October 12, 2018

Martha Kruhm, MS RAC
Head, Protocol and Information Office
Quality Assurance Section
CTEP, DCT, NCI
6130 Executive Blvd, EPN Room 7000
Bethesda, MD 20892

Dear Ms. Kruhm:

Enclosed is Addendum #16 to EAY131-Z1D, *Molecular Analysis for Therapy Choice (MATCH): MATCH Treatment Subprotocol Z1D: Nivolumab in Patients with Tumors with Mismatch Repair Deficiency*.

The following are ECOG-ACRIN's responses to CTEP's "Review of Amendment #23 of Protocol #EAY131-Z1D" dated October 5, 2018. Please note the Principal Investigator's comment appears in bold below:

I. Comments Requiring a Response– Administrative & Editorial Issues:

	Section	Comments
1.	Appendix V	As a reminder, Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy requires all persons participating in any NCI-sponsored clinical trial to register. Our records indicate: • Evisa Gjini, PhD (A-603314)- Registration will expire on October 10, 2018 and also needs to be advanced to Associate Plus (AP) person type. • Scott Rodig, MD, PhD (IVR- needs to be claimed on the ECOG-ACRIN roster. • Mariano Severgnini, PhD (A-558565) has a suspended registration status in RCR. This needs to be renewed and advanced to Associate Plus (AP) person type. **To register, please log into the NCI Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr/ using your CTEP-IAM username and password and complete the required information.** If you need to update your **CTEP-IAM account** information, please visit: https://ctepcore.nci.nih.gov/iam . **For guidance** on registering via RCR, please visit: https://ctep.cancer.gov/investigatorResources/default.htm . If you have questions or encounter difficulties, please contact the CTEP Registration and Credential Repository (RCR) Team at RCRHelpDesk@nih.gov at any time. Thank you for your cooperation. If you have any questions, please feel free to contact the Pharmaceutical Management Branch by phone at (240) 276-

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	Section	Comments
		6575 Monday through Friday from 8:30 am to 4:30 pm Eastern Time or by email at RCRHelpDesk@nih.gov . **PI Response: Updates to registrations listed above are in progress.**

II. Recommendations:

	Section	Comments
2.		Please include the recommended changes below at the earliest opportunity. The changes are based on information from the latest nivolumab IB (version 17).
3.	5.1.4	Add the following after the first sentence: The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours. **PI Response: The sentence has been added as requested.**
4.	5.1.5	Replace the current section with the following: Description: Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate dihydrate, sodium chloride, mannitol, diethylenetriaminepentacetic acid (pentetic acid), polysorbate 80 (Tween® 80), **and water for injection. Dilute solutions of hydrochloric acid and/or sodium hydroxide may be used for pH adjustment (pH 5.5-6.5).** How Supplied: change "butyl" with "fluoropolymer film-laminated". **PI Response: The requested changes have been made.**
5.	5.1.6	Replace section with the following: Preparation: Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose. When the dose is based on patient weight (i.e., mg/kg), nivolumab injection can be infused undiluted or diluted to protein concentrations as low as 0.35 mg/mL. When the dose is fixed (eg, 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of **160 mL. For patients weighing less than 40 kilograms (kg), the total volume of infusion must not exceed 4 mL per kg of patient weight.** During drug product preparation and handling, vigorous mixing or shaking is to be avoided. **PI Response: The section has been replaced as requested.**
6.	5.1.7	Add "Intravenous infusion over 30 minutes." **PI Response: The first sentence has been modified appropriately.**

Please see responses to the CIRB Early Phase Emphasis Review below.

December 11, 2018

Re**: CIRB Approval Pending Modification of Amendment Review**
Study ID, Study Title: **EAY131, Molecular Analysis for Therapy Choice (MATCH)**
Protocol Version Date: October 12, 2018

Study Chair: **Keith Flaherty, M.D., B.S.**

Dear NCI Adult CIRB - Early Phase Emphasis,

Thank you for reviewing EAY131-Z1D (Protocol Version Date 10/12/18) protocol at the meeting of the NCI Adult CIRB - Early Phase Emphasis held on December 4, 2018. The CIRB determined that the regulatory and CIRB SOP requirements for approval are met, but the CIRB requests minor, directed modifications as described below.

<u>Stipulations:</u>

	Section	Comments
1.	Appendix V	Appendix VI under the Methodology section revise the sentence, "All specimens will be de-identified/coded prior to distribution to the investigator" to remove reference to de-identification as it is inaccurate. Add information regarding the code and code-key process. **<u>PI Response:</u> The term "de-identified/coded" refers to the process of stripping PHI from the samples and inventories (de-identified) and replacing with codes (coded). The key to link the codes back to the patient identifiers are held by the BioBank, the ECOG-ACRIN Operations Office and the MATCH statisticians. So there is no confusion regarding the term "de-identified", the text has been replaced with the following:** **"No patient identifiers will be provided to investigators with the samples. The trial-specific patient ID will be replaced with a unique randomized number and specimens will be labeled with a barcode not related to any PHI. The following entities have access to the key: the ECOG-ACRIN biobank, the ECOG-ACRIN Operations Office, and the MATCH statisticians."**

<u>Additional Changes by Principal Investigator:</u>

	Section	Comments
1.	ICD: Page 1	Updated version date.
2.	Protocol Cover Page	Updated version date.
3.	Appendix V	Removed Dr. Gjini as a Principal Investigator of Correlative Proposal CS-MATCH-0005.

The following revisions to EAY131-Z1D protocol have been made in this addendum:

	Section	Change
1.	Cover Page	Updated version date and title.
2.	5.1.4	Updated storage information.
3.	5.1.5	Updated dosing and supply information.
4.	5.1.6	Updated preparation information.
5.	5.1.7	Updated drug administration information.

6.	Appendix V	Added Correlative Proposal CS-MATCH-0005.

The following revisions to EAY131-Z1D Informed Consent Document have been made in this addendum:

	Section	Change
1.	Page 1	Updated version date and title.

If you have any questions regarding this addendum, please contact kpollard@ecog-acrin.org or 857-504-2900.

We request review and approval of this addendum to EAY131-Z1D so ECOG-ACRIN may activate it promptly.

Thank you.

Sincerely,

Pamela Cogliano

Senior Director of Protocol Development

Enclosure

CC:
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ECOG-ACRIN cancer research group
Reshaping the future of patient care

EAY131-Z1D

Molecular Analysis for Therapy Choice (MATCH)

Rev. Add16

MATCH Treatment Subprotocol Z1D: Nivolumab in Patients with Tumors with Mismatch Repair Deficiency

NIVOLUMAB TREATMENT SUBPROTOCOL CHAIR: Nilofer Azad, MD
NIVOLUMAB TREATMENT SUBPROTOCOL CO-CHAIR: Michael James Overman, MD
NIVOLUMAB TRANSLATIONAL CHAIR: Jonathan Schoenfeld, MD

Version Date: December 11, 2018

NOTE: This subprotocol (EAY131-Z1D) should be used in conjunction with the MATCH Master Protocol (EAY131).

Rev. Add13

NOTE: As of 11/17, all protocol changes will be noted by addendum number. Please reference the activation memo for the addendum activation date.

SUBPROTOCOL ACTIVATION DATE

May 31, 2016 (Incorporated in Addendum #3)
Addendum #4 – 7/16
Addendum #5 – 12/16
Addendum #6 – 1/17
Addendum #7 – 3/17
Addendum #13
Addendum #15
Addendum #16

Agent	IND#	NSC#	Supply
Nivolumab			NCI Supplied

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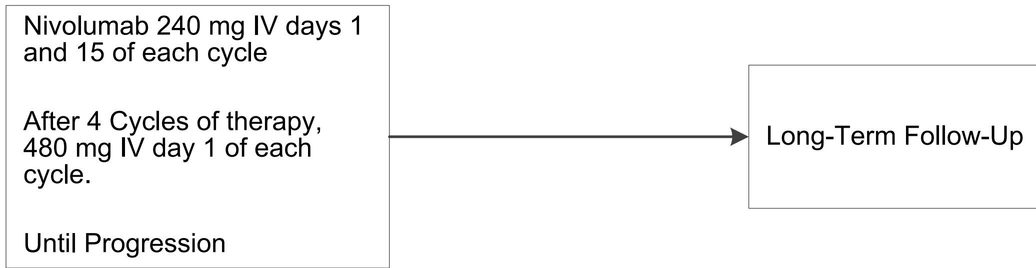
Rev. 3/17

Schema



Cycle = 28 days
Accrual Goal: 70

1. Introduction

1.1 Nivolumab (BMS-936558)

Nivolumab (also known as BMS-936558 or anti-PD-1) is a fully human, IgG4 (kappa) isotype monoclonal antibody (mAb) PD-1 receptor blocker.

1.1.1 Nonclinical Development of Nivolumab

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab alone was well tolerated (Investigator Brochure, 2014). Combination studies have highlighted the potential for toxicity when combined with ipilimumab, MDX-1408, and BMS-986016. Nivolumab bound specifically to PD-1 (and not to related members of the CD28 family such as CD28, ICOS, CTLA-4, and BTLA) with a Kd = 3.06 nM. A surrogate rat anti-mouse PD-1 antibody (4H2) was derived and expressed as chimeric IgG1 murine antibody. Antitumor activity was seen for several tumor models, including colon carcinoma and fibrosarcoma.

1.1.2 Clinical Development of Nivolumab

Nivolumab is being evaluated as monotherapy and in combination with cytotoxic chemotherapy, other immunotherapy (such as ipilimumab), anti-angiogenesis therapy, and targeted therapies in completed and ongoing BMS-sponsored clinical trials in NSCLC, melanoma, RCC, hepatocellular carcinoma (HCC), gastrointestinal (GI) malignancies including microsatellite instability (MSI) in colorectal cancer, and triple-negative breast cancer (TNBC) with an expanding group of indications (Investigator Brochure, 2014). In addition, two investigator-sponsored trials (ISTs) of nivolumab in combination with a peptide vaccine in melanoma are being conducted in the adjuvant setting and advanced disease.

Seven nivolumab studies were conducted in Japan, including six studies in advanced solid tumors and recurrent or unresectable stage III/IV melanoma sponsored by Ono Pharmaceuticals Co. Ltd., and one IST in recurrent or advanced platinum-refractory ovarian cancer.

1.1.3 Pharmacokinetics

Pharmacokinetics (PK) of nivolumab was linear in the range of 0.3 to 10 mg/kg, with dose-proportional increases in maximum serum concentration (Cmax) and area under the concentration-time curve from time zero to infinity (AUC0-∞), with low to moderate inter-subject variability observed at each dose level (Investigator Brochure, 2014). Clearance of nivolumab is independent of dose in the dose range (0.1 to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights. The mean terminal elimination half-life of BMS-936558 is 17 to 25 days consistent with the half-life of endogenous IgG4.

1.1.4 Efficacy

In a phase 1 (1, 3, and 10 mg/kg nivolumab doses) dose-escalation study the 3 mg/kg dose was chosen for expanded cohorts. Among 236 patients, objective responses (ORs) (complete or partial responses [CR or PR]) were seen in NSCLC, melanoma, and RCC. ORs were observed at all doses (Sznol et al., 2013). Median OS was 16.8 months across doses and 20.3 months at the 3 mg/kg dose. Median OS across all dose cohorts was 9.2 months and 9.6 months for squamous and non-squamous NSCLC, respectively (Brahmer et al., 2013). In the RCC cohort, median duration of response was 12.9 months for both doses with 5 of the 10 responses lasting ≥1 year (Drake et al., 2013).

In an advanced melanoma phase 1 study, nivolumab and ipilimumab were administered IV every 3 weeks for 4 doses followed by nivolumab alone every 3 weeks for 4 doses (concurrent regimen) (Wolchok et al., 2013). The combined treatment was subsequently administered every 12 weeks for up to 8 doses. In a sequenced regimen, patients previously treated with ipilimumab received nivolumab every 2 weeks for up to 48 doses. In the concurrent regimen (53 patients), 53% of patients had an OR at doses 1 mg/kg nivolumab and 3 mg/kg ipilimumab, with tumor reduction of 80% or more (modified World Health Organization [mWHO] criteria). In the sequenced-regimen (33 patients), the objective response rate (ORR) was 20%.

In a phase 1 study of nivolumab plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naïve NSCLC patients, 43 patients were treated with nivolumab + PT-doublet (Rizvi et al., 2013). No dose-limiting toxicities (DLTs) were reported and total/confirmed ORRs were 43/33%, 40/33%, and 31/31% in nivolumab/gemcitabine/cisplatin, nivolumab/pemetrexed/cisplatin, and nivolumab/carboplatin/paclitaxel arms, respectively.

In addition, an overall survival benefit has been demonstrated with nivolumab treatment in squamous cell NSCLC, non-squamous cell NSCLC, renal cell cancer, melanoma, and head and neck cancer.

1.1.5 Toxicology

A maximum tolerated dose (MTD) of nivolumab was not defined (Topalian et al., 2012). Serious adverse events (SAEs) occurred in 32 of 296 patients (11%) similar to the immune-related inflammatory events seen with ipilimumab: pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis (with noted pulmonary toxicity resulting in 3 deaths. Renal failure, symptomatic pancreatic and DM, neurologic events, and vasculitis have also been reported.). In combination with ipilimumab in the concurrent-regimen group (Wolchok et al., 2013), grade 3 or 4 treatment-related events were noted in 53% of patients. Skin rash represents the majority of these events.

1.1.6 Pharmacodynamics/Biomarkers

Tumor-cell expression (melanoma) of PD-L1 was characterized in combination with ipilimumab with the use of IHC staining and

pharmacodynamics changes in the peripheral-blood absolute lymphocyte count (Wolchok et al., 2013). With PD-L1 positivity defined as expression in at least 5% of tumor cells, biopsy specimens from 21 of 56 patients (38%) were PD-L1–positive. Among patients treated with the concurrent regimen of nivolumab and ipilimumab, ORs were observed in patients with either PD-L1–positive tumor samples (6 of 13 patients) or PD-L1–negative tumor samples (9 of 22). In the sequenced regimen cohorts, a higher number of overall responses was seen among patients with PD-L1–positive tumor samples (4 of 8 patients) than among patients with PD-L1–negative tumor samples (1 of 13) suggesting the possibility that these tumors have higher response rates to the combination. The relationship between PDL-1 expression and responses may not be present in patients treated with the combination. Tissue expression of PDL-2, interferon-γ (IFN- γ), IDO, and T cell CD8+ are of current interest. Until more reliable data based on standardized procedures for tissue collection and assays are available, PD-L1 status cannot be used to select patients for treatment at this time.

1.2 Supporting Preliminary Data

Rationale for anti-PD1 therapy in mismatch repair deficient tumors

Germline DNA repair defects, e.g. mismatch repair deficiencies, are associated with higher risk of cancers of certain organs. They have also been associated with higher number of mutations within tumors compared to those without mismatch repair deficiency (Timmerman et al. 2010, Eshleman et al. 1995 TCGA 2012). Although unselected colorectal cancer patients did not respond to PD-1 inhibitors, an exceptional responder was found to have a mismatch repair deficiency. A recent phase 2 study (Le et al., 2015) noted that pembrolizumab, and anti-PD1 agent, had an immune related and RECIST response rate of 62% (N=13) in colorectal tumors that had mismatch repair deficiency. In patients with tumors other than colorectal cancer, who had mismatch repair, the response rate was similar (6 out of 10 patients, including one CR). In this study, high somatic mutation loads assessed with whole exome sequencing correlated with prolonged progression free survival. The investigators determined mismatch repair status with the Promega MSI Analysis system, evaluating selected microsatellite sequences. In this study, the numbers of tumor infiltrating CD8+ T-cells and PD-L1 expression were not significantly associated with progression free survival or overall survival, though the study was not adequately powered to assess this.

Lynch syndrome is an autosomal dominant inherited condition caused by germline mutation in one of the mismatch repair genes (MLH1, MSH2, MSH6, PMS2, EPCAM). The germline mutations increase the risk of colorectal, endometrial, breast, prostate and other cancers, which usually occur at an earlier age than sporadic cancers. The mismatch repair phenotype can also occur in sporadic cancers, usually as a result of epigenetic silencing of MMR genes. The diagnosis of mismatch repair deficiency can be challenging, due in part to the fact that MMR proteins bind to one another, and there can be false positives with loss of MSH6. It is common for both PCR and IHC to be used to diagnose Lynch syndrome in clinical practice. This subprotocol will not restrict eligibility to those with Lynch syndrome, but will restrict eligibility to those with MMR deficiency.

The study mentioned above enrolled a small number of patients with presumed Lynch syndrome. Yet in this small cohort, 60% responded. This proposal will explore the activity of a different PD-1 inhibitor, nivolumab, in patients with tumors that have mismatch repair deficiency in order to ascertain the response rate and duration of response in these patients. IHC for MLH-1 and MSH-2 captures 90% of Lynch syndrome patients, as well as those with sporadic MSI.

Because several studies are underway testing anti-PD1 agents in colorectal cancer (CRC) with MSI, we will not enroll such patients in this study. About 15% of CRC, gastric and endometrial cancers will have MSI (Yamamoto et al. 2015). Hereditary MMR defects account for about 10% of epithelial ovarian cancer (Kobayashi et al. 2014). Other cancers have not been as well characterized as to their MMR status, but reports have documented adrenocortical tumors, peritoneal mesothelioma, pancreatic tumors, MFH, prostate cancer, anaplastic thyroid cancer, melanoma, various sarcomas, breast cancer and others as having lost expression of MLH1 or MSH2 with or without microsatellite instability (Karamurzin et al. 2012).

To date, there are no known effective therapies targeting patients with mismatch repair deficiencies. As mismatch repair deficiencies can be found across many solid tumors, if treatment with nivolumab is successful, this treatment would be a major advance. In addition, the responses observed in the early study of PD1 inhibition in MMR-deficient tumors demonstrated compelling durability of responses, with 5/6 responders maintaining continued response at the time of reporting [median not reached (Le et al. 2015)]. This speaks to the realistic possibility of checkpoint inhibitor therapy converting MMR-deficient cancers into truly chronic diseases.

Based on the preliminary clinical data described above, patients with mismatch repair deficiency as determined by the microsatellite instability assay incorporated in the MATCH diagnostic panel will be assigned to this treatment arm in preference to any other potential treatment assignment regardless of co-occurring actionable molecular alterations of interest.

1.3 Justification for the collection of research blood

There is great utility to identifying mechanisms of response and resistance to PD-1 blockade as well as evaluating potential biomarkers that may be associated with clinical benefit. Based on prior preliminary studies conducted in patients across several disease types, systemic immune responses engendered by immunologic checkpoint blockade therapy may be predictive of response (Herbst et al. Nature 2014; Posto et al. N Engl J Med 2012; Schoenfeld et al. Cancer Res 2010). However, it is unknown to what degree circulating factors such as specific T-cell populations, myeloid derived suppressor cells, and immunologic cytokines may change in response to PD-1 blockade in patients with mismatch repair deficient tumors, and to what degree these factors are associated with response and clinical benefit.

Performing serial blood collections will allow for the analyses of circulating immunologic factors and changes in these factors engendered over the course of treatment. Specific analyses will be reviewed and approved by the MATCH Correlative Science committee prior to inclusion for this study. Results of such studies may also serve as the basis for combinatorial immune therapies to further improve response rates.

2. Selection of Patients

Each of the criteria in the checklist that follows must be met, along with the eligibility in the MATCH Master Protocol, in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28.

ECOG-ACRIN Patient No. ____________________________

Patient's Initials (L, F, M) _______________________

Physician Signature and Date __________________

NOTE: Policy does not allow for the issuance of waivers to any protocol specified criteria (http://ctep.cancer.gov/protocolDevelopment/policies_deviations.htm). Therefore, all eligibility criteria listed in Section 2 must be met, without exception. The registration of individuals who do not meet all criteria listed in Section 2 can result in the participant being censored from the analysis of the study, and the citation of a major protocol violation during an audit. All questions regarding clarification of eligibility criteria must be directed to the Group's Executive Officer (EA.Execofficer@jimmy.harvard.edu) or the Group's Regulatory Officer (EA.RegOfficer@jimmy.harvard.edu).

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration/randomization by the treating physician.

NOTE: All patients must have signed the relevant treatment consent form

2.1 Registration to Treatment

_____ 2.1.1 Patients must fulfill all eligibility criteria outlined in Section 3.1 of MATCH Master Protocol (excluding Section 3.1. 4 and 3.1.6) at the time of registration to treatment step (Step 1, 3, 5, 7)

Rev. Add13

_____ 2.1.2 Patients must have mismatch repair deficiency as determined via the MATCH Master Protocol. See Appendix I for a list of the mismatch repair genes whose absence results in mismatch repair deficiency and corresponding Levels of Evidence.

_____ 2.1.3 Patients must not have known hypersensitivity to nivolumab or compounds of similar chemical or biologic composition.

_____ 2.1.4 No prior therapy with anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, anti-OX-40, anti-CD40 or anti-CTLA-4 antibodies (or any other antibody targeting T cell co-regulatory pathways).

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_____ 2.1.5 Patients with cancers for which nivolumab is approved or becomes approved are excluded (e.g: colorectal cancer, locally advanced or metastatic urothelial carcinoma, unresectable or metastatic melanoma, metastatic non-small cell lung cancer, advanced renal cell carcinoma, classical Hodgkin lymphoma, and recurrent or metastatic squamous cancer of the head and neck).

_____ 2.1.6 Must not have received any of the following therapies within four weeks prior to the first dose of the study drug: IL-2, interferon, or other non-study immunotherapy regimens or immunosuppressive agents.The master protocol eligibility criterion regarding wash-out period from prior therapy is also applicable (See Section 3.1.13 of the Master Protocol).

_____ 2.1.7 Must not have a history of toxic epidermal necrolysis (Stevens-Johnson syndrome).

_____ 2.1.8 Must not have received growth factors, including but not limited to granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), erythropoietin, etc. within 2 weeks of study drug administration. Use of such agents while on study is also prohibited. Prior use of growth factors should be documented in the patient's medical history.

_____ 2.1.9 Must not have a history of any autoimmune disease: inflammatory bowel disease, (including ulcerative colitis and Crohn's Disease), rheumatoid arthritis, systemic progressive sclerosis (scleroderma), systemic lupus erythematosus (SLE) autoimmune vasculitis (e.g., Wegener's Granulomatosis), CNS or motor neuropathy considered to be of autoimmune origin (e.g., Guillian-Barre Syndrome, Myasthenia Gravis, Multiple Sclerosis). Patients are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger (precipitating event). Entry of patients with autoimmune diagnoses not listed here must be approved by the protocol chair.

_____ 2.1.10 Must not be on supplemental home oxygen.

_____ 2.1.11 Must not have evidence of interstitial lung disease

Rev. 12/16

_____ 2.1.12 Patients with a requirement for steroid treatment or other immunosuppressive treatment: Patients will be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses >10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.

_____ 2.1.13 No history of severe hypersensitivity reaction to any monoclonal antibody.

Rev. 3/17

_____ 2.1.14 Women of childbearing potential (WOCBP) receiving nivolumab must agree to use adequate contraception (hormonal or double barrier method of birth control; abstinence) from one week prior to study treatment starting, during treatment, and for a period of 5 months after the last dose of nivolumab. Men receiving nivolumab and who are sexually active with WOCBP must agree to use adequate contraception (hormonal or double barrier method of birth control; abstinence) from one week prior to study treatment starting, during treatment, and for a period of 7 months after the last dose of nivolumab.

_____ 2.1.15 Patients with Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV) infection may be eligible provided they have the following:

- There must be no evidence of clinically significant hepatic injury from hepatitis virus infection.
- For HBV, patients must be on suppressive therapy and have undetectable HBV viral load.
- For HCV, patients must either be on suppressive therapy for HCV or have already completed therapy thought to have eradicated HCV.

_______________________________________ _________________________

Physician Signature Date

OPTIONAL: This signature line is provided for use by institutions wishing to use the eligibility checklist as source documentation.

3. Nivolumab Treatment Plan

Rev. 3/17
Rev. Add13

3.1 Administration Schedule

Nivolumab will be given Days 1 and 15 (every two weeks ± 2 days) at a dose of 240 mg. Patients may be dosed no less than 12 days from the previous dose of drug. After 4 cycles of therapy, patients will be transitioned to 480 mg flat dose every 4 weeks (± 2 days) for the remainder of their time on study.

Nivolumab is to be administered as an approximately 60-minute IV infusion (± 10 minutes) or can be safely infused over 30 minutes, using a volumetric pump with a 0.2-1.2 micron in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline for delivery but the total drug concentration of the solution cannot be below 0.35 mg/mL or not to exceed 120 mL. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

Repeat cycles every 28 days until progression.

3.2 Adverse Event Reporting Requirements

The Adverse Event Reporting Requirements for all EAY131 subprotocols are outlined in the MATCH MASTER protocol. Please refer to those guidelines when determining if an event qualifies as a Serious Adverse Event (SAE) and requires expedited reporting via CTEP's Adverse Event Reporting System (CTEP-AERS).

In addition, the following section outlines agent specific requirements and must be followed to ensure all reporting requirements are met.

3.2.1 Additional instructions, requirements and exceptions for protocol EAY131 – Subprotocol Z1D

Additional Instructions

For instructions on how to specifically report events that result in persistent or significant disability/incapacity, congenital anomaly, or birth defect events via CTEP-AERS, please contact the AEMD Help Desk at aemd@tech-res.com or 301-897-7497. This will need to be discussed on a case-by-case basis.

EAY131 – Subprotocol Z1D specific expedited reporting requirements:

- **Pregnancies**: Pregnancies and suspected pregnancies (including a positive or inconclusive pregnancy test, regardless of age or disease state) occurring while the subject is on nivolumab, or within 28 days of the subject's last dose of nivolumab, are considered immediately reportable events. The pregnancy, suspected pregnancy, or positive/ inconclusive pregnancy test must be reported via CTEP-AERS within 24 hours of the Investigator's knowledge. Please refer to Appendix VIII in MATCH Master Protocol for detailed instructions on how to report the occurrence of a pregnancy as well as the outcome of all pregnancies.

EAY131 – Subprotocol Z1D specific expedited reporting exceptions:

For Subprotocol Z1D, the adverse events listed below **do not** require expedited reporting via CTEP-AERS:

- If an AE meets the reporting requirements of the protocol, and it is listed on the SPEER, it should ***ONLY*** be reported via CTEP-AERS if the grade being reported exceeds the grade listed in the parentheses next to the event.

3.2.2 Second Primary Cancer Reporting Requirements

All cases of second primary cancers, including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), that occur following treatment on NCI-sponsored trials must be reported to ECOG-ACRIN using Medidata Rave

- **A second malignancy is a cancer that is UNRELATED to any prior anti-cancer treatment (including the treatment on this protocol). Second malignancies require ONLY routine reporting as follows:**
  1. Complete a Second Primary Form in Medidata Rave within 14 days.
  2. Upload a copy of the pathology report to ECOG-ACRIN via Medidata Rave confirming the diagnosis.
  3. If the patient has been diagnosed with AML/MDS, upload a copy of the cytogenetics report (if available) to ECOG-ACRIN via Medidata Rave.
- **A secondary malignancy is a cancer CAUSED BY any prior anti-cancer treatment (including the treatment on this protocol). Secondary malignancies require both routine and expedited reporting as follows:**
  1. Complete a Second Primary Form in Medidata Rave within 14 days
  2. Report the diagnosis via CTEP-AERS at http://ctep.cancer.gov

     Report under a.) leukemia secondary to oncology chemotherapy, b.) myelodysplastic syndrome, or c.) treatment related secondary malignancy
  3. Upload a copy of the pathology report to ECOG-ACRIN via Medidata Rave and submit a copy to NCI/CTEP confirming the diagnosis.
  4. If the patient has been diagnosed with AML/MDS, upload a copy of the cytogenetics report (if available) to ECOG-ACRIN via Medidata Rave and submit a copy to NCI/CTEP.

NOTE: The Second Primary Form and the CTEP-AERS report should not be used to report recurrence or development of metastatic disease.

NOTE: If a patient has been enrolled in more than one NCI-sponsored study, the Second Primary Form must be

submitted for the most recent trial. ECOG-ACRIN must be provided with a copy of the form and the associated pathology report and cytogenetics report (if available) even if ECOG-ACRIN was not the patient's most recent trial.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted via CTEP-AERS or by the Second Primary Form.

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3.3 Comprehensive Adverse Events and Potential Risks List (CAEPR) for BMS-936558 (Nivolumab, MDX-1106, NSC 748726)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 2069 patients.* Below is the CAEPR for BMS-936558 (Nivolumab, MDX-1106).

NOTE: If an AE meets the reporting requirements of the protocol, and it is listed on the SPEER, it should ***ONLY*** be reported via CTEP-AERS if the grade being reported exceeds the grade listed in the parentheses next to the event in the SPEER.

Version 2.3, June 18, 2018[1]

Adverse Events with Possible Relationship to BMS-936558 (Nivolumab, MDX-1106) (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
CARDIAC DISORDERS			
		Cardiac disorders - Other (cardiomyopathy)	
		Myocarditis	
		Pericardial tamponade[2]	
		Pericarditis	
ENDOCRINE DISORDERS			
	Adrenal insufficiency[3]		
	Hypophysitis[3]		
	Hyperthyroidism[3]		
	Hypothyroidism[3]		
EYE DISORDERS			
		Blurred vision	
		Dry eye	
		Eye disorders - Other (diplopia)[3]	
		Eye disorders - Other (Graves ophthalmopathy)[3]	
		Eye disorders - Other (optic neuritis retrobulbar)[3]	
	Uveitis		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Colitis[3]		

Adverse Events with Possible Relationship to BMS-936558 (Nivolumab, MDX-1106) (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Colonic perforation[3]	
	Diarrhea		***Diarrhea (Gr 3)***
	Dry mouth		***Dry mouth (Gr 2)***
		Gastritis	
		Mucositis oral	
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[4]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	Injection site reaction		***Injection site reaction (Gr 2)***
IMMUNE SYSTEM DISORDERS			
		Allergic reaction[3]	
		Autoimmune disorder[3]	
		Cytokine release syndrome[5]	
		Immune system disorders - Other (GVHD in the setting of allotransplant)[3,6]	
		Immune system disorders - Other (sarcoid granuloma)[3]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction[7]		
INVESTIGATIONS			
	Alanine aminotransferase increased[3]		***Alanine aminotransferase increased[3] (Gr 3)***
	Aspartate aminotransferase increased[3]		***Aspartate aminotransferase increased[3] (Gr 3)***
	Blood bilirubin increased[3]		***Blood bilirubin increased[3] (Gr 2)***
	Creatinine increased		
	Lipase increased		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 2)***
	Neutrophil count decreased		
	Platelet count decreased		
	Serum amylase increased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
		Hyperglycemia	***Hyperglycemia (Gr 2)***
		Metabolism and nutrition disorders - Other (diabetes mellitus with ketoacidosis)[3]	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
		Musculoskeletal and connective tissue disorder - Other (polymyositis)	

Adverse Events with Possible Relationship to BMS-936558 (Nivolumab, MDX-1106) (CTCAE 5.0 Term) [n= 2069]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Myositis	
		Rhabdomyolysis	
NERVOUS SYSTEM DISORDERS			
		Encephalopathy[3]	
		Facial nerve disorder[3]	
		Guillain-Barre syndrome[3]	
		Myasthenia gravis[3]	
		Nervous system disorders - Other (demyelination myasthenic syndrome)	
		Nervous system disorders - Other (encephalitis)[3]	
		Nervous system disorders - Other (meningoencephalitis)	
		Nervous system disorders - Other (meningoradiculitis)[3]	
		Nervous system disorders - Other (myasthenic syndrome)	
		Peripheral motor neuropathy	
		Peripheral sensory neuropathy	
		Reversible posterior leukoencephalopathy syndrome[3]	
RENAL AND URINARY DISORDERS			
		Acute kidney injury[3]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pleural effusion[3]		
	Pneumonitis[3]		
		Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia)[3]	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme[3]	
	Pruritus[3]		***Pruritus[3] (Gr 2)***
	Rash maculo-papular[3]		***Rash maculo-papular[3] (Gr 2)***
		Skin and subcutaneous disorders -Other (bullous pemphigoid)	
	Skin and subcutaneous disorders - Other (Sweet's Syndrome)[3]		
	Skin hypopigmentation[3]		
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Pericardial tamponade may be related to possible inflammatory reaction at tumor site.

[3]BMS-936558 (Nivolumab, MDX-1106) being a member of class of agents involved in the inhibition of "immune checkpoints", may result in severe and possibly fatal immune-mediated adverse events probably due to T-cell activation and proliferation. This may result in autoimmune disorders that can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune nephritis, autoimmune neuropathy, autoimmune thyroiditis, bullous pemphigoid, exacerbation of Churg-Strauss Syndrome, drug rash with eosinophilia systemic symptoms [DRESS] syndrome, facial nerve disorder (facial nerve paralysis), limbic encephalitis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, thyrotoxicosis, and adrenal insufficiency), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome.

[4] Pancreatitis may result in increased serum amylase and/or more frequently lipase.

[5]Cytokine release syndrome may manifest as hemophagocytic lymphohistiocytosis with accompanying fever and pancytopenia.

[6]Complications including hyperacute graft-versus-host disease (GVHD), some fatal, have occurred in patients receiving allo stem cell transplant (SCT) after receiving BMS-936558 (Nivolumab, MDX-1106). These complications may occur despite intervening therapy between receiving BMS-936558 (Nivolumab, MDX-1106) and allo-SCT.

[7]Infusion reactions, including high-grade hypersensitivity reactions which have been observed following administration of nivolumab, may manifest as fever, chills, shakes, itching, rash, hypertension or hypotension, or difficulty breathing during and immediately after administration of nivolumab.

Adverse events reported on BMS-936558 (Nivolumab, MDX-1106) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that BMS-936558 (Nivolumab, MDX-1106) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Leukocytosis

CARDIAC DISORDERS - Atrial fibrillation; Atrioventricular block complete; Heart failure; Ventricular arrhythmia

EAR AND LABYRINTH DISORDERS - Vestibular disorder

EYE DISORDERS - Eye disorders - Other (iridocyclitis); Optic nerve disorder; Periorbital edema

GASTROINTESTINAL DISORDERS - Constipation; Duodenal ulcer; Flatulence; Gastrointestinal disorders - Other (mouth sores); Vomiting

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Malaise; Pain

HEPATOBILIARY DISORDERS - Bile duct stenosis

IMMUNE SYSTEM DISORDERS - Anaphylaxis; Immune system disorders - Other (autoimmune thrombotic microangiopathy); Immune system disorders - Other (limbic encephalitis)

INFECTIONS AND INFESTATIONS - Bronchial infection; Lung infection; Sepsis; Upper respiratory infection

INVESTIGATIONS - Blood lactate dehydrogenase increased; GGT increased; Investigations - Other (protein total decreased); Lymphocyte count increased; Weight loss

METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Hypophosphatemia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain;

Musculoskeletal and connective tissue disorder - Other (musculoskeletal pain); Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Myalgia; Pain in extremity

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (histiocytic necrotizing lymphadenitis)

NERVOUS SYSTEM DISORDERS - Dizziness; Headache; Intracranial hemorrhage

PSYCHIATRIC DISORDERS - Insomnia

RENAL AND URINARY DISORDERS - Hematuria; Renal and urinary disorders - Other (tubulointerstitial nephritis)

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchospasm; Cough; Dyspnea; Hypoxia

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Pain of skin; Photosensitivity; Rash acneiform; Skin and subcutaneous tissue disorders - Other (rosacea)

VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Vasculitis

NOTE: BMS-936558 (Nivolumab, MDX-1106) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent

3.4 Dose Modifications

All toxicity grades below are described using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov).

Please refer to the Nivolumab Investigator Brochure or Appendix II to the protocol for toxicity management algorithms which include specific treatment guidelines. These algorithms should be followed unless there are specific clinical circumstances for which the treating physician decides an alternative treatment approach is clinically appropriate. Consultation with the study chair is recommended.

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In all of the tables for dose modification and holds in this section, the guidelines are for adverse events thought at least possibly attributed to study drug. Generally we strongly encourage early evaluation while withholding drug, and appropriate treatment as indicated in the management tables and event specific guidelines.

ALL OTHER EVENTS	Management/Next Dose for Nivolumab
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below)
Grade 3	Off protocol therapy (exceptions as noted below)
Grade 4	Off protocol therapy
Recommended management: As clinically indicated	

- Any grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment should go off protocol treatment
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued study drug dosing should go off protocol treatment.

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- Any grade 2-4 drug-related laboratory abnormality or electrolyte abnormality, that can be managed independently from underlying organ pathology with electrolyte replacement, hormone replacement, transfusion or insulin or that does not require treatment does not require hold/discontinuation.

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- Tolerable and clinically stable grade 2 toxicities attributed to study drug may remain on study with PI approval (e.g. thrombosis that is being anticoagulated)

Skin Rash and Oral Lesions	Management/Next Dose for Nivolumab
≤ Grade 1	No change in dose *
Grade 2	Treatment may continue at investigator discretion. A short course of steroids is permitted. If treatment is held, resume at same dose level.
Grade 3	Hold* until ≤ Grade 1. Resume at same level at investigator discretion
Grade 4	Off protocol therapy
* Patients with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphagoid. Pruritus may occur with or without skin rash and should be treated symptomatically if there is no associated liver or GI toxicity. Note skin rash typically occurs early and may be followed by additional events particularly during steroids tapering.	
Recommended management: AE management guidelines	

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Liver Function AST, ALT, Bilirubin	Management/Next Dose for Nivolumab
≤ Grade 1	Treatment may be continued at investigator discretion. Dose level is maintained.
Grade 2	Hold until UNL or baseline. Resume at same dose level.
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
* I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN Continued treatment of active immune mediated hepatitis may exacerbate ongoing inflammation. Holding drug to evaluate LFT changes and early treatment are recommended. ** LFT changes may occur during steroid tapers from other events and may occur together with other GI events including cholecystitis/pancreatitis.	
Recommended management: see Hepatic AE management algorithm	

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Diarrhea/ Colitis	Management/Next Dose for Nivolumab
≤ Grade 1	Symptomatic treatment but continue therapy. No change in dose
Grade 2	Hold until baseline. No change in dose
Grade 3	Hold until baseline. No change in dose.
Grade 4	Off protocol therapy
See GI AE Algorithm for management of symptomatic colitis. Patients with grade 2 symptoms but normal colonoscopy and biopsies may be retreated after resolution. Please evaluate pituitary function prior to starting steroids if possible without compromising acute care. Evaluation for all patients for additional causes includes *C. diff*, acute and self-limited infectious and foodborne illness, ischemic bowel, diverticulitis, and IBD.	
Recommended management: see GI AE management Algorithm	

Symptomatic Pancreatitis	**Management/Next Dose for Nivolumab**
≤ Grade 1	Continue therapy.
Grade 2	Continue therapy.
Grade 3	Patients who develop symptomatic pancreatitis or DM should be taken off treatment
Grade 4	Patients who develop symptomatic pancreatitis or DM should be taken off treatment
Patients with any grade lipase/amylase elevation may continue treatment at investigator discretion if asymptomatic. Patients may develop symptomatic and radiologic evidence of pancreatitis as well as DM and DKA. Lipase elevation may occur during the period of steroid withdrawal and with other immune mediated events or associated with colitis, hepatitis, and patients who have asymptomatic lipase elevation typically have self-limited course and may be retreated. For treatment management of symptomatic pancreatitis please follow the Hepatic Adverse Event Management Algorithm	

Pneumonitis	**Management/Next Dose for Nivolumab**
≤ Grade 1	Hold dose pending evaluation and resolution to baseline including baseline pO2. Resume no change in dose after pulmonary and/or ID consultation excludes lymphocytic pneumonitis.
Grade 2	Hold dose pending evaluation. Resume no change in dose after pulmonary and/or ID consultation excludes nivolumab and associated lymphocytic pneumonitis as the cause of the pneumonitis. Off study if steroids are required. ^
Grade 3	Hold dose pending evaluation. Refer to Appendix II for diagnostic and treatment guidelines.
Grade 4	Off protocol therapy
Distinguishing inflammatory pneumonitis is often a diagnosis of exclusion for patients who do not respond to antibiotics and have no causal organism identified including influenza. Most patients with respiratory failure or hypoxia will be treated with steroids. Bronchoscopy may be required and analysis of lavage fluid for lymphocytic predominance may be helpful. Patients with new lung nodules should be evaluated for sarcoid like granuloma. Please consider recommending seasonal influenza killed vaccine for all patients.	
Recommended management: See Pulmonary Adverse Event Management Algorithm	

Fatigue	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose.
Grade 2	No change in dose
Grade 3	Hold until ≤ Grade 2. Resume at same dose level
Fatigue is the most common adverse event associated with immune checkpoint therapy. Grade 2 or greater fatigue should be evaluated for associated or underlying organ involvement including pituitary, thyroid, and hepatic, or muscle (CPK) inflammation	

Neurologic events	Management/Next Dose for Nivolumab
≤ Grade 1	Hold dose pending evaluation and observation. Resume with no change in dose when resolved to baseline.
Grade 2	Hold dose pending evaluation and observation. # Hold until ≤ Grade 1. Off protocol therapy if treatment with steroids is required. Resume at same dose level for peripheral isolated n. VII (Bell's palsy)
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
Patients with any CNS events including aseptic meningitis, encephalitis, symptomatic hypophysitis, or myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), GB syndrome, myasthenia gravis should be off study.	
Recommended management: See Neurologic Adverse Event Management Algorithm	

Endocrine Hypophysitis Adrenal Insufficiency	Management/Next Dose for Nivolumab
≤ Grade 1	Asymptomatic TSH elevation * May continue therapy pending evaluation, consider endocrine consult
Grade 2	Hold until patients are on a stable replacement hormone regimen. If treated with steroids patients must be stable off steroids for two weeks. Resume at same dose level.
Grade 3	See Appendix II for diagnostic and management algorithm.
Grade 4	See Appendix II for diagnostic and management algorithm.
Note all patients with symptomatic pituitary enlargement, exclusive of hormone deficiency, but including severe headache or enlarged pituitary on MRI should be considered grade 3 events. Isolated thyroid or testosterone deficiency may be treated as grade 2 if there are no other associated deficiencies and adrenal function is monitored. Please evaluate pituitary function before beginning steroid therapy or replacement therapy of any kind. *Note patients with thyroiditis may be retreated on replacement therapy. Patients must be evaluated to rule out pituitary disease prior to initiating thyroid replacement.	
Recommended management: See Endocrine Management Algorithm	

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Renal	Management/Next Dose for Nivolumab combination
≤ Grade 1	May continue therapy per investigator discretion.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off treatment

Infusion reaction	Management/Next Dose for Nivolumab combination
≤ Grade 1	May continue therapy per investigator discretion.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off treatment
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever	

Fever	Management/Next Dose for Nivolumab combination
≤ Grade 1	Evaluate and continue at same dose level
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off treatment
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever	
See Section 5. Infusion reactions	

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Cardiac*	Management/Next Dose for BMS-936558 (Nivolumab) Cardiac Toxicities
≤ Grade 1	Hold dose pending evaluation and observation.** Evaluate for signs and symptoms of CHF, ischemia, arrhythmia or myositis. Obtain history EKG, CK (for concomitant myositis), CK-MB. Repeat troponin, CK and EKG 2-3 days. If troponin and labs normalize may resume therapy. If labs worsen or symptoms develop then treat as below. Hold pending evaluation
Grade ≥ 2 with suspected myocarditis	Hold dose.** Admit to hospital. Cardiology consult. Rule out MI and other causes of cardiac disease. Cardiac Monitoring. Cardiac Echo. Consider cardiac MRI and cardiac biopsy. Initiate high dose methylprednisolone. If no improvement within 24 hours, add either infliximab, ATG or tacrolimus. Resume therapy if there is a return to baseline and myocarditis is excluded or considered unlikely.
Grade ≥ 2 with confirmed myocarditis	Off protocol therapy. Admit to CCU (consider transfer to nearest Cardiac Transplant Unit). Treat as above. Consider high dose methylprednisolone. Add ATG or tacrolimus if no improvement. Off treatment.
* *Including CHF, LV systolic dysfunction, Myocarditis, CPK, and troponin* ** *Patients with evidence of myositis without myocarditis may be treated according as “other event”* Note: The optimal treatment regimen for immune mediated myocarditis has not been established. Since this toxicity has caused patient deaths, an aggressive approach is recommended.	

- Drug will be held for grade 2 cardiac dysfunction pending evaluation
- Drug will be permanently discontinued for grade 3 or 4 cardiac dysfunction and grade 2 events that do not recover to baseline or that reoccur
- Treatment with steroids as clinically indicated

If treatment is delayed >6 weeks for an adverse event, the study PI must be consulted for any consideration of further therapy.

Patients with grade 3 thyroiditis and skin rash may continue therapy as for grade 2 events with resolution and stable replacement treatment.

Patients with thyroiditis or hypopituitarism who are stable as above may be restarted with replacement hormones including thyroid hormone and physiologic doses of corticosteroids.

Please note that grading and for hypophysitis with symptoms of headache, visual or neurologic changes or radiologic evidence of pituitary enlargement and other CNS events such as aseptic meningitis or encephalitis should be considered grade 3 events.

Any patients who require additional immune suppressive treatment beyond steroids should go off study treatment

Patients requiring > two dose delays for the same event should go off protocol therapy.

Prior to starting corticosteroids or hormone replacement for any reason, appropriate endocrine testing including cortisol, ACTH, TSH and T4 should be obtained to document baseline. However, if urgent steroid use is clinically required, steroid treatment should not be delayed for bloodwork.

Please note that in some cases the treatment algorithms recommend steroids if symptoms do not resolve in 7 days. However, this recommendation is not meant to delay steroid treatment at any time it is clinically indicated.

Patients may be dose-delayed for evaluation and restarted depending on results.

Any patient started on corticosteroids initially who is determined to not require steroid treatment for an autoimmune adverse event may resume therapy after a 2 week observation period without further symptoms at the discretion of the PI or investigator.

3.5 Supportive Care

3.5.1 Appendix II details required supportive care and management guidelines for immune-related toxicity

3.6 Duration of Agent-specific treatment

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued. In this event submit forms according to the instructions in the MATCH Forms Packet.
- Patient withdraws consent.
- Patient experiences unacceptable toxicity.
- Non-protocol therapies are administered. Patients who require palliative radiation therapy may be considered to remain on study after discussion and approval by the subprotocol chair(s). Treatment with nivolumab should be halted during radiation therapy.

- Any dosing interruption lasting >6 weeks, with the following exceptions: Dosing interruptions >6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting >6 weeks, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted.
- Disease progression
- Please see Section 3.7 for details regarding treatment past progression

3.7 Treatment Past Progression

A minority of subjects treated with immunotherapy may derive clinical benefit either delayed responses, stable disease, or increased overall survival despite initial evidence of progressive disease (PD) with nivolumab or combination treatment.

Patients may be permitted to continue treatment beyond initial RECIST 1.1-defined PD occurring during 24 weeks as long as they meet the following criteria:

- No more than 4 new lesions total sum of the longest diameter (SHORT diameter for LN) cannot exceed 40% of the initial sum including new lesions
- Patients must be clinically stable with no change in performance status due to disease progression
- No indication for immediate alternative treatment
- Patient (assessed by the investigator) is showing clinical benefit and tolerates study drug. The assessment of clinical benefit should take into account whether the subject is clinically stable or deteriorating and likely or unlikely to receive further benefit from continued treatment.
- The time of progression is noted from the first assessment that exceeds standard criteria
- New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes, which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor burden measurement if the longest diameter increases to at least 10 mm (except for pathological lymph nodes, which must have an increase in short axis to at least 15 mm).

3.8 Duration of Follow-Up

Refer to the MATCH Master Protocol for specifics on the duration of follow-up.

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4. Study Parameters

4.1 Therapeutic Parameters for Nivolumab Treatment

NOTE: In addition to the study parameters listed in the MATCH Master Protocol, the below parameters must also be performed for patients receiving nivolumab treatment.

NOTE: All assessments required prior to registration to treatment should be done ≤ 4 weeks prior to registration to Steps 1, 3, 5, 7, excluding the radiologic evaluation and electrocardiogram (ECG).

Test/Assessment	Prior to Registration to Treatment	Treatment		End of Treatment	Follow Up[F]
		Every Cycle, prior to treatment	Every 2 Cycles		
H&P, Weight, Vital signs[A]	X	X[I]			X
Performance status	X	X[I]			X
CBC w/diff, plts[B]	X	X[I]			X
Serum chemistry[B]	X	X[I]			X
Thyroid function testing [TSH (with reflex to free T4 ± free T3 if abnormal)]	X	X			X
Radiologic evaluation[D]	X		X[D]		X[F]
β-HCG[C]	X				
Toxicity Assessment[G]		X		X	X[F]
ECG	X	X[H]			
ECHO	X[H]				
Tumor biopsy and blood sample for MATCH Master Protocol[E]			X	X	
Research Blood (See Section 6)[J]		X[J]		X[J]	
Archival tumor block *or* 20 (at least 5 micron thick) unstained slides, if available	X[K]				

A. History and physical, including vital signs and weight at the start of each cycle (up to 3 days before start of new cycle). A contraceptive, menstrual, and sexual history be obtained with each H&P for all women of childbearing potential and sexually active men.

B. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, creatinine, glucose, phosphorus, potassium, SGOT[AST], SGPT[ALT], sodium, magnesium, TSH (with reflexive Free T4 ± Free T3 if abnormal), serum tumor markers (prior to each cycle, not each dose). For eligibility purposes, participants with creatinine levels above eligibility, Cockcroft-Gault will be used to calculate creatinine clearance. CBC w/diff, platelets and serum chemistries should be performed on cycle 1, day 1 (or up to 7 days prior), and at the start of each subsequent dose (up to 3 days

before start of next dose) while tumor markers are performed prior to day 1 of a cycle (up to 3 days before). CBC with differential will be performed more frequently in patients with grade 4 neutropenia or thrombocytopenia until resolution to ≤ grade 3. CBC and serum chemistries are only required in follow-up until values return to pre-treatment levels or until progressive disease.

C. Blood pregnancy test (women of childbearing potential) required prior to beginning treatment.

D. Disease measurements are repeated every 2 cycles for the first 26 cycles, and every 3 cycles thereafter until PD or start of another MATCH treatment step. The baseline evaluation should be performed as closely as possible to the beginning of treatment and never more than 6 weeks before registration to treatment step. For multiple myeloma patients, please refer to Section 6.4 of the MATCH Master Protocol for additional information on myeloma response criteria and the required disease assessments. Documentation (radiologic) must be provided for patients removed from study for progressive disease.

E. Additional blood specimens and/or biopsies are to be submitted from consenting patients per Section 9.3.2 of the MATCH Master Protocol. Submit at the following time points, as applicable:

- Blood specimens are to be submitted at the end of Cycle 2 (prior to start of Cycle 3 treatment). If patient progresses or treatment is discontinued prior to Cycle 3, collect the blood at that time instead. On-treatment kits for blood sample collections will be automatically shipped to sites upon registration to the treatment step.
- Screening biopsies for additional aMOI assessments after registration to appropriate screening step, if applicable (Step 2 or Step 4).
- At end of all MATCH study treatments, blood specimens and/or research biopsy after consent and registration to Step 8

Please refer to Section 4 of the MATCH Master Protocol to determine whether the patient proceeds to the next screening step or to follow-up (with a potential end of treatment biopsy for research purposes on Step 8). Samples are to be submitted as outlined in Section 9 of the MATCH Master Protocol. To order Step 2/4 Screening or Step 8 kits, complete the EAY131 Collection and Shipping Kit Order Form (See Appendix XII of the MATCH Master Protocol) and fax to 713-563-6506.

F. Every 3 months if patient is < 2 years from study entry, and every 6 months for year 3. Toxicity assessments and radiologic evaluations are not required to be done during Follow Up if progression has been previously reported; however if an adverse event occurs post treatment that meets the SAE reporting requirements, it still must be reported via CTEP-AERS, even if progression has occurred.

G. Site personnel should evaluate for toxicity and discuss treatment compliance with the patient in order to ensure the medication is taken correctly; this evaluation may be conducted by telephone or in person. The Toxicity Assessment is not required prior to Cycle 1, but is required every subsequent cycle.

H. Evaluate cardiac function including EKG and ECHO for any patients with a history of CHF or at risk because of underlying cardiovascular disease or exposure to cardiotoxic drugs, as clinically indicated. For patients with evidence of CHF, MI, cardiomyopathy, or myositis cardiac evaluation including lab tests and cardiology consultations as clinically indicated including EKG, CPK, troponin, ECHO.

I. For Cycle 1, if the following tests/assessments occurred within 7 days of Day 1, they do not need to be repeated at this timepoint: H&P, Weight, Vital Signs; Performance Status; CBC w/diff, plts; Serum chemistry; Concomitant Medications.

J. From consenting patients, collection prior to start of treatment and end of cycle 2 (prior to start of cycle 3) and End of treatment.

K. MANDATORY: Submit per EAY131 Master Protocol (Section 9.3 and Section 9.4) prior to or within 8 weeks following registration to EAY131-Z1D.

Rev. Add13

5. Drug Formulation and Procurement

This information has been prepared by the ECOG-ACRIN Pharmacy and Nursing Committees.

Availability

NO STARTER SUPPLIES MAY BE ORDERED. Subjects must be enrolled and assigned to the treatment subprotocol prior to submitting the clinical drug request to PMB.

Drug Ordering: NCI supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that drug be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained – see general information) The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://ctepcore.nci.nih.gov/OAOP). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam/) and the maintenance of an “active” account status, a “current” password, and an active person registration status.

NCI Supplied Agent(s) – General Information

Questions about drug orders, transfers, returns, or accountability should be addressed to the PMB by calling 240-276-6575 Monday through Friday between 8:30 AM and 4:30 PM Eastern Time or email PMBAfterHours@mail.nih.gov anytime.

Drug Returns: All undispensed drug supplies should be returned to the PMB. When it is necessary to return study drug (e.g., sealed bottles remaining when PMB sends a stock recovery letter), investigators should return the study drug to the PMB using the NCI Return Agent Form available on the NCI home page (http://ctep.cancer.gov).

Drug Accountability: The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return of agent received from the PMB using the NCI Investigational Agent Accountability Record Form available on the NCI home page (http://ctep.cancer.gov). Maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator.

Rev. 12/16

Rev. 3/17

Investigator Brochure Availability: The current versions of the IBs for PMB-supplied agents will be accessible to site investigators and research staff through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an “active” account status, a “current” password, and active person registration status. Questions about IB access may be directed to the PMB IB coordinator at IBCoordinator@mail.nih.gov.

Rev. 3/17

5.1 Nivolumab (NSC #748726)

5.1.1 Other Names

BMS-936558, MDX1106

5.1.2 Classification

Anti-PD-1MAb

5.1.3 Mode of Action

Nivolumab targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Nivolumab inhibits the binding of PD-1 to PD-L1 and PD-L2. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens.

Rev. Add13
Rev. Add16

5.1.4 Storage and Stability

Storage:

Vials of Nivolumab injection must be stored at 2°-8°C (36°-46°F) and protected from light and freezing. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours.

If a storage temperature excursion is identified, promptly return Nivolumab to 2°C-8°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability:

Shelf-life surveillance of the intact vials is ongoing. The administration of undiluted and diluted solutions of Nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 24 hours in a refrigerator at 2°-8°C (36°-46°F) and a maximum of 8 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and room light. The maximum 8-hour period under room temperature and room light conditions includes the product administration period.

CAUTION: The single-use dosage form contains no antibacterial preservative or bacteriostatic agent. Therefore, it is advised that the product be discarded 8 hours after initial entry.

Rev. Add16

5.1.5 Dose Specifics

Description: Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate dihydrate, sodium chloride,

mannitol, diethylenetriaminepentacetic acid (pentetic acid) and polysorbate 80 (Tween® 80), and water for injection. Dilute solutions of hydrochloric acid and/or sodium hydroxide may be used for pH adjustment (pH 5.5-6.5).

Rev. Add13

How Supplied: Nivolumab is supplied by Bristol-Myers Squibb and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as 100 mg vials (10 mg/mL) with a 0.7mL overfill. It is supplied in 10 mL type I flint glass vials, with fluoropolymer film-laminated rubber stoppers and aluminum seals.

Rev. Add16

5.1.6 Preparation

Preparation: Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose. When the dose is based on patient weight (i.e., mg/kg), nivolumab injection can be infused undiluted or diluted to protein concentrations as low as 0.35 mg/mL. When the dose is fixed (eg, 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL. For patients weighing less than 40 kilograms (kg), the total volume of infusion must not exceed 4 mL per kg of patient weight. During drug product preparation and handling, vigorous mixing or shaking is to be avoided.

Rev. Add16

5.1.7 Route of Administration

Intravenous infusion over 30 minutes. Do not administer as an IV push or bolus injection.

Method of Administration:

Administer through a 0.2 micron to 1.2 micron pore size, low-protein binding (polyethersulfone membrane) in-line filter.

5.1.8 Potential Drug Interactions

The indirect drug-drug interaction potential of nivolumab was assessed using systemic cytokine modulation data for cytokines known to modulate CYP enzymes. There were no meaningful changes in cytokines known to have indirect effects on CYP enzymes across all dose levels of nivolumab. This lack of cytokine modulation suggests that nivolumab has no or low potential for modulating CYP enzymes, thereby indicating a low risk of therapeutic protein-drug interaction.

Rev. 12/16

5.1.9 Patient Care Implications: Women of childbearing potential (WOCBP) receiving nivolumab must agree to use adequate contraception (hormonal or double barrier method of birth control; abstinence) from one week prior to study treatment starting, during treatment, and for a period of 5 months after the last dose of nivolumab. Men receiving nivolumab and who are sexually active with WOCBP must agree to use adequate contraception (hormonal or double barrier method of birth control; abstinence) from one week prior to study treatment starting, during treatment, and for a period of 7 months after the last dose of nivolumab.

5.1.10 Side Effects

See Section 3.3 for side effects.

6. Specimen Submissions

Peripheral blood for research studies will be obtained from patients on EAY131-Z1D who consent "Yes" to "I agree to provide additional blood for research." These blood collections will allow for potential analyses of circulating immunologic factors and changes in these factors engendered over the course of treatment and other possible research studies.

Kits are available to order for the collection and submission of the peripheral blood samples and will contain the supplies and instructions for collecting, processing, and shipping the samples. To order kits, complete the EAY131-Z1D Collection and Shipping Kit Order Form (Appendix III) and Fax as instructed. Kits will arrive within 48-72 hours from when the order was placed.

Requirements and guidelines for the submission of archived FFPE tumor tissue for confirmation of MLH1 or MSH2 testing are in Section 9 of the MATCH Master Protocol as they are considered part of the screening assessments. Tissue is to be submitted prior to or within 8 weeks following registration to EAY131-Z1D. If the results of these additional assessments do not confirm the initial screening assessment and are received after registration to treatment on EAY131-Z1D, treatment on EAY131-Z1D can continue per physician discretion.

6.1 Specimen Submissions

If you have any questions concerning sample collection and shipment, please contact the ECOG-ACRIN CBPF at (844) 744-2420.

Logging and Tracking of Samples: It is required that all samples submitted on this EAY131-Z1D be entered and tracked using the ECOG-ACRIN Sample Tracking System (see Section 6.1.4). An STS shipping manifest form is to be included with every submission.

Labeling of Samples: All samples must be clearly labeled with the ECOG-ACRIN protocol number (EAY131-Z1D), ECOG-ACRIN five-digit patient case number, patient initials, date of collection and sample type.

6.1.1 Sample Collection and Submission Schedule

Peripheral blood samples are to be submitted at ambient on day of collection. Samples are to be collected at the following time points for each tube type:

- Prior to start of treatment
- Post-Cycle 2 (prior to start of Cycle 3)
- Discontinuation of treatment

6.1.2 Specimen Collection Guidelines

Peripheral blood samples are to be collected

- Draw 32mL of whole blood into four (4) CPT tubes (blue/black top, provided in the kit) – Note: Tube should be gently inverted 5-10 times following collection
- Draw 7mL of whole blood into one (1) red top tube (provided in the kit)

6.1.3 Shipping Procedures

The integrity of the blood samples requires they be processed within 24 hours of collection. Peripheral blood samples are to be shipped Monday-Thursday at ambient via overnight courier the day of collection. **Do not ship samples the day before a weekend or holiday.**

Ship using the ECOG-ACRIN CBPF's FedEx account using the FedEx on-line Ship Manager.

Access to the shipping account for shipments to the ECOG-ACRIN CBPF can only be obtained by logging into fedex.com with an account issued by the ECOG-ACRIN CBPF. For security reasons, the account number will no longer be given out in protocols, over the phone, or via email. If your site needs to have an account created, please contact the ECOG-ACRIN CBPF by email at eacbpf@mdanderson.org.

Ship to:

ECOG-ACRIN Central Biorepository and Pathology Facility
MD Anderson Cancer Center
Department of Pathology, Unit 085
Tissue Qualification Laboratory for ECOG-ACRIN
Room G1.3598
1515 Holcombe Boulevard
Houston, TX 77030
Toll Free Phone: (844) 744-2420 (713-745-4440 Local or International Sites)
Fax: (713) 563-6506
Email: eacbpf@mdanderson.org

6.2 ECOG-ACRIN Sample Tracking System

It is **required** that all specimens submitted on this trial be entered and tracked using the ECOG-ACRIN Sample Tracking System (STS). The software will allow the use of either 1) an ECOG-ACRIN user-name and password previously assigned (for those already using STS), or 2) a CTSU username and password.

When you are ready to log the collection and/or shipment of the samples required for this study, please access the Sample Tracking System software by clicking https://webapps.ecog.org/Tst.

Important: Please note that the STS software creates pop-up windows, so you will need to enable pop-ups within your web browser while using the software. A user manual and interactive demo are available by clicking this link: http://www.ecog.org/general/stsinfo.html

Please take a moment to familiarize yourself with the software prior to using the system.

An STS generated shipping manifest form should be shipped with all sample submissions.

Please direct your questions or comments pertaining to the STS to ecog.tst@jimmy.harvard.edu.

Study Specific Notes

Generic Specimen Submission Form (#2981v3) will be required only if STS is unavailable at time of sample submission. Notify the laboratory of the shipment by faxing a copy of the completed form to the laboratory.

- ECOG-ACRIN Central Biorepository and Pathology Facility

Retroactively enter all sample collection and shipping information when STS is available.

6.3 Use of Specimens in Research

From the heparinized whole blood, peripheral blood mononuclear cells (PBMC) will be prepared by Ficoll-Hypaque density gradient centrifugation by standard laboratory procedures, transferred to cryogenic vials, and stored in a liquid nitrogen freezer for use in approved research projects. Aliquots of plasma will also be stored in 1mL cryogenic vials.

Whole blood Serum Tube will be processed using standard laboratory procedures. Aliquots of 1 mL of serum will be transferred to cryogenic vials and stored at ≤ -70°C or below.

Specimens will be retained in an ECOG-ACRIN designated central repository. For this trial, specimens will be retained at the ECOG-ACRIN Central Biorepository and Pathology Facility.

Specific analyses will be reviewed and approved by the MATCH Correlative Science committee and NCI prior to inclusion for this study.

If consent for use in research is denied or withdrawn by the patient, the specimens will be removed from consideration for use in any future research study and the blood specimens destroyed per guidelines of the respective repository.

6.4 Sample Inventory Submission Guidelines

Inventories of all samples submitted from institutions will be tracked via the ECOG-ACRIN STS and receipt and usability verified by the receiving laboratory. Inventories of samples forwarded and utilized for approved laboratory research studies will be submitted by the investigating laboratories to the ECOG-ACRIN Operations Office - Boston on a monthly basis in an electronic format defined by the ECOG-ACRIN Operations Office - Boston.

7. References

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Molecular Analysis for Therapy Choice (MATCH)
MATCH Treatment Subprotocol Z1D: Nivolumab

Appendix I

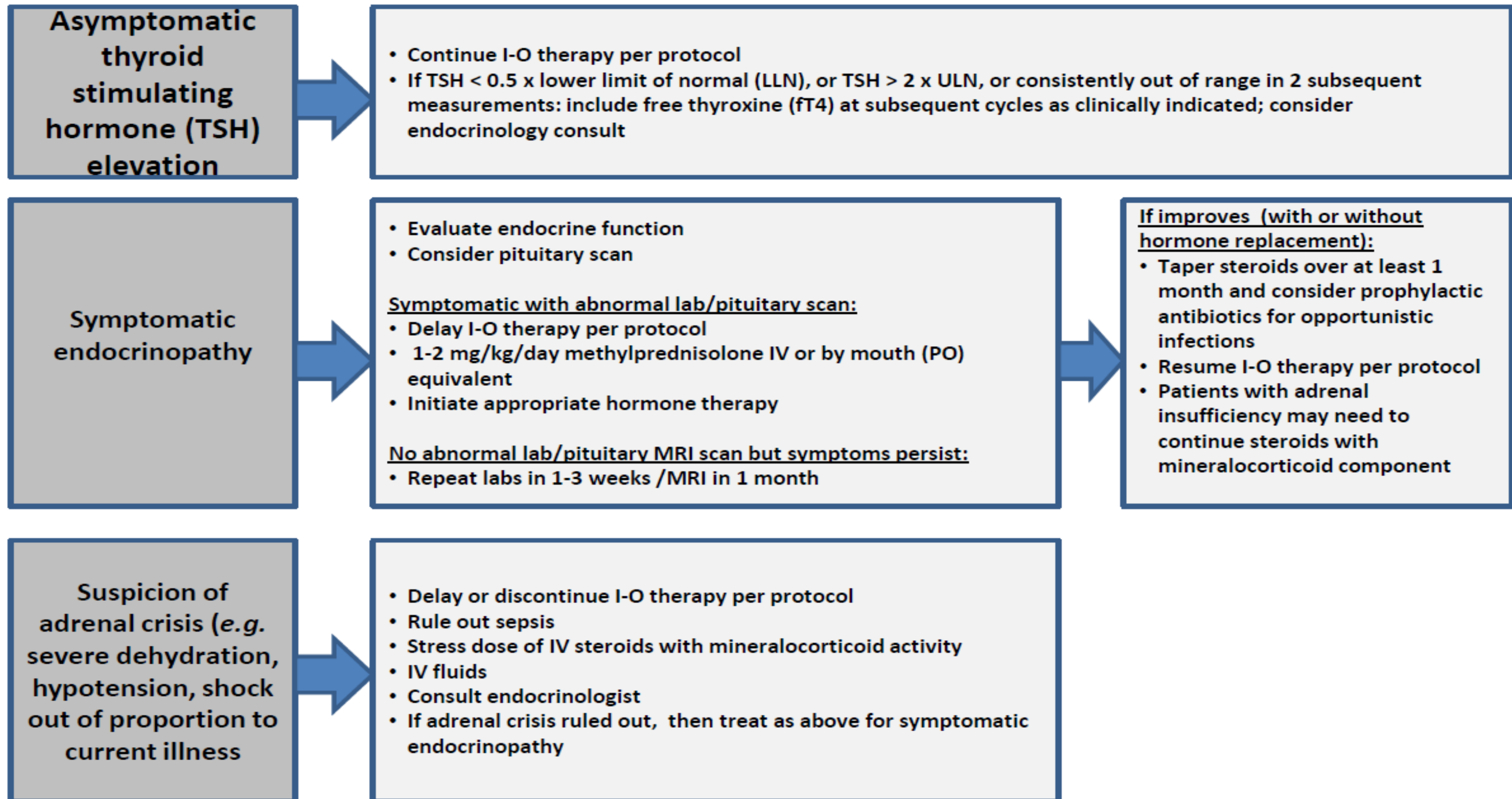
Actionable Mutations for Sub-Protocol EAY131-Z1D

1. Absence of MLH1 by immunohistochemistry or
2. Absence of MSH2 by immunohistochemistry

Molecular Analysis for Therapy Choice (MATCH)
MATCH Treatment Subprotocol Z1D: Nivolumab

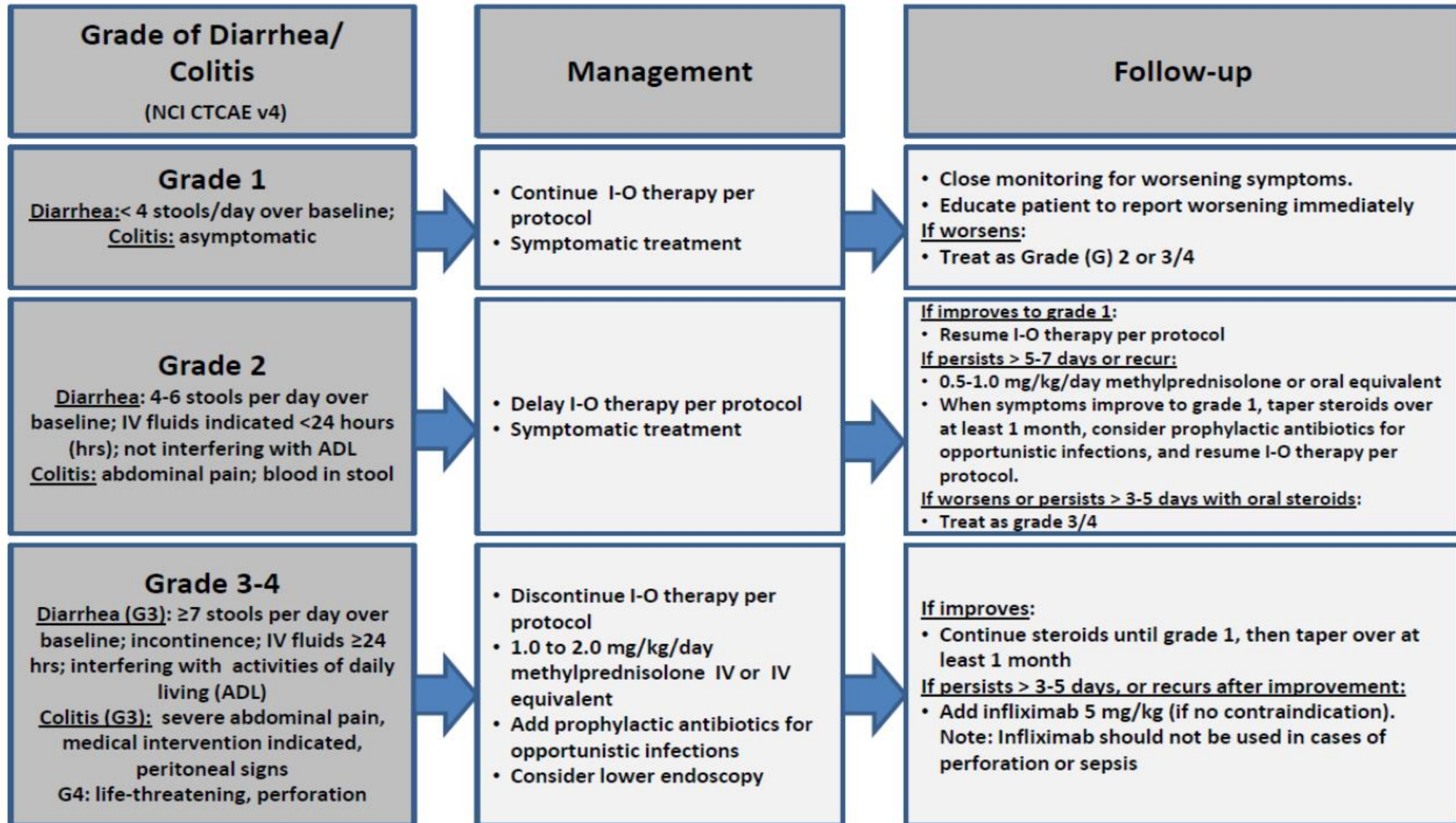
Appendix II

Management Algorithms For Endocrinopathy, Gastrointestinal, Hepatic, Neurological, Pulmonary, Renal, And Skin Adverse Events


Endocrinopathy Management Algorithm
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue immuno-oncology (I-O) therapy.
Consider visual field testing, endocrinology consultation, and imaging.
Asymptomatic thyroid stimulating hormone (TSH) elevation
• Continue I-O therapy per protocol
• If TSH < 0.5 x lower limit of normal (LLN), or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: include free thyroxine (fT4) at subsequent cycles as clinically indicated; consider endocrinology consult
Symptomatic endocrinopathy
• Evaluate endocrine function
• Consider pituitary scan
Symptomatic with abnormal lab/pituitary scan:
• Delay I-O therapy per protocol
• 1-2 mg/kg/day methylprednisolone IV or by mouth (PO) equivalent
• Initiate appropriate hormone therapy
No abnormal lab/pituitary MRI scan but symptoms persist:
• Repeat labs in 1-3 weeks /MRI in 1 month
If improves (with or without hormone replacement):
• Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections
• Resume I-O therapy per protocol
• Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component
Suspicion of adrenal crisis (e.g. severe dehydration, hypotension, shock out of proportion to current illness
• Delay or discontinue I-O therapy per protocol
• Rule out sepsis
• Stress dose of IV steroids with mineralocorticoid activity
• IV fluids
• Consult endocrinologist
• If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathy
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

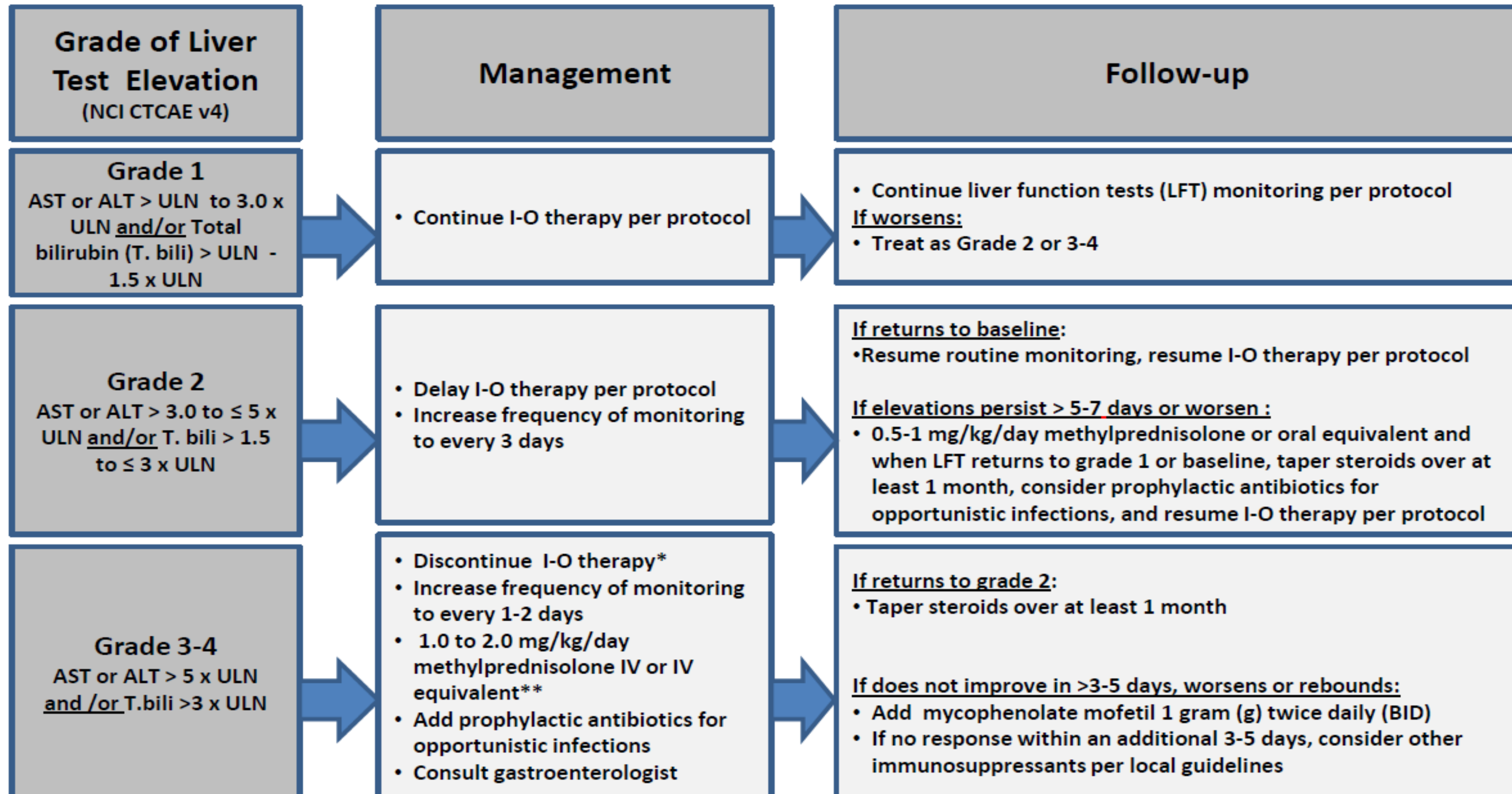


Grade of Diarrhea/ Colitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Diarrhea: < 4 stools/day over baseline; Colitis: asymptomatic	• Continue I-O therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately If worsens: • Treat as Grade (G) 2 or 3/4
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 hours (hrs); not interfering with ADL Colitis: abdominal pain; blood in stool	• Delay I-O therapy per protocol • Symptomatic treatment	If improves to grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • Treat as grade 3/4
Grade 3-4 Diarrhea (G3): ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; interfering with activities of daily living (ADL) Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: Infliximab should not be used in cases of perforation or sepsis

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.



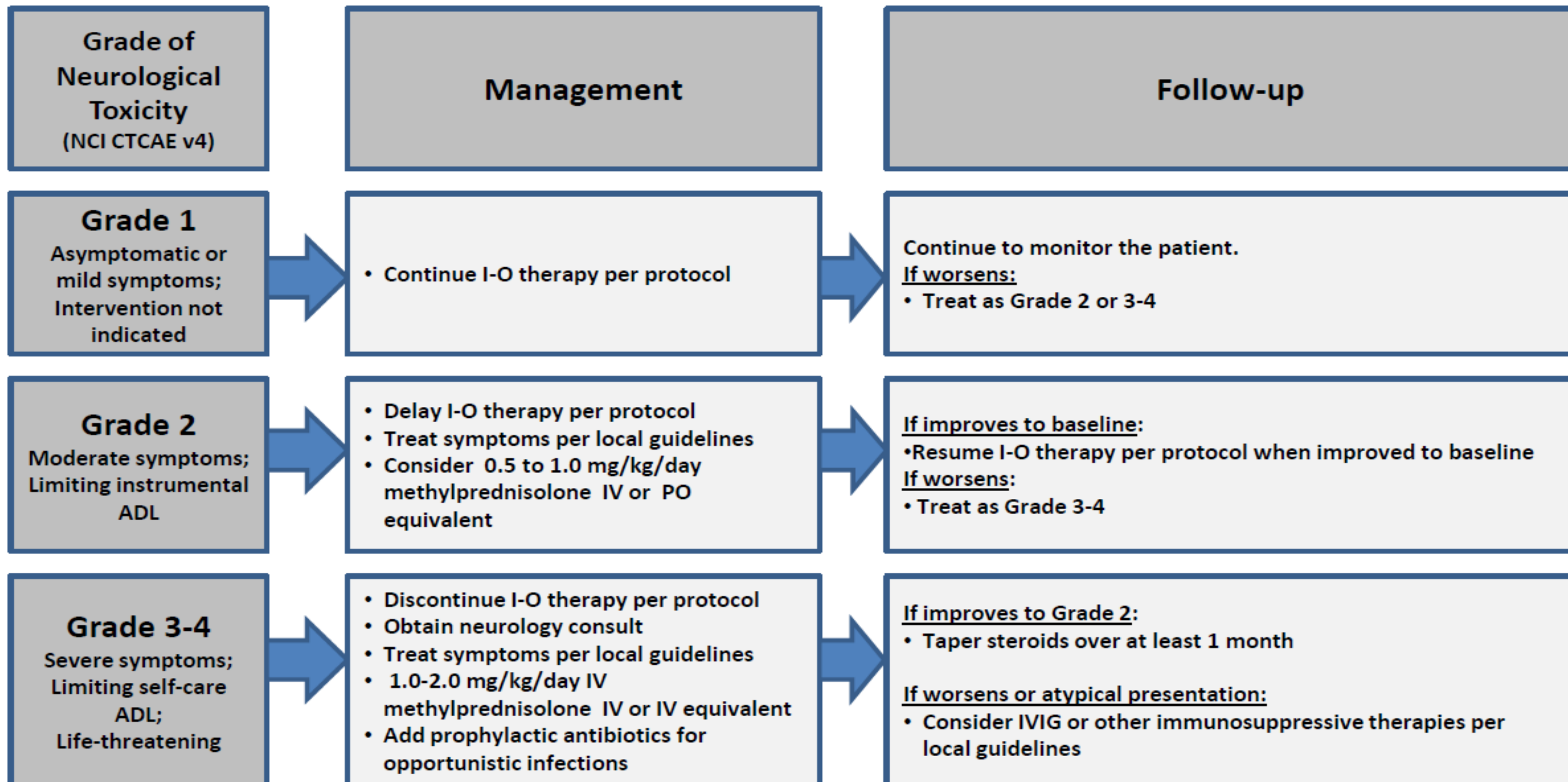
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

***I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.**

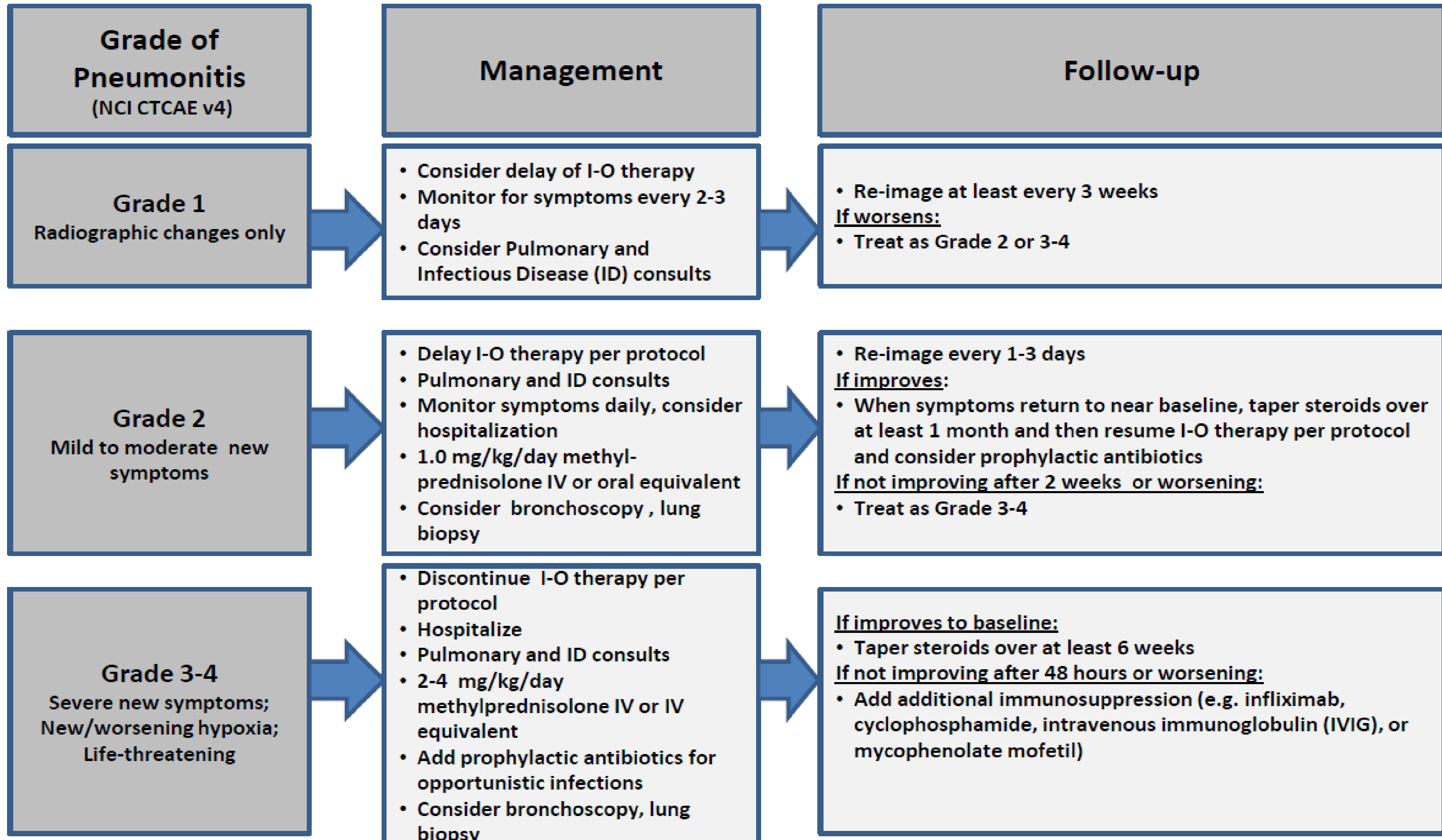
**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Neurological Adverse Event Management Algorithm

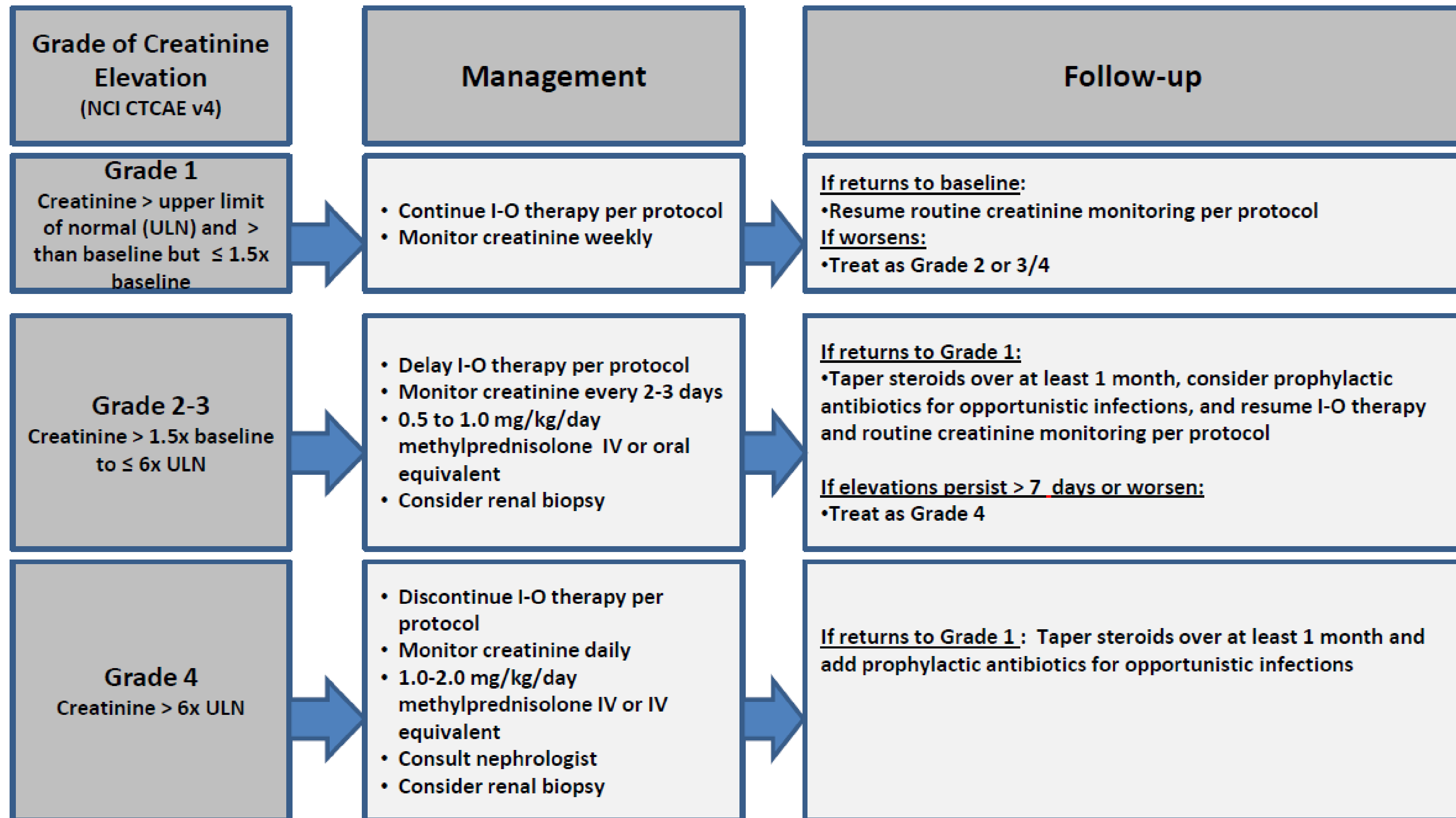
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.



Renal Adverse Event Management Algorithm

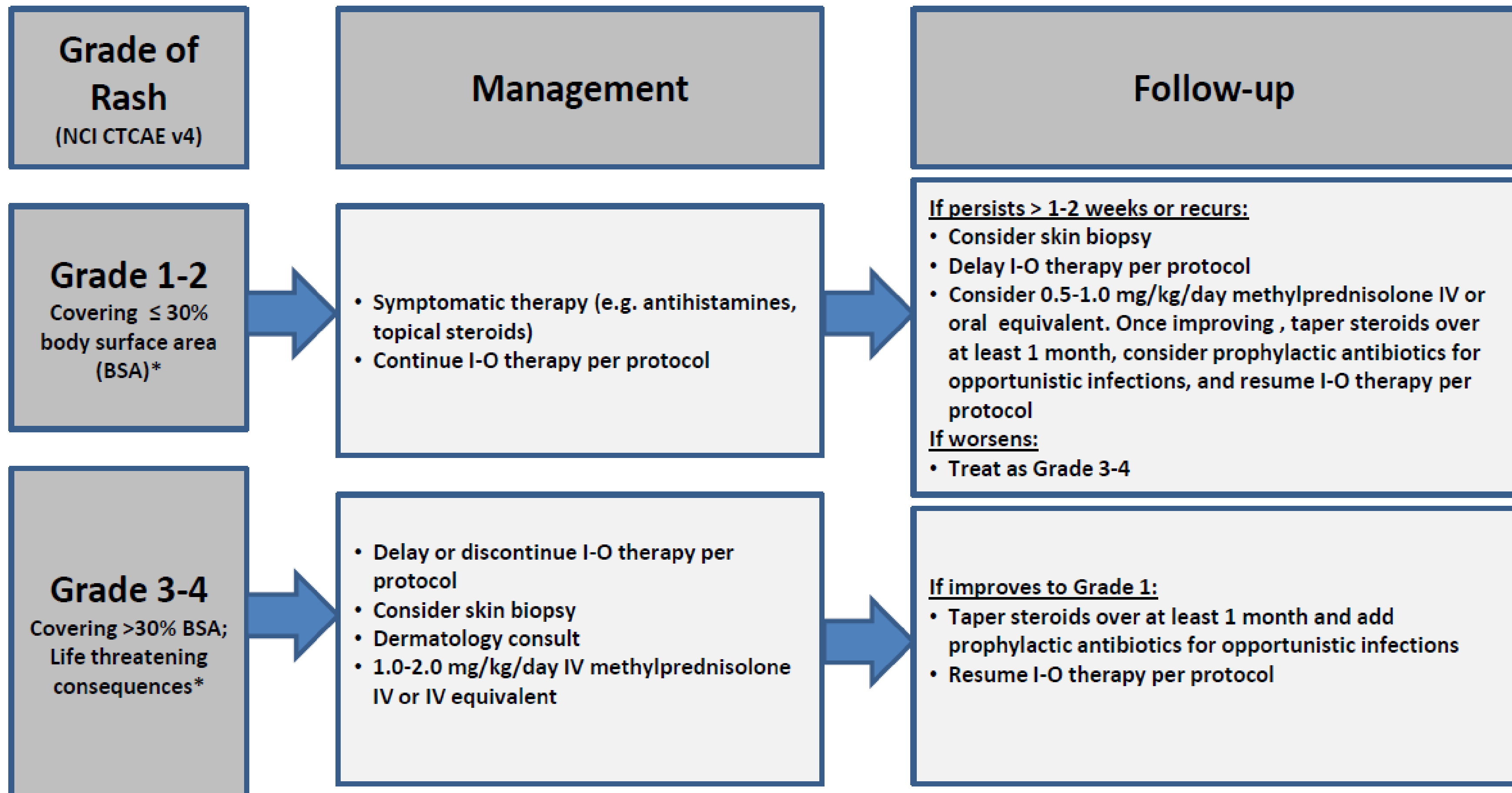
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy

Grade of Creatinine Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 Creatinine > upper limit of normal (ULN) and > than baseline but ≤ 1.5x baseline	• Continue I-O therapy per protocol • Monitor creatinine weekly	<u>If returns to baseline</u>: •Resume routine creatinine monitoring per protocol <u>If worsens:</u> •Treat as Grade 2 or 3/4
Grade 2-3 Creatinine > 1.5x baseline to ≤ 6x ULN	• Delay I-O therapy per protocol • Monitor creatinine every 2-3 days • 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent • Consider renal biopsy	<u>If returns to Grade 1:</u> •Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy and routine creatinine monitoring per protocol <u>If elevations persist > 7 days or worsen:</u> •Treat as Grade 4
Grade 4 Creatinine > 6x ULN	• Discontinue I-O therapy per protocol • Monitor creatinine daily • 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent • Consult nephrologist • Consider renal biopsy	<u>If returns to Grade 1</u>: Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.

Molecular Analysis for Therapy Choice (MATCH)
MATCH Treatment Subprotocol Z1D: Nivolumab

Appendix III

EAY131-Z1D Collection and Shipping Kit Order Form

Kit orders are to be made **after** patient has consented to participate in EAY131-Z1D.

Please complete this form correctly, including the valid ECOG-ACRIN case number and complete shipping address. If information is missing the kit processing will be delayed.

FAX to ECOG-ACRIN Central Biorepository and Pathology Facility at (713) 563-6506.

Date: ___________________

EAY131 Patient Case Number: ______________________

EAY131-Z1D Kit Requested: Kit will contain three (3) subkits (collection and shipping supplies) for (a) Prior to start of treatment, (b) Post-Cycle 2, and (c) End of Treatment

Ship Kit to:

Institution Contact: ____________________________________

Phone Number for Contact: ____________________________________

Fax Number for Contact: ____________________________________

Email for Contact: ____________________________________

Institution Address: ____________________________________

____

____

NOTE: Questions are to be directed to the ECOG-ACRIN CBPF

Phone: Toll Free 1-844-744-2420 (713-745-4440 Local or International Sites)
Fax: 713-563-6506
Email: eacbpf@mdanderson.org

Comments:

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Molecular Analysis for Therapy Choice (MATCH)
MATCH Treatment Subprotocol Z1D: Nivolumab

Appendix IV

Patient Drug Information Handout and Wallet Card

Information for Patients, Their Caregivers and Non-Study Healthcare Team on Possible Interactions with Other Drugs and Herbal Supplements

The patient ____________________________ is enrolled on a clinical trial using the experimental study drug, ***nivolumab****.* This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.

These are the things that you as a healthcare provider need to know:

- This agent can stimulate an autoimmune response, including but not limited no hypophysitis, hypothyroiditis, pneumonitis, colitis, hepatitis, and rash. These conditions can be life-threatening and require emergent intervention.

To the patient: Take this paper with you to your medical appointments and keep the attached information card in your wallet.

Nivolumab may interact with other drugs which can cause side effects. For this reason, it is very important to tell your study doctors of any medicines you are taking before you enroll onto this clinical trial. It is also very important to tell your doctors if you stop taking any regular medicines, or if you start taking a new medicine while you take part in this study. When you talk about your current medications with your doctors, include medicine you buy without a prescription (over-the-counter remedy), or any herbal supplements such as St. John's Wort. It is helpful to bring your medication bottles or an updated medication list with you.

Many health care providers can write prescriptions. You must tell all of your health care providers (doctors, physician assistants, nurse practitioners, pharmacists) you are taking part in a clinical trial.

Nivolumab has not been shown to have any effect on cytochrome P450 or other drug metabolizing enzymes and is not expected to interact with other medications that are impacted by cytochrome P450 or other drug metabolizing enzymes.

These are the things that you and they need to know:

Nivolumab must be used very carefully with other medicines. Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements you are taking.

- Please be very careful! Over-the-counter drugs (including herbal supplements) may contain ingredients that could interact with your study drug. Speak to your doctors or pharmacist to determine if there could be any side effects.
- Your regular health care provider should check a frequently updated medical reference or call your study doctor before prescribing any new medicine or discontinuing any medicine.

Your study doctor's name is

____

and he or she can be contacted at:

____________________________________.

<table>
<tr>
<td>
STUDY DRUG INFORMATION WALLET CARD
You are enrolled on a clinical trial using the experimental study drug nivolumab. This clinical trial is sponsored by the NCI. Nivolumab may interact with drugs. Because of this, it is very important to:
➢ Tell your doctors if you stop taking any medicines or if you start taking any new medicines.
➢ Tell all of your health care providers (doctors, physician assistants, nurse practitioners, or pharmacists) that you are taking part in a clinical trial.
➢ Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.
</td>
<td>
➢ Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that you are taking.
➢ Nivolumab has not been shown to have any effect on cytochrome P450 or other drug metabolizing enzymes and is not expected to interact with other medications that are impacted by cytochrome P450 or other drug metabolizing enzymes.
➢ Your study doctor's name is ____________________________ and can be contacted at ________________________________.
</td>
</tr>
</table>

Molecular Analysis for Therapy Choice (MATCH)
MATCH Treatment Subprotocol Z1D: Nivolumab

Rev. Add16

Appendix V

CS-MATCH-0005

Tissue / blood biomarker analysis of samples collected from patients with mismatch repair deficient tumors (non-colorectal cancers) treated with nivolumab (EAY131-Z1D)

Principal Investigators:

PRINCIPAL INVESTIGATORS:	Jonathan Schoenfeld, MD MPhil MPH
CO-INVESTIGATORS:	Nilofer Azad, MD
	F. Stephen Hodi, MD
	Michael Overman, MD
	Scott Rodig, MD PhD
	Mariano Severgnini, PhD
BIOSTATISTICS:	Paul Catalano, ScD
SUB-PROTOCOL PRINCIPAL INVESTIGATORS:	Nilofer Azad, MD
	Michael Overman, MD
	Jonathan Schoenfeld, MD MPhil MPH

I. Introduction

PD-1 inhibitors have demonstrated impressive response rates and improvements in survival across multiple solid tumor types, including melanoma, non-small cell lung cancer and renal cell carcinoma [1, 5, 17, 18] and preliminary results demonstrate activity across many additional cancers [19]. Response rates to PD-1 inhibitors have varied considerably across different disease types, from approximately 40% in melanoma patients, to single digit response rates in patients with ovarian cancer [20]. And there are few to no responders in other disease types, such as microsatellite stable colorectal cancer [9]. Thus, identifying biomarkers that predict response is of significant benefit, even more so because patients treated with immune checkpoint blockade may have atypical patterns of response that may initially mimic disease progression [21]. Additionally, patients that do respond can achieve durable clinical benefit.

One of the ligands for the PD-1 receptor, PD-L1, is known to be overexpressed in the setting of chronic viral infection and, more recently, ubiquitously across many tumor types as a mechanism of tumor immune evasion. Binding of PD-L1 to the PD-1 receptor can down regulate T-cell activation by recruiting SHP-2 to the phosphorylated tyrosine residue in the cytoplasmic region [22]. In addition to tumor cells, PD-L1 can also be expressed on tumor infiltrating lymphocytes, which may also lead to immune suppression in the tumor microenvironment.

PD-L1 overexpression was identified as one of the early potential markers of response to inhibitors of the PD-1 pathway [23]. In one of the initial studies testing PD-1 inhibitor therapy, none of the 17 patients with tumors that did not express PD-L1 responded to treatment with nivolumab (BMS-936558), while 9/25 patients (36%) with PD-L1 expressing tumors did (p=0.006) [3]. More recently, PD-L1 expression in pretreatment tumor samples has demonstrated promise as potential biomarkers in patients treated with the anti-PD-L1 antibody MPDL3280A [2]. In this study of 277 patients with a variety of tumor types including NSCLC, RCC, melanoma and HNSCC, PD-L1 expression on tumor infiltrating lymphocytes was associated with response (p=0.007), although the association between PD-L1 expression on tumor cells and response did not reach statistical significance (p=0.08). High PD-L1 expression on tumor cells or increasing percentage of infiltrating immune cells has also been found to be a predictive biomarker in patients with NSCLC [1, 13]. Membranous PD-L1 was also seen in the majority of patients with mismatch repair deficient tumors treated with pembrolizumab in a previous study, although expression was not significantly associated with outcome [9].

In addition to PD-L1 expression, the degree of tumor infiltrating lymphocytes has also been associated with benefit following PD-1 pathway blockade. This has been demonstrated for CD8+ T-cells present at the margin between tumor and stroma in patients with melanoma treated with the PD-1 inhibitor pembrolizumab [4] as well as in patients with mismatch repair deficient tumors, CD8+ T-cells, particularly at the tumor invasive fronts, were associated with a trend towards response or stable disease [9].

There is an expansive and rapidly growing literature supporting PD-L1, CD3/CD4 and other immune marker IHC staining across tumor types. For example, multiple meta-analyses of PD-L1 IHC describe IHC staining results in over 16,000 patients across almost 100 studies across numerous tumor histologies (Wu, Wu et al. 2015; Pyo, Kang et al. 2016). PD-L1 is not an antigen but a conserved mechanism of immune evasion whose expression has been demonstrated across multiple tumor types and specifically in MSI tumors (Le, Uram et al. 2015) and therefore would be expected to be detectable across the various tumor types included in this study. An additional meta-analysis

conducted over 5 years ago identified 52 studies each including over a hundred patients that evaluated the prognostic value of tumor infiltrating lymphocytes across multiple histologies using IHC for the markers CD3, CD8, CD4, FoxP3 and others (Gooden, de Bock et al. 2011).

In addition to PD-L1 and tumor infiltrating lymphocytes, other immune markers may also indicate tumor mediate immune regulation and could also predict response to immune therapy either alone or in conjunction with other markers. Additionally, while traditional immunohistochemistry generally only allows for the evaluation of one or two proteins at a time, multiplex immunofluorescence (IF) simultaneously evaluates expression and provides more quantifiable spatial resolution for multiple biomarkers. Our initial work has demonstrated the reproducibility of this technique in identifying various populations of tumor infiltrating lymphocytes, as well as PD-L1 expressing tumor cells and PD-L1 and PD-1 expressing infiltrating lymphocytes; therefore, we propose to use multiplex IF to explore associations between response and expression of PD-1/PD-L1 in the tumor versus tumor/stroma interface, as well as co-localized PD-L1 / PD-1 expression.

In terms of circulating biomarkers, various studies have suggested that patients treated with immune checkpoint blockade demonstrate changes that can be detected in the peripheral blood. Herbst et al. found treatment with the PD-L1 inhibitory antibody MPDL3280A to be associated with increases in IL-18, ITAC, and CD8+/HLA-DR+/Ki67+ T-cells, and a decrease in circulating IL-6 [2]. In patients treated for renal cell carcinoma, responders to MPDL3280A with prolonged overall survival demonstrated decreases in circulating VEGFA as well as various acute phase proteins [24]. In the previously mentioned study evaluating patients with mismatch repair deficient colorectal cancer treated with PD-1 inhibitors, decreases in the tumor markers CEA, CA19-9, and CA-125 were observed in the majority of responding patients depending on their underlying tumor type [9].

In summary, preliminary data demonstrates that tissue markers including PD-L1 expression and quantification of tumor infiltrating lymphocytes, as well as changes in circulating markers such as cytokines and T-cell subsets, may predict activity associated with PD-1 pathway inhibitors across tumor types and including patients with mismatch repair deficient tumors. Thus, these studies have the potential for clinical utility a method of predicting which patients with mismatch repair tumors would be more likely to respond to PD-1 directed therapy and then as an early marker of long term benefit in patients during the course of therapy.

II. Objectives

A. Primary objectives:

1. Studies in tissue: The primary objective of this study will be to determine associations between PD-L1 expression (quantitatively determined as a continuous variable) in tumor tissue at baseline and an objective response to protocol treatment.
2. Peripheral blood studies: To determine association between changes in percentages of circulating activated T-cells, specifically CD8+/HLA-DR+/Ki-67+ T cells and an objective response to protocol treatment.

B. Exploratory studies

1. To associate levels of tumor infiltrating CD8+ T-cells, and PD-L1 expression defined categorically, and also in specific cell populations such as tumor cells and tumor infiltrating lymphocytes with response
2. As tissue allows, to explore correlations between response and multiple immunologic biomarkers including PD-1, CTLA-4, MHC-1, MHC-2, Lag-3, B2M, Na channel and other exploratory multiplex IF to determine expression levels of these biomarkers in components of the tumor microenvironment and in relation to each other
3. To evaluate mechanisms of resistance by comparing immunologic markers in sequential biopsies and biopsies taken at progression if available
4. To explore correlations between PD-L1 expression and CD8 + T-cell tumor infiltration with overall tumor mutational burden as determined by whole-exome and targeted sequencing to be performed through the parent NCI MATCH study
5. To explore associations between changes in a panel of circulating immune populations and immunologic cytokines with response Methodology

III. Methodology

Summary:

Correlative science will involve immune evaluation of archival tissue, serum and PBMCs obtained from multiple time points. PD-L1 expression and CD8+ T-cell infiltration will be evaluated on cut FFPE slides as below to correlate with response. We will also examine changes in these biomarkers over time when sequential biopsy specimens are available. Additional tissue, when available will be used for multiplexed IF and assayed for other immunologic markers as described in detail below. CyTOF will primarily examine changes in circulating activated T-cells over the course of therapy as a predictor of response but will also explore other immune subsets and cytokines.

Patients enrolled on MATCH Z1D have loss of MLH1 or MSH2 as determined by IHC performed at the Central Pathologic lab for the NCI MATCH study. Other MSI testing is currently outside of the scope of the current correlative proposal, although additional testing can be considered if adequate tissue is remaining.

No patient identifiers will be provided to investigators with the samples. The trial-specific patient ID will be replaced with a unique randomized number and specimens will be labeled with a barcode not related to any PHI. The following entities have access to the key: the ECOG-ACRIN biobank, the ECOG-ACRIN Operations Office, and the MATCH statisticians.

Prioritization of tissue:

Formalin fixed-paraffin embedded (FFPE) tumor slides will be prepared and a slide H&E stained from all tumor samples. Immunohistochemical (IHC) and multiplex immunofluorsecent (IF) staining will be performed on FFPE tumor slides using some or all of the following antibodies/assays. Since there may not be sufficient tissue to perform all the assays in every sample we have prioritized the assays. In the order of priority based on the amount of tissue available, the assays we will perform include:

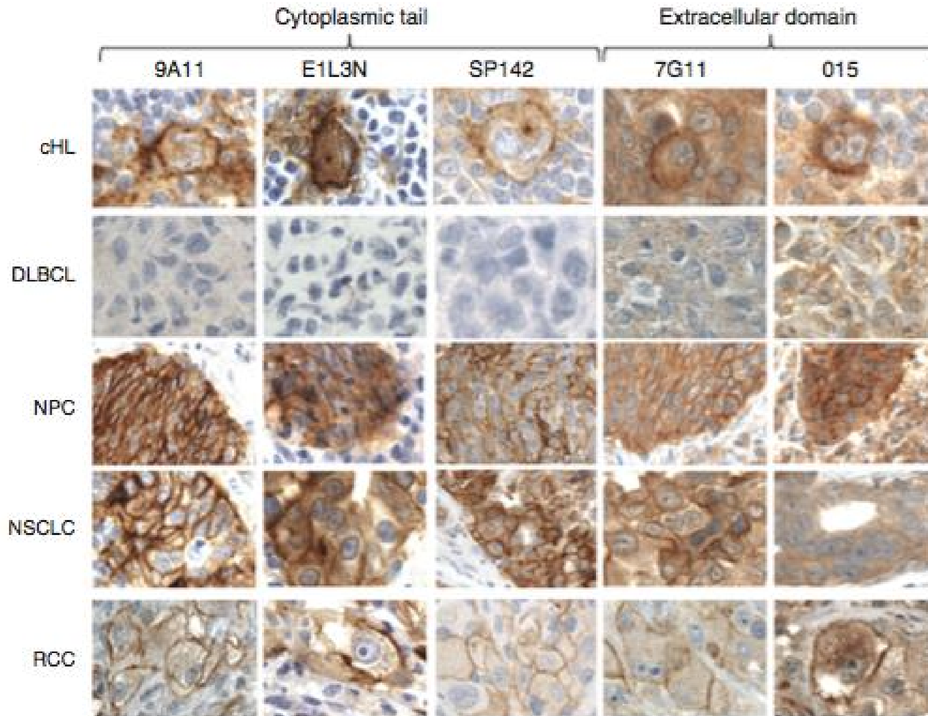
- IHC to determine intratumoral PD-L1 (CD274) expression

- IF to determine tumor infiltrating lymphocytes (TILs) using CD8 expression
- Exploratory multiplex IF and IHC to evaluate response and distribution of PD-L1 staining in conjunction with other markers such as tumor infiltrating lymphocytes and PD-1 expressionand, and other immunologic markers will be performed including CD3, CD8, CTLA-4, MHC-1, MHC-2, Lag-3, B2M, Na channel, (these will only be performed if there is sufficient tissue)

Materials and methods for studies in tissue:

Our Brigham and Women's Hospital Core Pathology Lab and Immune-oncology Core has developed significant expertise for immunohistochemical staining and multiplex immunofluorescence under the leadership of Dr. Scott Rodig, Core Director and is now part of the CIMAC network. PD-L1, CD3, and CD8 IHC testing, in particular, have been established in a CLIA approved setting, and the PD-L1 IHC will be performed in a CLIA-certified laboratory. Dr. Rodig's laboratory has experience with the IF analyses. We plan to use positive and negative controls to validate our results. Extensive validation is not feasible for these exploratory biomarkers given the variety of tumor types. We would plan for any hypothesis-generating results to be validated in future studies.

Regarding our primary endpoint of PD-L1 expression in tumor tissue, mouse monoclonal anti-PD-L1 antibody (clone 9A11) was generated in the laboratory of Dr. Gordon Freeman (Dana-Farber Cancer Institute) and has been licensed to Cell Signaling Technology (Beverly, MA) for commercial use. This antibody is human gene product and can recognize all isoforms. The specificity of these antibodies has been confirmed by western blotting in human cancer cell lines and comparing the results with orthogonal methods of analysis (i.e. flow immunophenotyping using a panel of anti-PD-L1 antibodies). The specificity of IHC staining was established using genetically defined cells lines, and critically, staining was detected in cells with endogenous or engineered to express PD-L1 and negative in PD-L1 deficient or knock-down cancer cells. Specifically, the 9A11 antibody clone was compared to 4 other antibodies commercially available and used in other immunotherapy studies as shown in the figure below (Mahoney, Sun et al. 2015) across multiple tumor types such as might be encountered on this trial. Additional reproducibility data can be found on the cell signaling technology website https://www.cellsignal.com/products/primary-antibodies/pd-l1-405-9a11-mouse-mab/29122



Additional data supporting the use of the 9A11 antibody, and/or the techniques to be applied in this study, are provided by Howitt et al. JAMA Oncol. 2016 (cervix and vulvar cancers), Howitt et al. JAMA Oncol 2015 (MSI high endometrial cancers), Derks et al. Oncotarget 2016 (gastric cancer), Roemer et al. JCO 2016 and Ansell et al. NEJM 2015 (Hodgkin Lymphoma), George et al. Immunity 2017 (leiomyosarcoma), and Strickland et al. Oncotarget 2016 (ovarian cancer)   Furthermore, a recently published quantitative comparison demonstrates high concordance between PD-L1 staining between 9A11 and 5 other commercially available antibodies directed against PD-L1 including the PD-L1 IHC 22C3 PharmDx kit (Dako) approved by the FDA as a companion diagnostic for pembrolizumab and the PD-L1 SP142 Ventana test (Ventana medical systems) approved by the FDA as a complimentary diagnostic for nivolumab and atezolizumab (Gaule et al. JAMA Oncol 2016). This study was performed using automated scoring using a PD-L1 index microarray including cell line and tissue controls as well as a commercially available, genetically defined PD-L1 engineered cell line array with a range of controlled protein expressing cell lines. These data support the feasibility of PD-L1 IHC, specifically using the 9A11 antibody, in a clinical trial setting across multiple disease types, including specifically among patients with MSI tumors and demonstrate that the various patterns of PD-L1 expression (tumor, stromal, tumor-stromal interface) can be reliably determined.

Below, we also provide reproducibility data generated in our labs regarding PD-L1 staining in colorectal and lung cancer. We demonstrate consistency of PD-L1 staining with the 9A11 antibody on 2 separate days on 2 sequential tissue slides across 10

colorectal cases and 7 lung cancer cases.

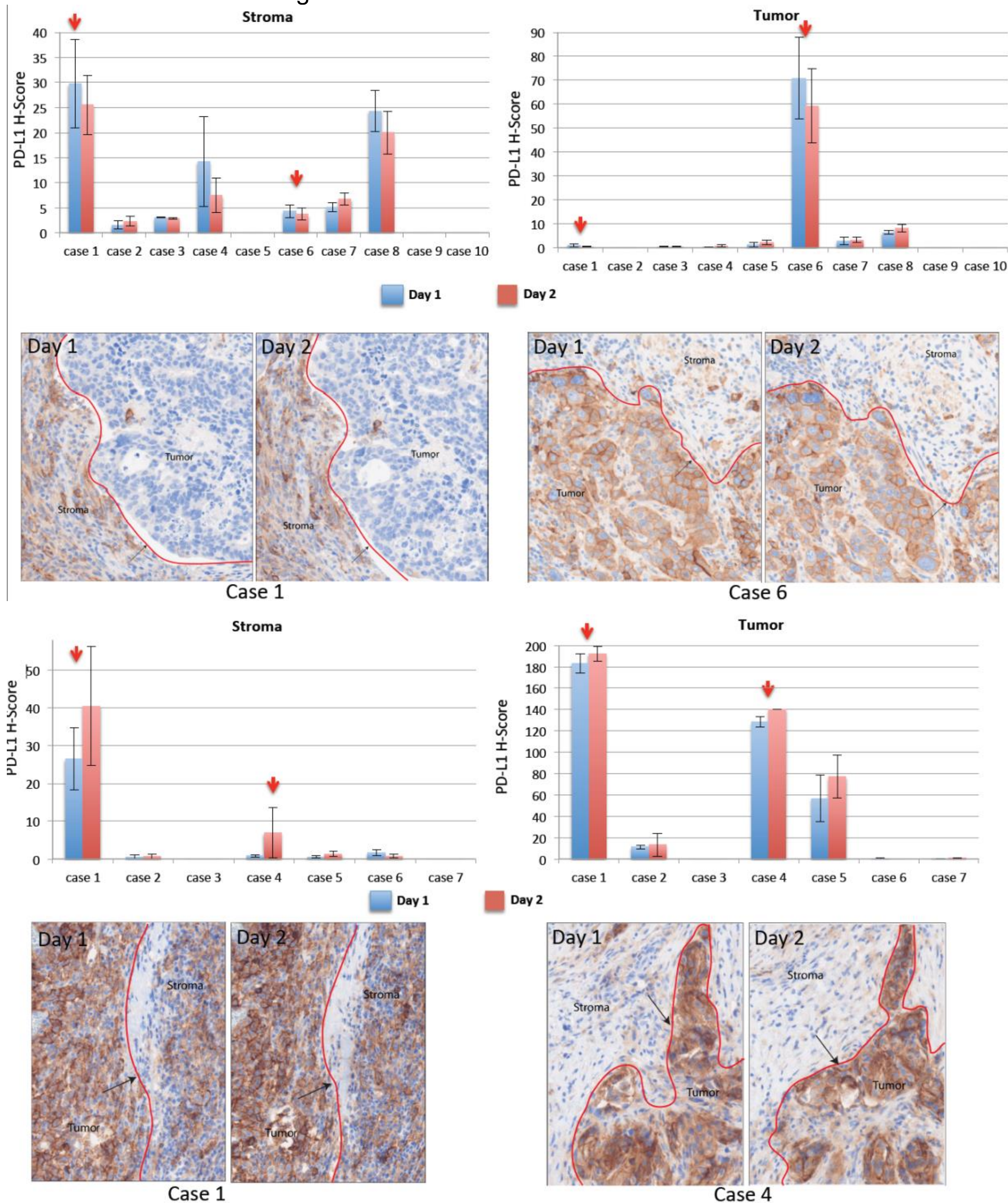
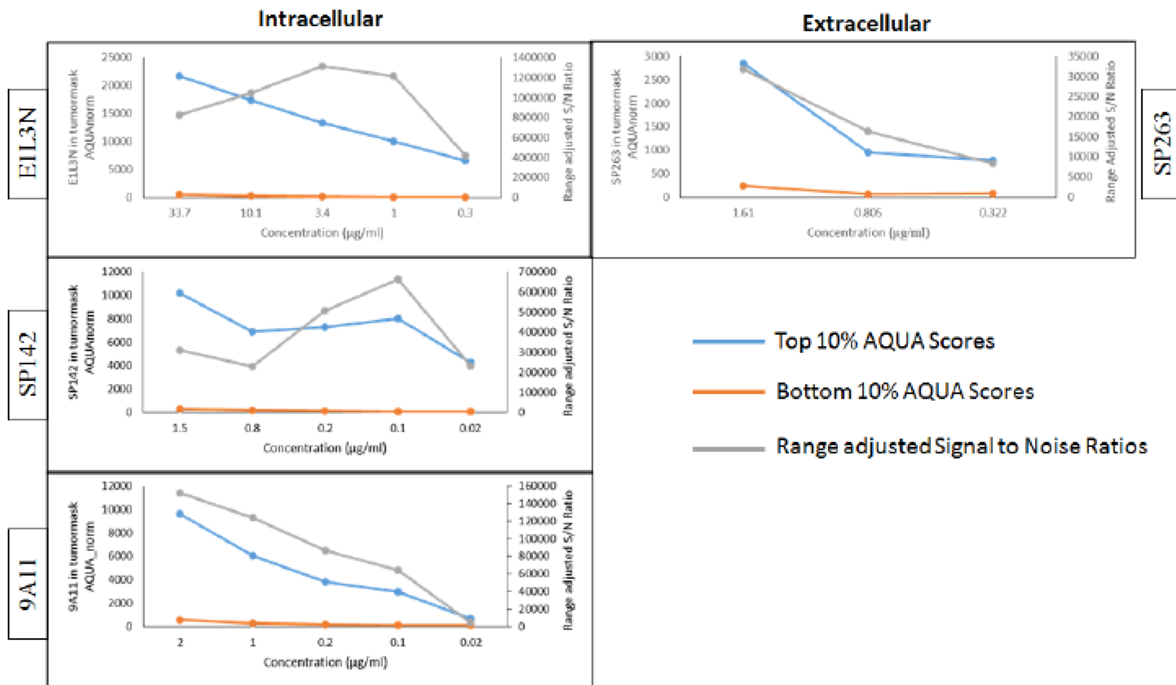


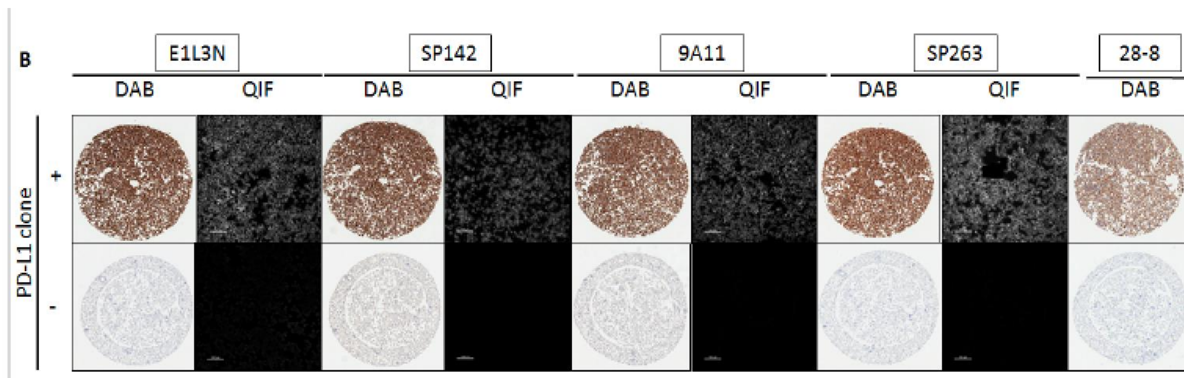
Figure: Analysis of PD-L1 staining in 10 randomly selected colorectal cancers (top) and 7 randomly selected NSCLC (bottom). IHC was performed on two different days and PD-L1 was analyzed in tumor and tumor surrounding stroma. Reproducibility was assessed on the 17 cases provided above demonstrating concordance in results obtained on different days.

Additional data confirming this antibody produced interpretable results on Western blot (suggesting specificity) and across a variety of samples including tissues known to be positive for PD-L1 such as tonsil, placenta, PD-L1 controls (HDLM-2 and PC-3), and a range of tumor tissue can be found here: https://media.cellsignal.com/pdf/29122.pdf.

The data presented from Gaule et al. also show specificity given concordance among PD-L1 antibodies specifically in the low PD-L1 scoring samples. The data obtained from Gaule et al. demonstrates highly concordant and reproducible findings with IHC performed using the 9A11 antibody compared to other commercially available and validated PD8L1 antibodies FDA approved as companion diagnostics (see figure below):



eFigure 3. Signal to Noise Curves Across Range of Concentrations Tested for Four PD-L1 Antibodies in Tumor Cases



eFigure 5. Schematic Layout of 15-Spot PD-L1 Reference TMA (A) and DAB Staining at Low Power Objective and QIF Staining at High Power Objective of 15-Spot reference PD-L1 IHC TMA for PD-L1 Antibodies (B)

Figures (above): Data demonstrating Signal to Noise ratios and DAB staining with the 9A11 antibody compared with other widely used PD-L1 antibodies. Copied from supplementary materials from Gaule et al. JAMA Oncol. 2016.

Beyond PD-L1 IHC, other tissue biomarkers are exploratory. As above, meta-analyses have demonstrated the feasibility of assessing many of these exploratory immune

markers across cancer types. Our pathology group has published the methods, protocols, and data for many of these antibodies including PD-L1 as above and other antibodies to be used in this series of studies such as PD-L2. Antibodies for many other markers such as CD3 and CD8 are commercial and are currently used in the CLIA-certified hospital laboratory of Brigham and Women's Hospital for diagnostic purposes. As part of the validation assays in a CLIA-certified laboratory, identical cases were stained multiple times and under a variety of staining conditions and the results reviewed by two certified pathologists. A positive control sample (such as classical Hodgkin Lymphoma for PD-L1 expression; primary mediastinal large B-cell lymphoma for PD-L2 expression) and negative control sample (benign lymph node) is stained with each experimental tissue biopsy sample. The controls are reviewed by a certified pathologist at the time of review of the experimental sample.

The primary analysis will be performed on PD-L1 expression as a continuous PD-L1 metric (% positive tumor cells). Digital, quantitative scoring of stained tissue is performed using a slide scanning and analysis platform. Quantitative assessment of positive staining uses the commercially provided algorithm for cell identification and positive pixels counting within a pre-defined DAB (brown, chromogenic) channel. We have shown that this method of analysis shows good correlation with pathologists' scoring: Mino-Kenudson M et al., Clin Cancer Res. 2010 Mar 1;16(5):1561-71. PMID:20179225. [31]. We have also used this method to score PD-L1 expression in tumor cells: Green MR et al., Blood. 2010 Oct 28;116(17):3268-77. PMID: 20628145.[32] The quantitative scoring method is summarized in the following steps:

1. The pathologist takes 3-5 representative pictures for each case at 200x magnification
2. Tissue classification of the images is performed, where tumor and stroma are distinguishably labeled.
3. The pathologists perform “cell segmentation” where the whole image is segmented into individual cells based on hematoxylin as a nuclear stain
4. The pathologist trains the software to identify PD-L1 (+) and PD-L1 (-) cells on a cell-to-cell basis, based on expression and comparison to control tissues (tonsil).
5. Thresholds are established and then applied to the whole image by the pathologist.
6. A second pathologist checks the threshold, and either approves or disapproves it. In case of disproval, the two pathologists reconcile between them.
7. Scoring for the positive cells is then performed in the tumor compartment.

Depending on tissue availability, other exploratory IHC analyses will be performed using semi quantitative scoring, although multiplex IF may be used as below to analyze multiple markers in tandem. Chen et al. [30] describes a semi-quantitative scoring method, which is in accordance with typical biomarker scoring in anatomic and surgical pathology [30]. Briefly, staining is scored according to intensity (0= no staining, 1= weak staining, 2= moderate staining, 3= strong staining), staining pattern (M= predominantly cell membrane; C= predominantly cell cytoplasm), and the percentage of cells showing positive staining (0-100%). The semi-quantitative scoring is performed for: 1) the neoplastic tumor cells and 2) the non-neoplastic infiltrating immune cells.

In the research setting, all cases are reviewed by two pathologists and any discordant results resolved by consensus review. Significantly discordant scoring results have been rare during case evaluations [30]. IHC scoring for markers (such as the PD-Ligands) that stain macrophages, dendritic cells, and other cells of heterogenous morphology will also be semi-quantitative and performed by a pathologist to capture 1) the percentage of neoplastic cells positive for biomarker expression, intensity of expression, and

membrane or cytoplasmic expression, and 2) the percentage of non-neoplastic cells (macrophages, dendritic cells, endothelial cells) positive for biomarker expression, intensity of expression, and membrane or cytoplasmic expression.

Scoring for PD-1+ (and other lymphoid markers) lymphocytes will be accomplished using a standard algorithm, developed for quantifying nuclear stains, but found to be applicable to quantifying membrane staining of cells with a very high N:C ratio, such as lymphocytes (Nuclear algorithm). The output is number of positive-staining cells per unit area (micron2).

For image analysis:

IHC stained slides will be digitally scanned using the Aperio ScanScope XT (Leica

Microsystems, Buffalo Grove, IL). The instrumentation is housed in the Tissue Microarray and

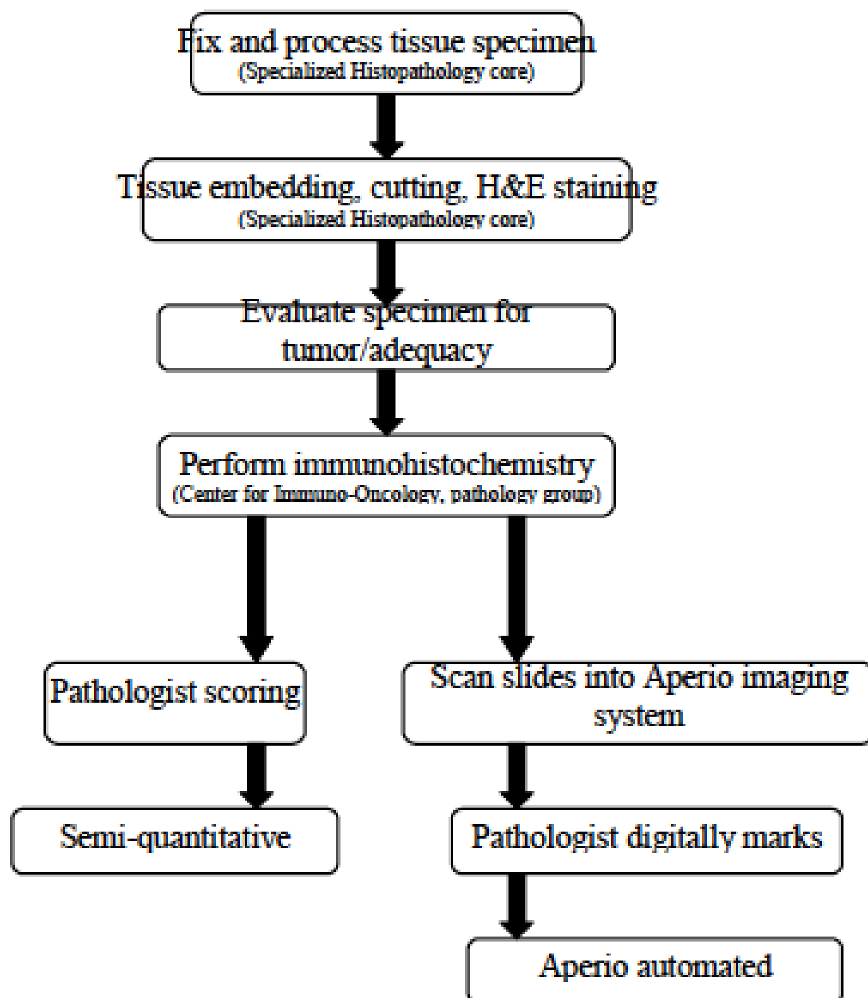
Imaging Core (TMI) facility of the Dana-Farber/ Harvard Cancer Center (DF-/HCC). This facility is located adjacent to the office of Dr. Scott Rodig in the Department of Pathology. All digital images are stored on servers owned by the TMI core facility and accessed via the internet using a password-protected log-in.

Digital images are viewed using ImageScope Software(version 10.0.35.1800; Leica) on standard PCs. Slides are digitally annotated by the pathologist (Dr. S. Rodig) to identify the region of interest and analysis.

Quantitative analysis is performed using analytical software (for PD-Ligands). Intensity of staining is also captured automatically using the above algorithms and assigned a score (0, 1, 2, or 3) based upon the average optical density of the region or cells. All results are exported into an excel spreadsheet.

Individual scoring data will be compared to clinical parameters to determine if there is an association with outcome. Scores using a combination of biomarker data will also be considered.

Below, we provide a schematic of the workflow for the tissue-based biomarker analysis:



The semi-quantitative scoring for this study is in accordance with those published previously [30, 33] and will include scores for both the neoplastic and non-neoplastic cells within the tumor microenvironment. Data derived from pathologist visual scoring (semi-quantitative, but with increased specificity for delineating neoplastic and non-neoplastic cells) and pathologist-assisted, automated scoring (quantitative, but without accurately delineating neoplastic and non-neoplastic cells) for each marker of interest will be assessed for its clinical value. As necessary, the data from combinations of markers will also be considered.

All IHC staining will be performed in the Center for Immuno-Oncology Pathology Core at Dana-Farber/Harvard Cancer Center Specialized Histopathology Core which is a designated Cancer Immune Monitoring and Analaysis Center (CIMAC). Standard EnVision two-step (indirect) staining method will be utilized. Four-micrometer-thick sections will be cut, deparaffinized, rehydrated and subjected to heat mediated antigen retrieval in citrate buffer (pH 6) (Invitrogen) by steaming for 30 minutes. After cooling, tissue sections will be incubated with peroxidase block (DAKO, Carpinteria, CA) for five minutes, then serum free protein block (DAKO) for 20 minutes. Slides will be incubated at room temperature for one hour with a primary antibody. Antibodies will be diluted in Da Vinci Green Diluent (Biocare Medical, Concord, CA). EnvisionTM anti-mouse HRP-labeled polymer (DAKO) will be applied to the sections for 30 minutes, followed by

visualization by using the chromogen 3, 3-diaminobenzidine (DAKO). All the sections will then be counterstained with haematoxylin, dehydrated, mounted and coverslipped. Positive and negative controls shall be included in each staining. Known positive stained Hodgkin lymphoma (CD274) will be used as external control (separate slides). Stained slides will be stored at room temperature.

For multiplex immunofluorescence, we use the Opal™ Multiplex Immunohistochemistry kit, which is a practical workflow for simultaneous detection of up to 6 tissue biomarkers plus nuclear counterstain, within a single image. Antibodies for simultaneous IHC may be selected based on performance, rather than species. This kit provides the tools to interrogate multiple pathways while retaining context provided by tissue images.

Methods: Step1. Overnight baked FFPE slides are de-paraffinized and then placed in AR6 solution (PerkinElmer) for 15min using a microwave. AR6 solution was developed for use in the multicolor immunohistochemistry protocol in microwave treatment, and provides antigen retrieval for FFPE tissues, quenches endogenous peroxidase, and removes antibodies after epitopes have been labeled with Opal dyes. Step 2. After the antigen retrieval step, the slides are cooled in room temperature for 20 min and then washed with distilled water and then with Tris buffer. Step 3. Afterwards, the first antibody for Protein 1 (example: PD1) is applied for 40 minutes. Step 4. HRP-linked secondary antibody (Dako Envision kit) is applied for 30 minutes. Step 5. After a few washes in Tris buffer (3x2minutes) the 1st Opal Fluorophore is applied (1:50, diluted in Amplification Diluent) for 5 minutes. Step 6. The slides are washed in Tris buffer and placed in pre-heated AR6 solution for 15 minutes using a microwave. Step 7. After this, the first antibody for Protein 2 (example: CD30) is applied and all the Steps 2-6 are repeated. Currently, using the Opal-7 Color Immunohistochemistry Kit, we can stain for 6 different proteins and Dapi.

To accurately visualize, analyze and quantify the different biomarkers in tissue sections, we use InForm®, which is an automated image analysis software package (PerkimElmer). inForm enables accurate per cell quantification of specific biomarkers defined within multiple tissue types including tumor and stroma and the phenotyping of immune cells and other cells in situ in solid tissue. It also enables the quantitation of weakly expressing and overlapping biomarkers within cells, nuclei and membranes, which cannot be identified by eye. This sensitive approach provides researchers with the confidence to discover indicators of disease and uncover relationships between biomarkers, improving results reliability.

Assay performance

As above, validation has been performed on retrospective data sets. Protocols have been optimized and standardized to minimize staining variance. Positive control and negative controls were used and stained separately with each batch of slides for PD-L1 staining. The IHC staining of PD-L1 has been performed in two different labs by three different technicians on whole tissue sections of Hodgkin lymphomas, melanomas, lung cancers and renal cell carcinomas. Three readers were involved, confirming the good reproducibility of the assay. Consistency of automated PD-L1 scoring has been confirmed on 10 colorectal and 7 lung cancer cases.

Laboratory information

The IHC and IF staining will be conducted in a research laboratory with GLP standard under the direction of Dr. Scott Rodig. Dr. Scott Rodig is the overall supervisor of the lab, and is a hematopathologist at the Brigham and Women's hospital with prior expertise evaluating PD1, PDL1 and other immunologic markers in paraffin embedded tumor

samples [30]. PD-L1 IHC and the multiplex IF panel examining CD8+ T-cell infiltration will be performed under the auspices of the CIMAC. Other exploratory IF panels, specifically those exploring hypothesized mechanisms of resistance (CTLA-4, MHC-1, MHC-2, Lag-3, B2M, Na channel) will be performed in the same laboratory but are not currently planned to be incorporated as core CIMAC assays.

Laboratory methods for evaluating serum and PBMCs:

Peripheral blood mononuclear cells (PBMC's) will be analyzed using CyTOF via the CIMAC. DFCI CIMAC reference document is in development and validation data will be sent to NCI under the CIMAC vetting process. Existing analytical validation data is available from Klensteuber et al.: Standardization and Quality Control for High-Dimensional Mass Cytometry Studies of Human Samples Cytometry A. 2016 Oct; 89(10): 903–913. As an example of the capabilities of the CyTOF assay in general to be offered through the CIMAC, please see attached reference document provided by Human Immune Monitoring Ceter at Mt. Sinai. As described in this reference document, "CyTOF offers single-cell level detection of 40+ markers with inter-channel cross talk levels <4% and very low background signals (<2 integrated dual counts ion per cell). The accuracy of individual antibodies is expected to be comparable to the analogous antibody clones used for conventional flow cytometry, and the accuracy of defined cell population identification is comparable to, if not better than flow cytometry."

Cytokine / serum analysis:

Cytokines will be analyzed using Olink via the CIMAC (Mt. Sinai). Please see attached reference document provided by Human Immune Monitoring Center at Mt. Sinai. As described in this reference document, O-link is "a 92-parameter targeted soluble factor immunoassay" that allows for "quantification and correlation of multiplexed cytokine levels." Precision, analytical sensitivity, and analytical specificity are described in more detail in this document.

A. Facilities & personnel

PD-L1 IHC and multiplex IF including CD8+ T-cell analysis will be performed in the laboratory of Dr. Scott Rodig at the DFCI as part of the DFCI CIMAC. Other multiplex IF panels described above examining mechanisms of resistance will be performed in the same laboratory, but are not currently CIMAC assays.

CyTOF will be performed via the DFCI CIMAC under the direction of Dr. Mariano Severgnini.

Olink will be performed via the Mt. Sinai CIMAC - Human Monitoring Core / Dr. Miriam Merad MD PhD, Director.

IV. Statistical Considerstions

A. Endpoints

1. Primary

a) PD-L1 positivity

b) Percent of circulating, activated CD8+/HLA-DR+/Ki-67+ T-cells

2. Secondary

a) PD-L1 positive cases

b) Infiltration of lymphocytes per high powered field

c) Exploratory analyses of expression levels of various serum cytokine and tumor immune biomarkers

B. Case selection and Sample Size

All consenting cases with adequate biospecimen available from the Z1D subarm.

Sample size estimate: 44 patients for tissue analyses, 37 patients for blood analyses

C. Analysis plan:

Regarding the primary endpoint of PD-L1 expression in tumor tissue, an unpaired t test will be used to compare the percent positive tumor cells in responders vs. non-responders. Responders will be determined using the primary endpoint of the trial – objective tumor response. Using the data provided in the supplementary appendix to Le et al. as a basis for the power calculations (Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509-20) it is assumed that PD-L1 expression, measured as a percentage of positive cells, will have a mean percentage of roughly 20% and a standard deviation of about 10%. Using these figures and assuming also that roughly 24% of all patients in this subarm will be responders, with approximately 44 patients with tissue available (roughly 11 responders and 33 non-responders) there is at least 80% power to detect a mean difference of 10% (e.g., 29% versus 19%) in PD-L1 expression between responders and non-responders using a two-sided 0.05 level t-test. The 24% figure used for the calculations was chosen based on the results of this arm recently presented at SITC.

Given the small sample size of this subarm, a categorical analysis to correlate objective response with PD-L1 positivity (for example via Fisher's exact test using a 5% cutpoint for PD-L1 positivity) will not have meaningful power, hence the proposed analyses will be done on the continuous PD-L1 metric and any categorical analyses will be considered exploratory. Following the logic of the power analyses above, there is 80% power to detect a 1.00 standard deviation difference in the means of any quantitative biomarker between responders and non-responders (assuming the overall response rate above) using a two-sided 0.05 level t-test.

The primary objective is to examine the association between a single biomarker (PD-L1 expression) and response. Thus, no statistical adjustments are planned for this analysis. Other planned IF and IHC testing is exploratory and largely based on mechanistic hypotheses (e.g. exploring for treatment resistance as a consequence of defects in the MHC I or MHC II expression and antigen presentation pathways). Therefore, exploratory analyses will be descriptive in nature with no planned adjustment for multiple testing.

When genomics data are available from the NCI MATCH study we may perform exploratory analyses to examine associations with protein expression across the markers tested. Specifically, there are immediate plans to attempt to look at numbers of MOIs based on the NCI MATCH assay as a proxy for high mutational burden for arm Z1D and correlate this with response. We will correlate this measure with our primary endpoint of PD-L1 expression as well as with our other exploratory endpoints.

The primary endpoint of studies to be conducted on peripheral blood will evaluate changes in percentage of circulating CD8+/HLA-DR+/Ki-67+ T cells and response. We will compare the change in percentage of this cell population between responder and non-responder patients. To give a sense of power for studies to be done on paired tissue and blood samples (baseline versus progression), and blood samples (paired within patient comparing baseline vs progression) for analyses of various quantitative biomarkers being proposed here, differences will be taken for all measures and the hypotheses is that biomarkers will change (increase or decrease) post therapy. PD-L1 expression on tumor cells, infiltrating and circulating T-cell populations, myeloid derived suppressor cells, and immunologic cytokines and other immunologic markers. Assuming that within this cohort we will obtain paired samples on conservatively 40 patients, using a two-sided 0.05 level paired t-test there is at least 80% power to detect a 0.46 SD change in mean of a quantitative correlative parameter using a two-sided test with 5% type I error. Quantitative measure will be transformed to attain normality or appropriate non-parametric tests will be conducted. With this level of consent for these studies in this cohort there is sufficient power for meaningful analyses. Power for these tests will be further improved if a greater percentage of the cohort of 47 patients consent and provide post-baseline (progression) samples for analysis.

D. Rationale for the sample size estimate:

As described above, assuming that roughly 24% of all patients in this subarm will be responders, with 44 patients (expected distribution to be roughly 11 responders and 33 non-responders) there is at least 80% power to detect a mean difference of 10% (e.g., 29% versus 19%) in PD-L1 expression between responders and non-responders using a two-sided 0.05 level t-test.

For the blood studies, assuming approximately 40 patients have usable specimens pre and post treatment, using a two-sided 0.05 level paired t-test there is at least 80% power to detect a 0.46 SD change in mean of a quantitative correlative parameter using a two-sided test with 5% type I error.

We propose to evaluate associations with PD-L1 expression measured as a continuous metric of positivity (as described above) as opposed to a single cutoff. Exploratory analyses will assess specific cutpoints of PD-L1 positivity (e.g. 1%, 5%, 10%, etc.) as potential biomarkers, as these cutpoints have been described previously in the literature. To our knowledge there are unfortunately no on-treatment pathologic specimens available and therefore magnitude of change on treatment cannot be assessed. As mentioned in the above statistical analysis plan, assuming that within this cohort we will obtain paired samples on conservatively 40 patients that yield evaluable data for our flow-cytometric biomarkers, using a two-sided 0.05 level paired t-test there is at least 80% power to detect a 0.46 SD change in mean of a quantitative correlative parameter using a two-sided test with 5% type I error. Quantitative measures will be transformed to attain normality or appropriate non-parametric tests will be conducted as appropriate. We will also compare changes between responder and non-responder groups for the purpose of our analyses.

E. Statistical considerations for secondary objectives (if applicable)

Our secondary objectives for testing multiple biomarkers in tissue and serum are exploratory. However, these objectives are based on strong biologic rationale

and may also identify future targets for combinations with PD-1 inhibitors in patients with mismatch repair deficiency or other tumors.

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