an appendix instead of as a separate document
2.0	Full protocol	Version date was updated to July 20, 2023, throughout the text.	To reflect the updated protocol version date
2.0	Full protocol	Formatting and grammatical changes were made throughout the text	To improve readability
2.0	Title Page	Version number and NCT number were added	Version date and NCT number were not previously present on the title page. Inclusion of the NCT number is required for eventual results posting on clinicaltrials.gov.
2.0	Table of Contents	The table of contents was updated	To reflect accurate page numbers in the updated protocol
2.0	Section 6.1	The Schedule of Events was updated to allow screening labs (CBC and serum chemistries) that were collected within 3 days of treatment visit 1 to also be used as baseline labs and not require repeating.	This prevents participants from undergoing additional blood draws that are unnecessary.
2.0	Section 6.1	Added a statement that CBCs and serum chemistries drawn at Treatment Visits 1 and 2, and biomarker blood draws at Treatment Visit 1, must be completed prior to administering investigational product	This is standard clinic practice but was not explicitly stated previously in the protocol.
2.0	Section 6.1	"Off-Study" was removed from the column describing Long-Term Follow-Up.	This is a correction because participants in Long-Term Follow-Up are off-treatment and are no longer receiving study-specific procedures, but because they are not off-study as their data is still

			being collected.
2.0	Section 6.1	The Schedule of Events table header was updated to define the short-term vs long-term follow-up periods more clearly. Additionally, assessments that may be abstracted from medical records, but are not required study procedures were removed from the checked column. A clarifying statement was added to the table footer.	To more clearly define the follow-up phase of the trial and prevent confusion about what are required study procedures and what is data that will be abstracted from the medical record as available
2.0	Section 6.1.3	The description of the study long-term follow-up phase duration was updated from "study until recurrence or death, whichever occurs first" to "until death, or two years post-surgical resection, whichever occurs first". Specifically, recurrence was removed and a maximum length of two years from surgical resection was added.	Recurrence was removed because even if a participant has a recurrence, they should still be followed for mortality and subsequent treatment data. Additionally, there was no specified maximum duration period for long-term follow-up, so a maximum period of 2 years was added.
2.0	Section 6.1.3.4	This section was updated to reflect the updated Schedule of Events for Long-Term Follow-Up.	To ensure clarity and consistency throughout the protocol
2.0	Section 14	The DSMP has been updated to clarify that a WCM DSMC periodic report will be submitted after enrollment of the first 6 participants and then every six months following the first DSMC Periodic Report submission.	Previously the DSMP stated that "A WCM DSMC data and safety analysis will be performed after enrollment of the first 6 participants and every six months following the accrual of the first study participant." The first 6 participants were enrolled before 6 months from accrual of the first participant with no safety concerns. The updated language clarifies the required submission timeline for future DSMC submissions.

*Protocol Version 4.0 (dated April 11, 2024) included expansion of Cohort B and the addition of Cohort D. Although this version was approved by the IRB, these cohort modifications were not approved for implementation following internal institutional review and were never implemented at the site. Subsequent protocol versions referenced these cohorts administratively. Protocol Version 7.0 removes these references to accurately reflect the study as conducted.