PILOT STUDY OF NIVOLUMAB IN PEDIATRIC PATIENTS WITH HYPERMUTANT CANCERS

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PROTOCOL SYNOPSIS

Protocol Title:	**Pilot Study of Nivolumab in Pediatric Patients with Hypermutant Cancers**
Protocol Number:	SickKids Protocol No.: 1000053649 Ozmosis Protocol No.: OZM-075
Sponsor:	Dr. Eric Bouffet, The Hospital for Sick Children
Phase of Development:	Pilot Study
Methodology:	Single arm pilot study (proof of concept study)
Study Duration:	Maximum duration of treatment: 24 months. Follow-up: 12 months. Duration of enrollment: 24 months. Estimated duration of the whole protocol: 60 months.
Objectives:	**Primary Objective:** 1. To evaluate the objective response rate (ORR) to nivolumab in paediatric patients with refractory or recurrent hypermutated malignancies, including patients with replication repair deficiencies (RRD), such as constitutional mismatch repair deficiency (CMMRD). **Secondary Objectives:** 1. To determine the progression free survival (PFS) and overall survival (OS) of paediatric patients with progressive or recurrent hypermutated malignancies, including RRD patients such as CMMRD, treated with nivolumab. 2. To evaluate safety and tolerability of nivolumab administered as a single agent at the adult recommended dose of 3 mg/kg every 2 weeks. To define and describe the toxicities in paediatric patients

	with progressive or recurrent hypermutated malignancies including RRD patients, such as CMMRD. **<u>Companion Biomarker Exploratory Objectives:</u>** 1) To explore associations between tumour mutation burden (TMB) and response to nivolumab. 2) To discover biomarkers predicting response of hypermutant cancers undergoing PD-1 blockade by investigating tumour neoantigen formation, specific T-cell receptor rearrangements (TCRR) of tumour infiltrating lymphocytes (TILs) and detailed characterization and activation of the immune infiltrations including the TILs. 3) To explore the use of minimally invasive methods to monitor and predict response to immune checkpoint inhibition in hypermutant cancers by investigating TCRR, immuno-phenotypic profiling of specific immune cells and their activation as a prognostic factor and variances throughout treatment as a response to therapy. In addition, investigating circulating tumour DNA (ctDNA) from serial peripheral blood samples as a surrogate marker of response.
Sample Size:	50 patients with hypermutant refractory or recurrent tumours across all diagnoses will be recruited, including those with RRD, such as CMMRD.
Investigational Product:	**Nivolumab** (also referred to as Opdivo® or BMS-936558 or MDX1106) is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets and inhibits the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. **Regimen:** Nivolumab will be administered every 14 days until confirmed disease progression or treatment discontinuation due to unacceptable toxicities. Treatment may extend up to 2 years in patients who show clinical and radiological benefit.

	Dose: 3 mg/kg intravenously (IV) as a continuous infusion over 60 min (+/-10 min window).
Overview of Study Design:	This is an open-label, single arm, multi-center, pilot study of nivolumab in paediatric patients with recurrent or refractory hypermutant malignancies aged 12 months to <25 years of age. Local centres are only obligated to treat/ admit patients in accordance their age range capabilities. The purpose of this study is to assess response of treatment with nivolumab in children with hypermutated cancers, including those with RRD, such as CMMRD syndrome. This study will be performed in two parts: **Part I – Molecular Profiling** and **Part II – Treatment and Companion Biomarker Studies**. In order to participate in **Part I**, patient's cancer specimen must undergo a gene sequencing panel to determine TMB or else have proof of RRD (for those patients for whom it is not possible to obtain a TMB level). Once TMB or proof of RRD is established, and **Part II** eligibility is confirmed, patients will be stratified into cohorts based on levels of TMB or RRD status (as outlined below). The TMB assay must be performed in a Study Chair or Co-chair specified, CLIA-certified laboratory. Proof of RRD status may be established by assays performed in either a CLIA or ISO 15189-certified laboratory in accordance with local regulations and at the discretion of the Study Chair or Co-chair. **<u>Part I - Molecular Profiling</u>** Patients with recurrent or relapsed paediatric cancers whom are suspected to be hypermutant (as defined in *Section 4.2.1*) will be consented to **Part I**. Those patients will submit a specimen (as outlined in the *Lab Manual*) and undergo either the study-specific, next-generation sequencing (NGS) targeted gene panel to determine TMB level or, only when sufficient neoplastic specimen is unavailable, provide proof of or tissue for diagnosis

	of an RRD disorder (see **Part I – Molecular Profiling** Inclusion Criteria in *Section 4.2.1).* **<u>Part II - Treatment and Companion Biomarker Studies</u>** Patients with cancers that have been confirmed as hypermutant based on a report by a specific-TMB assay (acquired via **Part I** participation or previously) or have proof of RRD will be consented and enrolled into to **Part II**. Cohort stratification of patients enrolled in **Part II** is based on their identified TMB levels or RRD status: • Cohort A: TMB ≥5 but <10 mutations/Mb (max. 20 patients); • Cohort B: TMB ≥10 mutations /Mb (max. 30 patients); • Cohort C: unobtainable TMB level in a patient with RRD. N.B.: *Patients stratified to Cohort C will be reallocated to Cohorts A or B if a TMB value subsequently becomes available.* All eligible patients will receive nivolumab intravenously (IV) at a dose of 3mg/kg administered every 14 days (two weeks). Two doses comprise one cycle (28 days or four weeks). Evaluations will be performed according to the schedule provided. N.B: *We strongly recommend that all patients with confirmed or suspected RRD syndromes undergo screening for possible concurrent malignancies (see Section 8.0).* Samples to perform *Companion Biomarkers* research to further our understanding of paediatric hypermutant cancer response to nivolumab will be obtained (see *Lab Manual* for details). Patients will be monitored for toxicity using standard National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03. Response assessment will use iRECIST criteria for solid

	tumours (modified for neuroblastoma using the revised INRC), iRANO criteria for CNS malignancies, RECIL 2017 criteria for lymphomas, revised criteria according to Creutzig, et al. (2012) for acute myeloid leukemia (AML; see *Section 10.13*), and criteria as specified in *Section 10.14* for acute lymphoblastic leukemia (ALL). The Safety visit will be completed when the patient comes off treatment and prior to entering the Follow-Up Period.
Inclusion Criteria:	**<u>Part I</u>** **1. Consent/ Assent:** Patient and/or Legally Acceptable Representative (LAR; such as a parent or guardian, as applicable) must be willing and able to provide written informed consent/assent for the trial as per local requirements. **2. Age:** patients must be ≥ 12 months and <25 years of age at time of **Part I** enrollment. Local centres are only obligated to treat/ admit patients in accordance their age range capabilities. **3. Recurrent or relapse paediatric cancer patients suspected to be hypermutant,** including those exhibiting evidence of one or more of the following: a. high microsatellite instability (MSI-H) in current or previous tumour; b. a mutation causing loss of mismatch repair gene (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM* or *MSH3*) expression; c. hypermutation by local sequencing in current or previous tumour; d. a history of CMMRD, Lynch syndrome, xeroderma pigmentosum (XP), or other established disorder associated with an elevated tumour mutation rate; e. a functional mutation of polymerase genes (*POLE* or *POLD1*) in current or previous tumour; f. a functionally impaired RRD pathway by other means; g. a temozolomide (TMZ) treated current or previous CNS tumour;

h. a predisposing hypermutant cancer signature (i.e. dysregulation of an apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deamination or UV-associated);

i. other factors, which may predicate an elevated mutation burden at the discretion of the Study Chair or Co-Chair.

4. **Diagnosis:** patients must have histologic or cytologic confirmation of malignancy at the time of initial diagnosis or relapse (as specified above). Patients with multiple concurrent and/or sequential neoplasms are eligible, including CNS and haematological malignancies.
5. **Specimen availability:** patients must be able to provide specimen (archival or newly obtained biopsy) of a tumor lesion, appropriately obtained and preserved in a manner compatible for TMB analysis or applicable IHC staining for MMR gene protein expression, if applicable (as described in the *Lab Manual*). Only those with an already ascertained TMB level report from the laboratory specified in the *Lab Manual* or those with proof of RRD as outlined in the *Lab Manual* will be exempt from mandatory tissue submission.
   If tissue (including archival) is not available, a new tissue specimen may be obtained if deemed clinically appropriate. Any such biopsy will *not* be considered a trial-related procedure.

Part II

1. **Consent/ Assent:** Patient and Legally Acceptable Representative (LAR; such as a parent or guardian, as applicable) must be willing and able to provide written informed consent/assent for the trial as per local requirements.
2. **Confirmation of hypermutation or Proof of RRD:** patient must have completed and verified a sufficient TMB level or have proof of RRD diagnosed in the appropriate lab, as outlined in the *Lab Manual.*

	3. **Age:** patients must be ≥ 12 months and < 25 years of age at the time of **Part II** enrollment. Local centres are only obligated to treat/ admit patients in accordance their age range capabilities. 4. **Diagnosis:** patients must have had histologic verification of malignancy at the time of initial diagnosis or at relapse (as specified above). Patients with multiple concurrent and/or sequential neoplasms are eligible, including CNS and haematological malignancies. 5. **Disease status:** patients must have either measurable or evaluable disease in accordance with criteria as outlined in *Section 10*. Tumour lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions. 6. **Treatment options:** patient's current disease state must be one for which there is no known curative therapy or therapy proven to prolong survival with an acceptable quality of life. Chemotherapy-naïve patients will be eligible in cases where first-line therapy does not include chemotherapy (e.g. surgery alone for management of ependymoma). 7. **Performance status**: Karnofsky ≥ 50% for patients > 16 years of age or Lansky ≥ 50 for patients ≤ 16 years of age. Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score. 8. **Previous treatment:** patients must have fully recovered from the acute toxic effects of all prior anti-cancer therapy. a. **Myelosuppressive chemotherapy:** at least 21 days after the last dose of myelosuppressive chemotherapy (42 days if prior nitrosourea). b. **Hematopoietic growth factors**: at least 14 days after the last dose of a long-acting growth factor (e.g. Neulasta) or 7 days for short-acting growth factor. For agents that have known adverse events occurring beyond 7 days after administration, this period must be

extended beyond the time during which adverse events are known to occur. The duration of this interval must be discussed with the Study Chair or Co-Chair.

c. **Biologic (anti-neoplastic agent):** at least 14 days after the last dose of a biologic agent. For agents that have known adverse events occurring beyond 14 days after administration, this period must be extended beyond the time during which adverse events are known to occur. The duration of this interval must be discussed with the Study Chair or Co-Chair.

d. **Monoclonal antibodies:** at least three (3) half-lives of the antibody after the last dose of a monoclonal antibody.

e. **Radiation Therapy (XRT):** at least 14 days after local palliative XRT (small port). At least 150 days must have elapsed if prior Total Body Irradiation, craniospinal XRT or if ≥ 50% radiation of pelvis. At least 42 days must have elapsed if other substantial BM radiation.

f. **Stem Cell Infusion without Total Body Irradiation (TBI):** no evidence of active graft vs. host disease and at least 56 days must have elapsed after transplant or stem cell infusion. Patients with prior allogeneic transplants (including solid organ) are not eligible.

9. Organ Function Requirements:

a. Adequate BM Function Defined as

i. Peripheral absolute neutrophil count (ANC) ≥0.75 x 10^9/L or 750/mm^3.

ii. Platelet count ≥75 x 10^9/L or 75,000/mm^3 (transfusion independent, defined as not receiving platelet transfusions for at least 7 days prior to enrollment.

iii. Hemoglobin ≥ 90g/L (transfusion permitted).

iv. Patients with known BM metastatic disease or haematological malignancies will be eligible for study provided they meet haematological criteria. These patients may receive transfusions (e.g. to achieve platelet threshold) provided they are not known to be refractory to platelet transfusions but will not be evaluable for hematologic toxicity.

b. Adequate Renal Function Defined as:

A serum creatinine based on age/gender as provided in *Table 3* (see *Section 4.2.2*)

c. Adequate Liver Function Defined as:

i. Bilirubin (sum of conjugated + unconjugated or total bilirubin) ≤1.5x institutional upper limit of normal (ULN) for age (except for patients with Gilbert's Syndrome, when bilirubin of < 51 µmol/L or 3.0 mg/dL is permitted).

ii. ALT/AST:

1. ≤ 2.5 x institutional ULN for patients without liver metastases.
2. ≤ 5 x institutional ULN for patients with liver metastases.

d. Adequate Pulmonary Function Defined as:

No history of chronic pulmonary disease (such as Cystic Fibrosis) and no evidence of dyspnea at rest, no exercise intolerance due to pulmonary insufficiency and a pulse oximetry > 92% on room air.

e. Adequate Pancreatic Function Defined as:

Serum lipase ≤ ULN. Patients with glucose intolerance should be on a stable regiment and be monitored.

10. For patients with brain tumors, debulking surgery prior to treatment with nivolumab should be considered when appropriate to reduce the risk of pseudoprogression-associated toxicities. Such debulking surgery is not mandatory for trial enrollment. Patients should be recovered from surgery and wait at least 7 days from surgery before first dose.

Exclusion Criteria **(Part II only):**	**1. Women who are pregnant or breastfeeding and men who are sexually active with women of childbearing potential (WOCBP)* who are not willing to use effective contraception, or to practice abstinence if this is the usual lifestyle and preferred contraception for the patient. **** • Pregnant or breast-feeding women will not be entered on this study due to risks of fetal and teratogenic adverse events as there is yet no available information regarding human fetal or teratogenic toxicities. • WOCBP must have a negative pregnancy test every 4 weeks. During **Part II** screening, WOCBP must have a negative serum pregnancy test. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab administration. WOCBP who are sexually active, must be willing to adhere to effective contraception during treatment and for 5 months after the last dose of nivolumab. • Men who are sexually active with WOCBP must be willing to adhere to effective contraception during treatment and for 7 months after the last dose of nivolumab. • Women who are surgically sterile, as well as azoospermic men do not require contraception. **"Women of childbearing potential" is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal.* *** List of contraception methods is provided in the Appendix II* **2. Concomitant Medications** **a. Corticosteroids:** Patients requiring systemic steroid therapy or any other form of immunosuppressive therapy within seven (7) days prior

to first dose of trial therapy or while on trial are not eligible. The use of physiologic doses of corticosteroids (up to 5mg/m^2/day prednisone equivalent) is permitted following discussion with the Study Chair or Co-Chair.

Note: *Use of topical, ocular, intra-articular, intra-nasal or inhaled corticosteroids will not render a patient ineligible. A brief course of corticosteroids for prophylaxis (e.g. contrast dye allergy) or for treatment of non-autoimmune conditions (e.g. delayed-type hypersensitivity reaction caused by contact allergen) is permitted if completed at least 7 days prior to initiation of therapy.*

b. **Investigational Drugs:** Patients who are currently receiving another investigational drug are not eligible.

c. **Anti-cancer Agents:** Patients who are currently receiving other anti-cancer agents are not eligible.

3. Patients with a History of Autoimmune Disease

- Patients with a history of autoimmune disorder that has required systemic treatment in the previous two (2) years are not eligible. Asymptomatic laboratory abnormalities (e.g. ANA, rheumatoid factor, altered thyroid function studies) will not render a patient ineligible in the absence of a diagnosis of an autoimmune disorder. Replacement therapy (e.g. thyroxine, insulin or physiologic corticosteroid replacement therapy) is not considered a form of systemic treatment.

4. Infection: Patients who have an uncontrolled infection are not eligible.

5. HIV and/or Hepatitis B/C patients: Patients with known HIV/AIDS or acute/chronic Hepatitis B or C are excluded.

6. Transplant patients: Patients who have received prior allogeneic Bone Marrow (BM) transplants or prior solid organ transplantation are not eligible.

7. Non-Compliance: Patients who in the opinion of the investigator may not be able to comply with the safety monitoring requirements of the study are not eligible.

	8. **Previous anti-PD-1 and/or anti-PD-L1 therapy:** Patients who have received prior anti-PD-1 and/or anti-PD-L1 directed therapy (mAb or small molecule) are not eligible. 9. **Live vaccines:** Patients who have received a live vaccine within 30 days of start of study treatment are not eligible.
Criteria for Efficacy Evaluation:	**Primary efficacy outcome measures:** Tumour disease evaluation, includes objective response rate (ORR = complete response [CR] and partial response [PR]) as defined by iRECIST response criteria for solid tumours (revised for neuroblastoma using the revised INRC), iRANO response criteria for CNS malignancies, RECIL 2017 response criteria for lymphomas, revised criteria according to Creutzig, *et al.* (2012) for acute myeloid leukemia (AML; see *Section 10.13*), and response criteria as specified in *Section 10.14* for acute lymphoblastic leukemia (ALL). Patients with response of 'stable disease' (SD) will also be reported as part of the final analysis of clinical benefit, but will not contribute to the primary efficacy outcome measure. **Secondary efficacy outcome measures:** PFS is defined as the time from the first dose of the study drug administration to the occurrence of disease progression or death from any cause during the study. OS is defined as time from first dose of study drug to death from any cause.
Criteria for Safety Evaluation:	A safety evaluation by the Safety Committee will be performed after Patient 1-Cycle 1, Patient 4-Cycle 1, Patient 7-Cycle 1, and Patient 10-Cycle 1. Enrollment will be held after each time point until a safety evaluation of adverse events (AE) and serious adverse events (SAE) confirms it is safe to resume enrollment.

	Toxicities will be described and defined in paediatric patients, focusing on SAEs, using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03. Toxicities (drug-related AEs) of any grade, plus all Grade ≥ 3 AEs, all Grade ≥ 3 laboratory toxicities and all SAEs as defined in *Section 12* will be collected for this study.
Statistical Methods:	The initial aim for this pilot study was to accrue 20 paediatric patients with biallelic mismatch repair disease (bMMRD) tumours. Following amendment, the study inclusion criteria are expanded to include those with hypermutant tumours (as determined by estimation of TMB by targeted next-generation gene sequencing), as well as patients with CMMRD tumours. Consequently, the total enrollment will be increased to 50 patients. The primary goal of this study is to determine the ORR of nivolumab in patients with hypermutant tumours. The study is predicated on the hypothesis that patients with hypermutated tumours will derive greater benefit from nivolumab than those without; however, the relevant threshold of TMB is not yet established. Consequently, in this pilot study, patients will be recruited into two cohorts depending on TMB. This will provide the opportunity to treat patients with a relatively low TMB (≥5 to <10/Mb, estimated ~15% of tumors) to assess for responses to nivolumab, while a separate cohort for patients with TMB (≥10/Mb, estimated ~5% of tumors) will ensure that overall study enrollment is not dominated by patients with lower TMB. A Simon two-stage design will be used within each cohort. For cohort A (TMB (≥5 to <10/Mb), the null hypothesis that the true response rate is only 10% will be tested against a one-sided alternative. In the first stage, 10 patients will be accrued. If there are 1 or fewer responses in these 10 patients, the cohort will be closed. Otherwise, 10 additional

	patients will be accrued for a total of 20. The null hypothesis will be rejected if 5 or more responses are observed in 20 patients. This design yields a type I error rate of 0.05 and power of 85% when the true response rate is 35%. Accrual to this cohort will stop if evidence accumulates that the efficacy is lower than the specified acceptable levels. If five or more (≥5) patients experience objective responses (CR+PR), then we will conclude that nivolumab is sufficiently active in patients with TMB ≥5 to <10/Mb to warrant recommendation for continued investigation. For cohort B (TMB ≥10/Mb), in the first stage, 10 patients will be accrued. If there are 1 or fewer responses in these 10 patients, the cohort will be closed. Otherwise, 20 additional patients will be accrued for a total of 30. The null hypothesis will be rejected if 6 or more responses are observed in 30 patients. This design yields a type I error rate of 0.05 and power of 80% when the true response rate is 30%. Accrual to this cohort will stop if evidence accumulates that the efficacy is lower than the specified acceptable levels. If six or more (≥6) patients experience objective responses (CR+PR), then we will conclude that nivolumab is sufficiently active in patients with TMB ≥10/Mb to warrant recommendation for continued investigation. Patients already recruited to study at the time of amendment will be retrospectively allocated to the appropriate cohort based on TMB. It is anticipated that most of these patients with CMMRD will have a TMB ≥10/Mb and will therefore fall into cohort B. Any patients with CMMRD for whom TMB is not available will be analyzed separately (Cohort C). In order to make decisions about further exploring the use of nivolumab in this patient population, a likelihood Bayesian analysis will also be used to provide estimates of likely effect sizes based on results from this pilot study. Posterior probability distributions for response rate will be plotted, using non-informative priors.

	For example, in Cohort A, a response in 5 of 20 patients (estimate response rate 0.25), indicates a probability that the true RR is >0.2 of 0.77; while there is only a 0.01 probability that the true RR is <0.1. Similarly, for Cohort B, a response in 6 of 30 patients (estimated RR 0.2), indicates a probability that the true RR is >0.2 of 0.57; while there is a 0.03 probability that the true RR is <0.1.

TABLE OF CONTENTS

PROTOCOL SYNOPSIS **4**
1. BACKGROUND **23**
1.1 Hypermutant Cancers Including Constitutional Mismatch Repair Deficiency Syndrome **23**
1.2 Tumour Mutation Burden and Hypermutant Cancers **26**
1.3 Checkpoint Inhibitors as a New Class of Immunotherapeutic Agents **27**
1.4 The Programmed Cell Death 1 (PD-1) Pathway **29**
1.5 Nivolumab **30**
1.6 Anti-Tumour Activity Studies **31**
1.6.1 Animal Toxicology **32**
1.6.2 Pre-Clinical Pharmacokinetic Studies **32**
1.6.3 Clinical Trials in Adults **32**
1.6.4 Pharmacology/Pharmacokinetic Studies in Adults **35**
1.6.5 Clinical Studies in Paediatrics **37**
1.6.6 Clinical Safety **37**
1.7 Study Rationale **39**
1.8 Companion Biomarkers Exploratory Studies **40**
2 TRIAL OBJECTIVES **42**
2.1 Primary Objective **42**
2.2 Secondary Objectives **42**
2.3 Exploratory/Biology Objectives **42**
3. STUDY DESIGN **43**
3.1. Study Description **43**
3.1.1 Study Overview **43**
3.2 Part II - Treatment and Companion Biomarker Study Duration **45**
3.3 Independent Review Committee **45**
3.4 Data Monitoring Committee **45**
3.5 Study Flow Diagram **46**
4. PATIENT SELECTION **47**
4.1 Informed Consent **47**
4.2 Patient Eligibility **47**
4.2.1 Part I - Molecular Profiling Inclusion Criteria **47**
4.2.2 Part II - Treatment and Companion Biomarker Inclusion Criteria **49**
4.2.3 Part II (ONLY) Exclusion Criteria **52**
4.3 Patient Registration Procedures **54**
5. TREATMENT PROGRAM **57**
5.1 Treatment Plan **57**
5.2 Criteria for Starting Subsequent Cycles **58**
5.3 Criteria to Resume Treatment **59**
6. DOSE MODIFICATION FOR ADVERSE EVENTS **60**
6.1 Dose Modifications for Haematological Toxicity **60**
6.2 Dose Modifications for Non-Haematological Toxicity **60**
6.3 Dose Modifications for Infusion-Related Reactions **62**
6.4 Dose Modifications for Skin Rash and Oral Lesions **64**
6.4.1 Recommended Skin Adverse Event Management Algorithm **65**
6.5 Dose Modifications for Hepatic/Pancreatic Adverse Events **66**
6.5.1 Recommended Hepatic Adverse Event Management Algorithm **67**
6.6 Dose Modifications for Gastrointestinal Adverse Events **69**
6.6.1 Recommended GI Adverse Event Management Algorithm **70**
6.7 Dose Modifications for Pneumonitis **71**
6.7.1 Recommended Pulmonary Adverse Event Management Algorithm **72**
6.8 Dose Modifications for Fatigue **73**
6.9 Dose Modifications for Neurologic Adverse Events **73**
6.9.1 Recommended Neurologic Adverse Event Management Algorithm **74**
6.9.2 Special Considerations for Corticosteroid Administration in Patients with CNS Tumour Pseudo-progression. **75**
6.10 Immunotherapy Continuation Pending Confirmation of Progression **75**
6.11 Dose Modifications for Endocrine Adverse Events **76**
6.11.1 Recommended Endocrine Adverse Event Management Algorithm **77**
6.12 Dose Modification for Fever **79**
6.13 Dose Modifications for Renal Adverse Events **79**

6.13.1 Recommended Renal Adverse Event Management Algorithm 80
7. SUPPORTIVE CARE AND OTHER CONCOMITANT THERAPY 81
7.1 Concurrent Anti-Cancer Therapy 81
7.2 Investigational Agents 81
7.3 Supportive Care 81
7.4 Skin Related Toxicity 81
7.5 Pneumonitis 82
7.6 Ocular Toxicity 82
7.7 Gastrointestinal Toxicity 83
7.8 Hepatotoxicity 84
7.9 Pancreatic Toxicity 84
7.10 Endocrinopathies 85
7.11 Neurologic Toxicity 86
7.12 Renal Toxicity 86
7.13 Autoimmune or Immune System Disorders Effecting Other Organ Systems 86
7.14 Other Immune-Mediated Adverse Events 87
7.15 Growth Factors 87
8. EVALUATION SCHEDULE AND DATA TO BE collected 88
8.1 Study Calendar: Part I – Molecular Profiling 89
8.2 Study Calendar: Part II – Treatment and Companion Biomarker Studies 90
8.3 Radiological Studies 92
8.4 Biological and Companion Biomarker Studies 92
8.4.1 Tumour Tissue 92
8.4.2 Blood Tissue 93
9. EXPERIMENTAL AGENT 94
9.1 Nivolumab 94
9.2 Structure and Molecular Weight 94
9.3 Supply 94
9.4 Formulation 94
9.5 Storage 94
9.6 Solution Preparation 94
9.7 Stability 95
9.8 Administration 95
9.9 Agent Ordering and Agent Accountability 95
9.10 Accountability and Destruction of Agent 95
10. RESPONSE EVALUATION CRITERIA 97
10.1 Common Terminology Criteria for Adverse Events (CTCAE) 97
10.2 Response Criteria for Patients with Solid Tumours 97
10.2.1 Definitions 98
10.2.1.1 iRECIST Definition 98
10.2.1.2 Evaluation for Objective Response 98
10.2.2 Disease Parameters 98
10.2.2.1 Measurable Disease 98
10.2.2.2 Malignant Lymph Nodes 99
10.2.2.3 Non-measurable Disease 99
10.2.3 Assessment of Target Lesions 99
10.2.4 Assessment of Non-Target Lesions 100
10.2.5 Assessment of New Malignant Lesions 100
10.2.6 Protocol Exceptions for Delayed Response ('Pseudo-progression') 102
10.2.7 Confirming Progression 102
10.3 Methods for Evaluation of Measurable Disease 104
10.3.1 Clinical Lesions 104
10.3.2 Chest X-Ray 104
10.3.3 Conventional CT and MRI 105
10.3.4 PET-CT 105
10.3.5 Tumour Markers 105
10.3.6 Cytology, Histology 105
10.3.7 FDG-PET 106
10.4 Response Criteria for Patients with Solid Tumour and Measurable Disease 106
10.4.1 Time Point and Best Overall Response 106
10.4.2 Evaluation of Target Lesions 107
10.4.3 Evaluation of Non-Target Lesions 108

10.5 Response Criteria for Patients with Solid Tumours and Evaluable Disease 109
10.6 Response Criteria for Neuroblastoma Patients 110
10.6.1 Neuroblastoma Patients with Primary and Metastatic Soft Tissue Disease 110
10.6.2 Primary Tumour 111
10.6.3 Metastatic Soft Tissue and Bone Disease 112
10.7 Response Criteria for Neuroblastoma Patients with Metastatic Bone Marrow Involvement 115
10.7.1 Best Overall Response (iBOR) 116
10.7.2 Duration of Response 120
10.8 Response Criteria for CNS Tumours 120
10.8.1 Special Considerations for Tumour "Pseudo-progression" Phenomena 120
10.8.2 Selection of Target and Non-Target Lesions 121
10.9 Response Criteria for Target Lesions 121
10.9.1 New Lesions 123
10.9.2 Confirmation of Radiographic Progression 125
10.9.3 Non-Target Lesions 127
10.9.3.1 Tissue Acquisition to Aid Response Assessment 127
10.9.3.2 Response Criteria for Non-Target Lesions 127
10.10 Response Criteria for Tumour Markers (if available) 127
10.11 Response Evaluation for Patients Receiving Therapeutic Corticosteroids 127
10.12 Overall Response Assessment 128
10.13 Response Criteria for Patients with AML 128
10.14 Response Criteria for the Patients with ALL 130
10.15 Response Criteria for Patients with Lymphoma 132
10.15.1 Assessment of Tumour Burden 132
10.15.2 Imaging and Baseline Scans 132
10.15.3 Response Assessment of Lymphoma 133
10.15.4 Small Responsive Lymph Nodes 135
10.15.5 Appearance of a New Extra-nodal Lesion 135
10.15.6 Measurement of Splitting Lesions 136
10.15.7 Spleen Measurement 136
10.15.8 Integrating Target and Non-Target Lesions 136
10.16 Response Criteria for Patients with More than One Tumour 137
11. STATISTICAL CONSIDERATIONS 138
12. SAFETY AND REPORTING PROCEDURES 140
12.1 Adverse Event 140
12.2 Adverse Event Documentation 141
12.3 Serious Events 141
12.4 Reporting Serious Adverse Events 143
12.5 Procedures for Expedited Reporting 143
12.5.1 Responsibility for Reporting Serious Adverse Events to Regulatory Authorities 144
12.5.2 Responsibility for Reporting Serious Adverse Events to Drug Provider 144
12.5.3 Reporting Serious Adverse Events to Local Research Ethics Boards 144
12.6 Follow up on Adverse and Serious Adverse Events 145
12.7 Relationships 145
13. PROCEDURES FOR DISCONTINUATION OF A PATIENT FROM INVESTIGATIONAL PRODUCT 147
13.1 Procedures for Withdrawal from Study 147
13.2 Premature Withdrawal/Discontinuation Criteria 147
13.3 Off Study Criteria 148
14. ETHICS 149
14.1 Informed Consents 149
14.2 Ethics Board Approval 149
15. DOCUMENTATION, RECORD ACCESS AND MAINTENANCE OF STUDY RECORDS 151
15.1 Documentation of Patients Participation 151
15.2 Regulatory Requirements 151
15.3 Patient Confidentiality and Access to Source Data 151
15.4 Confidentiality of the Study 152
15.5 Registration of Clinical Trial 152
15.6 Data Reporting 152
15.7 Case Report Forms 153
15.8 Maintenance of Study Records 153
16. QUALITY ASSURANCE AND QUALITY CONTROL 154
16.1 On Site Monitoring/Auditing 154

17. ADMINISTRATIVE PROCEDURES **155**
17.1 Amendments to the Protocol **155**
17.2 Protocol Deviations/Violations **155**
17.3 Premature Discontinuation of the Study **155**
18. LEGAL ASPECTS **156**
18.1 Publication Policies and Disclosure of Data **156**
19. APPENDIX I: PERFORMANCE SCALES **157**
20. APPENDEX II: CONTRACEPTION METHODS **158**
21. APPENDIX III: TANNER STAGING **159**
22. REFERENCES **160**

1. BACKGROUND

1.1 Hypermutant Cancers Including Constitutional Mismatch Repair Deficiency Syndrome

Accuracy of DNA replication is a vital cellular function necessary to avoid the introduction and perpetuation of potentially harmful mutations. Although remarkably accurate, DNA replication during cell division introduces multiple errors that must then be corrected by replication repair mechanisms, including the mismatch repair system (Nebot-Bral, 2017). The mismatch repair (MMR) system is a conserved and complex evolutionary pathway. Its important function includes degradation of the error-containing sections of the newly synthesized DNA strand and providing DNA polymerases the opportunity to produce a new error-free copy of the template sequence (Nebot-Bral, 2017; Tubbs, 2017; Supek, 2015). DNA replication-associated mutations are repaired by two constituents; DNA polymerase proofreading and mismatch repair (Nebot-Bral, 2017; Shlien, 2015). Loss of MMR brings about a mutator phenotype, which causes a predisposition to cancer (Tubbs, 2017; Jiricny, 2006). DNA replication repair deficiencies (RRD) predispose afflicted individuals to cancer and profoundly affect their responses to therapies (Tubbs 2017; Carreras Puigvert, 2015; Curtin, 2012; Middleton, 2015).

Constitutional mismatch repair deficiency (CMMRD; OMIM database accession no. 2763000, also known as biallelic mismatch repair deficiency [bMMRD]), is the most aggressive human cancer predisposition syndrome and results from biallelic mutations in one of the four MMR genes (*MSH2*, *MSH6*, *MLH1* or *PMS2*) and rarely in *MSH3* and *EPCAM* in the germline (Tabori, 2017; Durno, 2017; Adam, 2016; Wimmer, 2014; Tutlewska, 2013). Cancers in these patients have the highest mutational load amongst all human cancers and are often referred to as ultra-hypermutant (Shlien, 2015). Additionally, other secondary germline and somatic mutations, such as those in genes coding for DNA polymerase ε or δ subunits have also been reported to cause this hypermutant phenotype (Wimmer, 2017; Wimmer, 2016; Bouffet, 2016; Palles, 2013; Briggs, 2013). According to Shlien, *et al.* (2015), all bMMRD cancers (sampled from high-grade brain tumours) acquired early somatic driver mutations in DNA polymerase ε or δ (*POLE* and *POLD1*) in addition to the biallelic mutations in the four main MMR genes resulting in an ultra-hypermutated phenotype (Nebot-Bral, 2017). They demonstrated that bMMRD/polymerase-mutant cancers rapidly accumulate excessive simultaneous mutations (approximately 600

mutations/cell division) reaching up to 20,000 exon mutations in less than six months (Shlien, 2015). Individuals with heterozygous mutations in one of the MMR genes (i.e. an inherited deficiency of only one allele) have a different cancer predisposition syndrome known as Lynch syndrome (previously called hereditary non-polyposis colorectal cancer). These patients may develop tumours in which there is a somatic loss of the remaining functional MMR gene allele, leading to tumours that are functionally mismatch repair deficient and also hypermutant.

Relapsing and recurrent cancers of patients who do not possess germline MMR mutations may also have secondary somatic mutations acquired at random or as a result of MMR deficiency–associated resistance to previous therapies, such as purine analogs or nitrosureas, resulting in the same hypermutant response (Hunter, 2006, van Thuijil, 2015). A reported example of this includes gliomas treated with temozolimide (TMZ) acquiring secondary *MSH6* mutations (van Thuijil, 2015). This is of clinical importance in patients with CMMRD. Temozolomide is often used in the standard treatment of GBM, and since it has become clear that alkylating agents are less effective in MMR-deficient tumors, this may prove to be a growth advantage for the commonly affiliated GBM tumor cells (Scott, 2007; Fedier, 2004; Westdorp, 2017).

Identifying and confirming that two MMR mutations exist on separate alleles can be difficult, and MMR gene variants of unknown functional significance also have been reported (Durno, 2017). Often these patients are identified with either homozygous biallelic alterations and/or compound heterozygous alterations (Durno, 2017). Furthermore, the majority of CMMRD patients harbor *PMS2* mutations, which is complicated by the presence of 20 non-functional *PMS2* pseudogenes obscuring the identification of true mutations (Durno, 2017; Durno, 2015; Lavoine, 2015).

CMMRD syndrome was first recognized in 1999 (Riccardone, 1999; Wang, 1999) and since then several series have reported variable incidences of cancers in those patients (Felton, 2007; Wimmer, 2008; Wimmer 2014; Amayiri, 2015). The hallmark of CMMRD is early onset cancer, most often in childhood or young adulthood with the median age of onset of for the first tumor being 7.5 years old (range 0.4–39 years; Wimmer, 2014). The median survival after diagnosis of the primary tumor is less than 30 months (Lavoine, 2015). In contrast to patients with Lynch syndrome (heterozygous MMR mutation carriers), who develop colon and genitourinary cancers later in adulthood (Vasen, 2013), those with CMMRD develop multiple cancers at an early age. These are most typically CNS tumours and haematological malignancies, in addition to

carcinomas typical of Lynch syndrome, occurring in the first decades of life and consequently these patients rarely reach adulthood (see *Table 1*; Durno, 2017; Wimmer, 2014). Approximately one third of CMMRD patients develop leukemias or lymphomas (Vasen, 2014).

In contrast to Lynch syndrome, loss of MMR protein staining by IHC is apparent in non-malignant and malignant tissue alike due to the constitutive biallelic inactivation of the respective gene (Ripperger, 2016). Following allogenic BM transplantation, graft lymphocytes can be used as a control for IHC (Ripperger, 2010). Brain tumours are the most prevalent (36-48%), with high grade gliomas (HGGs) comprising three-quarters of all CNS malignancies, low grade gliomas (LGGs) accounting for up to 16% and primitive neuroectodermal tumours/ medulloblastomas reported at 10-18% (Wimmer, 2008; Bakry, 2014). The most commonly observed haematopoietic malignancies are non-Hodgkin lymphomas (NHL), particularly T-lymphoblastic NHL (Attarbaschi, 2016, Westdorp, 2017). T-cell acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML) have also been reported (Ripperger, 2016; Wimmer, 2014). Other tumours, such as neuroblastoma, rhabdomyosarcoma, osteosarcoma, melanoma, Wilms tumour, ovarian neuroectodermal tumour, hepatic adenoma, breast cancer pilomatricoma, or infantile myofibromatosis, have been reported in fewer than 5 cases in the literature thus far (Durno, 2017; Kratz, 2009).

Table 1. Estimated Penetrance and Age of Onset of Neoplasms in bMMRD (Durno, 2017; Aronson, 2016; Layoine, 2015; Vasen, 2014; Wimmer, 2014; Herkert, 2011).

Organ	***Estimated penetrance, %***	***Age at diagnosis (range)***
Small-bowel adenoma[i]	*50*	*12(10-20)*
Colorectal adenoma[i]	*>90*	*9(6-15)*
Small-bowel cancer	*10*	*28(11-42)*
Colorectal cancer[ii]	*70*	*16(8-48)*
Low-grade brain tumour	*Unknown*	*Unknown*
High-grade brain tumour[iii]	*70*	*9(2-40)*
Lymphoma	*20-40*	*5(0.4-30*
Leukemia	*10-40*	*8(2-21)*
Endometrial cancer	*<10*	*(19-44)*
Urinary tract cancer	*<10*	*(10-22)*
Other sites[iv]	*<10*	*(1-35)*

I Low and high-grade adenomas with probable rapid progression.
Ii Patients undergo subtotal colectomy and ileal–rectal anastomosis, resulting in a decreased risk of CRC
iii High-grade glioma, medulloblastoma, and primitive neuroectodermal tumors.
Iv Fewer than 5 cases of each of the following neoplasms have been reported: neuroectodermal tumor, neuroblastoma, Wilms tumor, rhabdomyosarcoma, pilomatricoma, osteosarcoma, breast cancer, melanoma, ovarian, and hepatic adenoma.

For CMMRD, consanguinity often plays a significant role in inheritance depending on the patient country of origin (Tabori, 2017; Bakry, 2014). If the patient originated from a population with a high rate of familial consanguinity, the cases are most often to be homozygous (Vasen, 2014); however, in most cases stemming from Western countries, composite heterozygous mutations were reported (Lavoine, 2015).

The hallmark of CMMRD disease is early onset of these hypermutant cancers, most often in childhood or young adulthood. Many non-neoplastic manifestations of CMMRD are also of diagnostic importance. The majority of the patients have neurofibromatosis type 1 associated findings; mainly café-au-lait macules and other hyper and hypo-pigmented skin alterations, especially in children (Wimmer, 2017; Bakry, 2014; Wimmer, 2008). Other features include developmental venous anomalies, agenesis of the corpus callosum, and mild immunodeficiency with decreased levels of immunoglobulins IgG2/4 and IgA, among others (Wimmer, 2014). The treatment of childhood cancers is dependent on the specific location and the type of cancer. Since CMMRD is very rare, there is limited information on optimal therapeutic strategies (Westdorp, 2017).

1.2 Tumour Mutation Burden and Hypermutant Cancers

Tumour mutation burden (TMB) measures the number of mutations within a tumor genome (Goodman, 2017; Frampton, 2016). Numerous reports have denoted that tumors harbouring more mutations have been shown to have a greater response to immunotherapy (Goodman, 2017; Salem, 2017; Chalmers, 2017). Researchers have reported that an increased mutation rate leads to an increased number of neoantigens on tumor cell surfaces capable of eliciting an immune response (Shlien, 2015; Schumacher, 2015; Brown, 2014). It is this immune-potential that is hypothesized to determine whether patients may derive benefit from immunotherapy.

Previous reports have shown that TMB predicts response to immunotherapy when measured using whole exome (coding regions of the genome) sequencing (WES; Shlien, 2015; Chalmers, 2017). This study tested whether TMB could be accurately measured by a targeted comprehensive genomic profiling (CGP) panel of 315 genes and found it to be highly correlated with measurement by WES (Frampton, 2016). To elucidate the applicability of TMB as a biomarker for a variety of cancer types, it was further investigated using >100 000 patients with multiple neoplasms. Characterization of the TMB level distribution, thereby identified those which

may be good candidates for clinical trials testing of immunotherapies (Chalmers, 2017; Frampton, 2016; Rosenberg, 2016; Márquez-Rodas, 2015; Rizvi, 2015; Snyder, 2014; Wolchok, 2013; Frampton, 2013).

Whole genome and exome sequencing of the malignant brain tumours from hypermutant, CMMRD patients have demonstrated a dramatic increase in the number of point mutations compared to non-CMMRD paediatric and adult tumours (Shlein, 2015; Campbell, 2017). Rapid mutation accumulation at the rate of >250 mutations per Mb (approximately 600 mutations per cell division) was observed in high-grade CMMRD tumours resulting in ultra-hypermutated genome (Shlien, 2015). Interestingly, those cancers were lacking copy number abnormalities and demonstrated microsatellite stability, as opposed to Lynch Syndrome associated cancers, which are microsattelite unstable (Shlein, 2015; Boland, 1998).

Recently, Campbell, *et al.* (2017) described an extensive assessment of mutation burden through a sequencing analysis of more than 81 000 paediatric and adult tumours. These included tumour with hypermutation derived from a number of sources not previously associated with and elevated TMB. These sources were clustered in tumour mutation signature groupings by chemotherapy, carcinogens, or germline alterations including, but not limited to high microsatellite instability (MSI-H), replication repair deficiency genes, xeroderma pigmentosum (XP), alkylating agent exposure, dysregulation of an apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deamination and UV-exposure associated (Campbell, 2017; Nebot-Bral, 2017). Hypermutation was found in 1 in 20 childhood cancers and 1 in 6 adult cancers. They further reported that enrichment for RRD and continuous long-term exposure to genotoxic agents could be used to identify tumours that should be susceptible to immune checkpoint inhibition (Campbell, 2017; Nebot-Bral, 2017). In some laboratories (mostly in Europe), analysis of MSI and tolerance to methylating or thiopurine agents in EBV-immortalized lymphoblastoid cells is used as a functional assay diagnostic tool (Bodo, 2015; Ripperger, 2016).

1.3 Checkpoint Inhibitors as a New Class of Immunotherapeutic Agents

Children with childhood cancer who present in many high-risk groups, including those who are diagnosed with metastatic disease and those with disease recurrence after frontline therapy, have poor survival rates, which have not changed substantially in many years. Current standard regimens for high-risk paediatric tumours already employ dose intensive cytotoxic therapy with

significant short-term and long-term toxicity, thus further dose escalation of such regimens is unlikely to improve outcomes, but will almost certainly increase toxicity. For these reasons, there is an urgent need to develop new classes of therapeutics to treat childhood cancer, based upon biologic insights into the tumour itself or the host response to cancer.

Immunotherapy approaches have changed the landscape of cancer treatment, providing benefit to numerous diseases once considered untreatable and extending the lives of many. These effects have been observed in a constantly growing number of cancer types, such as melanoma, non-small cell lung cancer (NSCLC), and bladder cancer, leading to multiple FDA approvals (Mellman, 2011; Topalian, 2012; Bracarda, 2015). However, currently most patients will not respond to these new therapies, with response rates ranging from 15-40% (Chalmers, 2017). Paired with the high cost and risk of serious immune-related side effects, an urgent need to identify reliable, quantitative biomarkers capable of identifying those most likely to benefit from immunotherapy exists.

Immune checkpoint inhibitors (ICI) are immunomodulatory mAbs that confront immune escape by tumor cells by inhibiting normal immunosuppressive mechanisms usually present to prevent autoimmunity and tissue damage in response to acute infection of healthy patients, but promote tumor progression in cancer patients (Morrissey, 2016). ICIs induce anti-tumour effects by blocking inhibitory immune receptors such as PD-1 and CTLA-4 (Weber, 2010). Development of this class of agents for cancer is based on the hypothesis that anti-tumour immune responses exist in patients with cancer, but are inhibited by tonic activation of inhibitory pathways. Blockade of inhibitory receptors uncovers anti-tumour immune responses that can mediate anti-tumour effects. Anti-CTLA-4 inhibitor (ipilimumab), the first of a new class of immunomodulatory agents has been FDA approved for the treatment of metastatic melanoma including paediatric patients (12 and older) with unresectable metastatic melanoma (Mallard, 2013; Eggermont, 2015) and is currently being investigated in numerous other clinical trials. Anti-PD-1 is a second-generation checkpoint inhibitor, comprised of monoclonal antibodies (mAbs) targeting PD-1. Anti-PD-1 have shown anti-tumour effects in melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), classical Hodgkin's lymphoma (cHL), squamous cell carcinoma of the head and neck (SCCHN), urothelial carcinoma (UC), and colorectal cancer (Topalian, 2012; Hamdi, 2013; Grosso, 2015; Ferris, 2016; Fuereder, 2016; Morrissey, 2016; Emens, 2016; Sharma, 2017;

Benson, 2017; Nebot-Bral, 2017). It is currently under clinical investigation in multiple paediatric and adult tumours (Postow, 2015; Errico, 2015).

1.4 The Programmed Cell Death 1 (PD-1) Pathway

The programmed cell death 1 (PD-1) pathway is a negative feedback system that represses cytotoxic immune responses and that, if unregulated, can damage the host (Nishimura, 1999; Nishimura, 2001; Chen, 2004). PD-1 is known to play a major role in inducing T-cell exhaustion caused by chronic antigen stimulation due to infections and cancer (Wherry, 2011). PD-1 is an inhibitory receptor expressed on exhausted T-cells, B-cells, and activated functional T-cells (Dong, 2003). PD-L1 is the ligand of PD-1 and is constitutively expressed in hematopoietic and non-hematopoietic cells, including some types of cancer (Dong, 2013). It can be induced by cytokine stimulation (Dong, 2003).

PD-1/PD-L1 inhibitors have shown a significant activity against multiple types of adult cancers (Hamid, 2013; Ansell, 2015; Brahmer, 2015; Garon, 2015; McDermott, 2015). The expression of PD-1 ligands (PD-L1 or PD-L2) on the surface of tumour cells or immune cells is an important but not a definitive predictive biomarker of response to PD-1 blockade Herbst, 2014; Powels, 2014; Taube. 2014; Topalian, 2014; Ansell, 2015).

Recent studies have shown an association between a higher TMB and/or defined tumor-associated neoantigen , signatures and the potential for clinical benefit, radiological response, and progression-free survival (PFS) in adults with non-small-cell lung cancer (NSCLC) treated with ICIs, such as PD-1 inhibitors (Garon, 2015; Rizvi, 2015; Shlien, 2015; Bouffet, 2016). Pembrolizumab has recently been approved by the FDA for the treatment of relapsed for patients with microsatellite instability high (MSI-H) or mismatch repair deficient (dMMR) progressive solid tumours regardless of histological subtype. MSI-H are high numbers of (mainly insertion deletion) mutations at repetitive DNA sequences known as microsatellites. Repeated DNA structures are prone to DNA polymerase slippage during DNA replication (Westdorp, 2017). As a result of these insertions and deletions, the length of the repeating sequences increases or decreases leading to microsatellite instability (MSI). When these occur in gene coding regions, it can cause inactivation of the gene products causing truncated or non-functional proteins (Maby, 2015; Westdorp, 2017). Essential MMR system components include the heterodimer of MSH2 and

MSH6 (MutSα), which detects base mismatches or ½ base-pair insertion/deletion loops (a type of MSI) and the heterodimer of MSH2 and MSH3 (MutSβ) that identifies larger IDLs (Nebot-Bral, 2017). Similar results of enhanced efficacy of another ICI, nivolumab, have also been reported in patients with MSI-H colorectal carcinoma (Overman, 2017) and patients with elevated tumour-mutation burden in the context of recurrent non-small-cell lung cancer (Carbone, 2017).

1.5 Nivolumab

Nivolumab (Opdivo®, BMS-936558, MDX1106, ONO-4538 or anti-PD-1) is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor (Investigator Brochure, 2017). PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes (Sharpe, 2007). Binding of PD-1 to its ligands, programmed death–ligands 1 and 2 (PD-L1 and 2), results in the down-regulation of lymphocyte activation and inhibition of such binding promotes immune and antigen-specific T-cell responses to both foreign self-antigens as well as self-antigens (Investigator Brochure, 2017).

Nivolumab is a sterile solution (pH 5.5 – 6.5) for parenteral administration comprised of a soluble protein consisting of 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains (Investigator brochure, 2017). The molecular weight of the compound is 146 221 daltons and its appearance is clear to opalescent, colorless to pale yellow liquid with few particulates (Investigator Brochure, 2017).

Opdivo® is approved for the treatment of several cancer types, such as non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), classical Hodgkin lymphoma (cHL), squamous cell cancer of the head and neck (SCCHN), urothelial carcinoma; and melanoma as monotherapy or in combination with ipilimumab (an anti-CTLA-4 inhibitor) in multiple regions including the United States (Dec. 2014), the European Union (Jun. 2015), and Japan (Jul. 2014) [7]. It is being investigated in various other types of cancer as monotherapy or in combination with other therapies for cancers and as monotherapy for the treatment of sepsis (Investigator Brochure, 2017). In addition, the use of nivolumab has been validated in special populations, such as the elderly, hepatic and renally impaired; however, the use of nivolumab in all paediatric populations has yet to be established Investigator Brochure, 2017).

1.6 Anti-Tumour Activity Studies

PD-1 is the second immune checkpoint receptor utilized for cancer immunotherapy. Unlike CTLA-4, which is expressed on nearly all regulatory T-cells and appears to be important in controlling T-cell proliferation during T-cell development, PD-1 is upregulated on peripheral T-cells following chronic activation. PD-1 signaling on T-cells is induced following binding to either PD-L1 (B7-H1, CD274, considered widely expressed, especially on macrophages and some tumours) or PD-L2 (B7-DC, CD273, more limited expression, on antigen presenting cells). Murine studies showed impressive effects when blocking antibodies to PD-1 were administered to mice with chronic viral infection, resulting in recovery of antiviral immunity and reversal of "T-cell exhaustion" (Barber, 2006). Furthermore, mice genetically induced to be deficient in PD-1 developed a variety of autoimmune-like diseases. Hence, PD-1 signaling has been associated with chronic T-cell activation and T-cell exhaustion. Current concepts hold that blocking PD-1 may augment responses in the setting of chronic immune activation. The differences in the biology between CTLA-4 and PD-1 leads to the prediction that PD-1 blockade is less likely to induce *de novo* autoimmunity and more likely to restore responses in the setting of chronic antigen exposure (Intlekofer, 2013).

Several preclinical studies demonstrated anti-tumour effects of anti-PD-1 in tumour models. A landmark manuscript by Dong *et al*. (2002) demonstrated robust tumour expression of PD-L1 as well as expression of PD-L1 on tumour-associated macrophages, but not on other normal tissues. This group further demonstrated that interferon gamma (IFN-γ) induced upregulation of PD-L1 on tumour cell lines, thus providing a means for tumour immune escape through signaling of PD-1 on activated T-cells. This in turn, induces suppressive signaling pathways. PD-1 signaling has been demonstrated to contribute to immune escape *in vivo* in murine myeloma (Iwai, 2002), Sa1N fibrosarcoma MC38 colorectal adenocarcinoma, and B16 melanoma (Woo, 2012). PD-1 has not been extensively studied in preclinical models of paediatric cancer. However, recent work demonstrated expression of PD-L1 in two murine embryonal rhabdomyosarcoma cell lines including M3-9-M, which is derived from an HGFTgp53+/- genetically engineered mouse and 76-9, a spontaneous rhabdomyosarcoma (Highfill, 2014). In immunocompetent mice inoculated with either line, treatment with anti-PD-1 prevented tumour growth if administration coincided with tumour inoculation. However, when anti-PD-1 was administered in the presence of established tumours, anti-PD-1 therapy had anti-tumour effects, but was not curative. This work demonstrated

that anti-PD-1 therapy did augment immune responses to tumour antigens expressed on M3-9-M mice. Interestingly, this work demonstrated that co-treatment with anti-PD-1 and anti-CXCR2 antibodies, which prevent trafficking of myeloid derived suppressor cells into the tumour bed were more effective than treatment with anti-PD-1 alone. These results implicate myeloid derived suppressor cells in tumour immune escape in rhabdomyosarcoma and suggest that a future clinical approach that combines anti-PD-1 with other immunomodulators holds promise. PD-1 signaling has also been implicated in immune escape in acute myeloid leukemia (Zhou, 2010), and unpublished work has demonstrated PD-L1 expression on tumour infiltrating myeloid cells in medulloblastoma tissue section and cell lines.

1.6.1 Animal Toxicology

None is pertaining to this trial. The anti-PD-1 mAb does not bind murine PD-1 or non-human primate PD-1 and therefore these are not informative for toxicity in humans.

1.6.2 Pre-Clinical Pharmacokinetic Studies

Such studies do not significantly inform this trial as the pharmacokinetic studies from the adult early phase trials are more informative.

1.6.3 Clinical Trials in Adults

Results of early phase clinical testing of two checkpoint inhibitors that block PD-1/PD-L1 interactions in adult cancer patients have been published. In general, these have demonstrated impressive anti-tumour activity in melanoma, lung cancer, renal cell cancer, classic Hodgkin's lymphoma, head and neck cancer, urothelial carcinoma (UC), and colorectal cancer (CRC) with less toxicity than that observed with ipilimumab. (Topalian, 2012; Hamdi, 2013; Grosso, 2015; Ferris, 2016; Morrissey, 2016; Emens, 2016; Sharma, 2017; Benson, 2017).

Topalian *et al*. (2012) reported on 296 adult patients treated on a Phase I study of nivolumab (then called BMS-936558) a fully human IgG4 blocking mAb. Patients received drug every two weeks for up to two years, unless they had a complete response (CR), unacceptable side effects, progressive disease (PD) or they declined to continue therapy. Response was assessed every 8 weeks and due to concerns regarding pseudo-progression seen with other checkpoint

inhibitors, patients were allowed to remain on study if they were clinically stable despite progression on routine restaging unless progression was confirmed on a subsequent restaging 8 weeks later. Doses tested were 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg and 10 mg/kg using a standard 3+3 design. No MTD was identified.

A total of five expansion cohorts were then studied at 10 mg/kg comprising melanoma, non-small cell lung cancer, renal cell carcinoma, castration resistant prostate cancer and colorectal cancer. Because signs of clinical activity were observed, additional cohorts of melanoma, squamous and non-squamous lung cancer and renal cell cancer were studied. Five percent of patients discontinued treatment due to adverse events, Grade 3 or 4 treatment-related adverse events occurred in 14% and drug related serious adverse events (SAEs) occurred in 11%. Most common drug related immune adverse events (AE) were pneumonitis, vitiligo, colitis, hepatitis, hypophysitis and thyroiditis. In general, adverse events were similar in nature, severity and reversibility to that seen with ipilimumab, except that the incidence appeared to be less, and pneumonitis was not observed with a significant frequency in studies with ipilimumab. There was no evidence that pneumonitis was more common in lung cancer or any other particular histology. There were three (3) deaths associated with pneumonitis, two (2) in patients with lung cancer and one (1) in a patient with CRC.

In July 2013, Hamid *et al.* reported results of a Phase I trial of lambrolizumab (previously MK-3475) a humanized IgG4 mAb that blocks PD-1 (Hamid, 2013) Using RECIST as response criteria, a 38% response rate was observed, combining all dose levels with a higher response rate (52%) in patients that received 10 mg/kg every 2 weeks. Overall, 77% of patients had a reduction in tumour burden during the study. Responses were durable in the majority of patients. Most common adverse events were fatigue, rash, pruritus and diarrhea and most were low grade. Patients (13%) with higher-grade events (grades 3-5), included pneumonitis in 4%, which indirectly led to the death of one patient, a 96-year-old man. Two cases of grade 3 renal failure were observed as well, both of which improved with discontinuation of therapy plus glucocorticoids. The pharmacokinetics (PK) studies showed linear relationships with dose and the half-life of the agent was judged to be 2-3 weeks later. Biopsy of lesions from responding patients showed dense infiltration with CD8+ cytotoxic lymphocytes. There is an ongoing trial randomizing dosing between 10 mg/kg administered every three vs every two weeks.

Table 2. Response rates associated with histology and dose (Topalian, 2012).

Histology	*Dose*	*Response rate (%)*	*Number*
Melanoma	0.1 mg/kg	29	14
Melanoma	0.3 mg/kg	19	16
Melanoma	1 mg/kg	30	27
Melanoma	3 mg/kg	41	17
Melanoma	10 mg/kg	20	20
Melanoma	All	28	94
Lung cancer	1 mg/kg	6	18
Lung cancer	3 mg/kg	32	19
Lung cancer	10 mg/kg	18	39
Lung cancer	All	18	76
Renal cell cancer	1 mg/kg	24	17
Renal cell cancer	10 mg/kg	31	16
Renal cell cancer	All	27	33

A substantial fraction of patients with objective responses followed for one year after initiation of therapy showed a prolonged duration of response (8/14 with lung cancer had responses lasting at least 24 weeks, 13/26 with melanoma had responses lasting at least one year and 5/8 with renal cancer had responses lasting at least one year).

Brahmer *et al.,* (2012) reported results of anti-PD-L1 blocking antibody therapy in 207 patients with a variety of cancers. The agent is an IgG4 subtype antibody, and therefore it is presumed that its effects would be mediated by blockade of PD-1/PD-L1 interactions, rather than by induction of ADCC or complement mediated cytotoxicity. Results were similar to that observed with anti-PD-1. ORR were observed in 6-17% of patient groups including melanoma, RCC and NSCLC. Several patients also showed prolonged stabilization of disease and grade 3 or 4 toxic effects occurred in 9% of patients, and were primarily autoimmune in nature. No significant anti-tumour activity was observed in cohorts of patients (n=16 each) with ovarian cancer, colorectal cancer, pancreatic cancer, breast cancer or gastric cancer.

With regards to biomarkers previously associated with responses to anti-CTLA-4 or anti-PD-1, investigators evaluated PD-L1 expression in tumours and absolute lymphocyte counts and looked for relationships with response. In patients treated concurrently, 6/13 patients with PD-L1^{+} tumours responded whereas 9/22 patients with PD-L1^{-} tumours responded (P>0.99 by Fisher's exact). Interestingly, however in the sequential group, 4/8 patients whose tumours were PD-L1^{+} responded, whereas only 1/13 who had PD-L1- tumours responded. Absolute lymphocyte counts at weeks 5-7 were not associated with response in this study. In summary, the combination anti-CTLA-4 plus anti-PD-1 administered concurrently, induces impressive durable response rates in metastatic melanoma, which are higher than that reported with any previous therapy. Given that this is a non-randomized study, these results must be interpreted with caution.

In 2015, Sampson *et al.* evaluated the safety/tolerability of the ICIs, nivolumab and ipilimumab in patients with a first recurrence of glioblastoma multiforme (GBM) in the first randomized controlled study. Twenty (20) patients were treated and divided equally into two arms: nivolumab only and nivolumab plus ipilimumab. All patients (median age of 57 years) had prior surgical resection, radiation, and TMZ. Median time from first GBM diagnosis was 9 months. Drug-related AEs in ≥3 patients were fatigue (n=3) and nausea (n=3) with nivolumab, and fatigue (n = 8), diarrhea (n = 7), elevated AST and lipase (n = 5 each), vomiting and elevated ALT (n=4 each), and elevated amylase, headache, hyperthyroidism, nausea and maculopapular rash (n=3 each) with nivolumab plus ipilimumab arm patients. All nivolumab adverse events were grade 1 or 2. No drug-related AEs leading to discontinuation of treatment occurred in nivolumab arm. There were no drug-related deaths. Overall survival (OS) at six months were 70% for the nivolumab treated arm.

Nivolumab has demonstrated clinical activity in NSCLC, melanoma, RCC, cHL, SCCHN, UC as approved indications, and other tumor types currently under investigation as monotherapy or in combination with ipilimumab or other therapeutics (Investigator Brochure, 2017). The majority of responses in patients were durable and exceeded six months. In randomized, controlled studies, nivolumab monotherapy demonstrated statistically significant improvement in OS over standard of care in patients with advanced or metastatic melanoma, patients with advanced or metastatic NSCLC, patients with advanced RCC, and patients with recurrent or metastatic SCCHN.

1.6.4 Pharmacology/Pharmacokinetic Studies in Adults

Pharmacokinetic studies in adults in the Topalian, *et al.* study (2012) demonstrated a median time to peak concentration of 1-4 hours after the start of the one-hour infusion and a dose proportion increase in peak concentration and area under the curve with increasing dose across the dose range 0.1 mg/kg-10 mg/kg. Eight half-lives are 2-4 weeks, similar to other therapeutic antibodies. Minimum concentrations 15 days after the first dose of 3 mg/kg of nivolumab from 34 adults treated on CA209003 ranged from 6.9 – 38.1 mcg/mL (median 17.6). Since then, the PK, clinical activity, and safety of nivolumab have been assessed in approximately 75 clinical studies (Investigator Brochure, 2017). Approximately, 1600 patients have received nivolumab monotherapy in single or multiple-dose Phase I/II/III studies or studies with nivolumab in

combination with other therapeutics, such as ipilimumab, cytotoxic chemotherapy, anti-angiogenics, and targeted therapies.

Pharmacodynamic (PD) analysis of PD-1 receptor occupancy revealed 64-70% occupancy across the range of dose levels tested. Tumours were analyzed for PD-L1 expression from archival samples in 42 patients. Of these, samples from 25 patients showed PD-L1 expression by immunohistochemical analysis (cut-off for positivity being > 5% of cells expressing PD-L1). Responses were observed in 9/25 patients with PD-L1 expression by IHC, whereas 0/17 patients without PD-L1 expression showed anti-tumour responses with anti-PD-1 therapy. More recent data from Grosso and colleagues, assessed ORR to nivolumab in patients with melanoma using a cut-off of 5% PD-L1 expression (Grosso, 2013). In this study, 14% of patients with PD-L1 negative tumours had objective anti-tumour responses compared to 41% of patients with PD-L1+ tumours (Grosso, 2013).

Other PD effects of nivolumab were studied in cancer patients by assessing receptor occupancy (RO), peripheral immune cell population modulation, systemic cytokine modulation, and change in absolute lymphocyte count (ALC; Brahmer, 2010; Topalian, 2012; Giannakis, 2017). The peripheral RO of PD-1 is saturated at doses greater to or equal than 0.3 mg/kg dose when measured affixed to $CD3^+$ derived from peripheral blood mononuclear cells (PBMCs) and nivolumab demonstrated no clinically meaningful changes in activated T-cells in peripheral blood or mean ALC at any dose. Additionally, baseline measurements of select immune cell subsets nor ALC were not associated with response to nivolumab (Brahmer, 2010; Topalian, 2012; Giannakis, 2017). From baseline to post-dose, the median percent increase chemokines (CXCL9 and CXCL10) were consistent with immunomodulatory activity derived from nivolumab.

Preliminary studies in adults have demonstrated higher response rates in patients with tumours expressing of the PD-L1, the major ligand for PD-1, although anti-tumour responses have also been seen in patients with PD-L1 negative tumours. There are currently no completely adopted biomarkers in clinical practice to predict tumour response to anti-PD-1 therapy. However, recent reports have shown that patients whose tumors harbour a high mutation load and/or neoantigen (tumor-associated antigen)/ signatures derive enhanced clinical benefit from ICI therapy (Soo, 2015; Gubin, 2015; Bouffet, 2016). Through WES of tumors, investigators have recently described that mutational burden in NSCLC was associated with response to pembrolizumab (an anti-PD-1 inhibitor; Rivzi, 2015). In two, independent patient cohorts, it was reported a high somatic non-

synonymous mutation burden was associated with a greater clinical benefit, higher ORR and a longer PFS to either a CTLA-4 or PD-1 treatment (Snyder, 2014; Rivzi, 2015). In addition, clinical efficacy was associated with a molecular smoking signature, certain DNA repair mutations and the burden of neoantigens (Soo, 2015).

1.6.5 Clinical Studies in Paediatrics

Outside of the young adults included in the Overman, *et al.* (2017) trial, little published data exist for the use of nivolumab in younger children, but a number of cases in the literature and a few ongoing trials have shown promise (Naumann-Bartsch, 2016; Shad, 2016; Bouffet, 2016; Foran, 2017; Wagner, 2017).

Currently open trials include a Phase I/II study of nivolumab in children, adolescents, and young adults with recurrent or refractory solid tumours as a single agent and in combination with ipilimumab opened by COG (ADVL412). This study has confirmed the RP2D and is currently accruing expansion cohorts. Patients with CNS malignancies are not eligible in this trial. Another trial entitled the *European Proof-of-Concept Therapeutic Stratification Trial of Molecular Anomalies in Relapsed or Refractory Tumours (ESMART)*, is a multi-centre trial, Phase I/II non-randomized, open label treatment trial, using molecular profiling protocols have been launched in Europe (MOSCATO-01 (Harttrampf, 2017), MAPPYACTS, INFORM, iTHER, SM-PAEDS, etc.) to determine multiple actionable alterations in pediatric recurrent cancers using a dozen different anti-cancer therapeutic agents including nivolumab. The purpose of this basket trial is to cover the targeting of several survival pathways in oncogenesis that are currently not adequately employed for paediatric patients solely in Europe.

1.6.6 Clinical Safety

The overall safety experience with nivolumab, as a monotherapy or in combination with other therapeutics, is based on experience in approximately 16 900 patients treated to date (Investigator brochure, 2017). For monotherapy, the safety profile is similar across tumor types with no maximum tolerated dose (MTD) reached at any dose tested up to 10 mg/kg and no pattern of AEs affiliated with dose level (Investigator Brochure, 2017). The only exception is pulmonary inflammation AEs, which may be greater in patients with NSCLC, possibly because it can be

difficult to distinguish between nivolumab-related and unrelated causes of pulmonary symptoms and radiographic changes (Investigator Brochure, 2017). The most frequently reported treatment-related AE is fatigue, which is usually of low grade. In rare instances, there have been reports of cerebral edema in patients treated with nivolumab. Additionally, one patient treated on this study (OZM-075) developed hydrocephalus, considered probably related to nivolumab by investigators.

There is no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. In Phase III controlled studies, the safety profile of nivolumab monotherapy is acceptable in the context of the observed clinical efficacy, and manageable using established safety guidelines (Investigator Brochure, 2017). Clinically relevant AEs typical of stimulation of the immune system were reported as infrequent and manageable by delaying or stopping nivolumab treatment and timely immunosuppressive therapy or other supportive care.

No unexpected safety findings to have been reported date who received a single dose of nivolumab monotherapy or in combination with other therapeutics such as ipilimumab, cytotoxic chemotherapy, anti-angiogenics, and targeted therapies; however, most studies are ongoing and, as such, the safety profile of nivolumab combinations continues to evolve. Most related AEs are thought to be due to the effects of inflammatory cells on specific tissues. The majority of these AEs have been managed successfully with supportive care and, in more severe cases, a combination of dose delay, permanent discontinuation, and/or use of corticosteroids or hormone replacement therapy (for endocrinopathies; Investigator Brochure, 2017).

As with all therapeutic proteins, there is a potential for immunogenicity. With nivolumab there is reported risk of prolong QT potential (CA209010), and drug-induced hepatotoxicity injury. A hepatic AE management algorithm that has been established (see below; Investigator Brochure, 2017). As well, some patients may require prolonged treatment with high-dose corticosteroids or alternative immunosuppressants for the treatment of nivolumab-related AEs. In these rare cases, opportunistic infections have occurred, though not in the OZM-075 study patients to date.

The non-clinical findings of increased late-stage pregnancy loss and early infant deaths/euthanasia in nivolumab-exposed pregnant monkeys suggest a potential risk to human pregnancy; however, cases of human in-utero exposure to nivolumab (involving the fetuses of female and partners of male subjects receiving nivolumab) were reported. Given the potential risk suggested by preliminary data from nonclinical and clinical data, dosing during pregnancy is prohibited in this trial. In addition, women of childbearing potential (WOCBP) and men who are

sexually active with WOCBP receiving nivolumab will be instructed to adhere to contraception to be on study.

1.7 Study Rationale

The biomarkers that may predict response to ICI therapy have yet to be fully characterized and implemented clinically; however, there is growing evidence that tumours with increased mutation burden and hence increased expression of neoantigens may be more responsive to ICIs. Recent reports indicate that subsets of malignant melanomas, lung, bladder and microsatellite-unstable gastrointestinal cancers, harbouring high mutation load are responsive to immune checkpoint inhibitors (Powles, 2014; Snyder, 2014; Garon, 2015; Le, 2015). This pre-existing immune potential is a major factor that may determine whether patients derive benefit from immunotherapy. Overall, the median number of non-synonymous coding-region mutations in paediatric tumors (such as medulloblastoma and neuroblastoma) are significantly lower than that seen in adult cancers (Alexandrov, 2013). As such, this may account for the disappointing reported efficacy of ICIs in paediatric cancers to date. As a hallmark representation of high TMB in children, CMMRD represents the ideal malignancy to elucidate the efficacy of prototypical ICIs like nivolumab in paediatric cases. Additionally, ICIs may prove to be the curative resolution for this particularly difficult to treat population. Numerous individual case reports suggest that this theory may be true for these rare patients and other hypermutant cancer patients alike (Bouffet, 2016).

CMMRD cancers harbor the highest mutation load among human cancers (Shlien, 2015). Germline mutations in the four most common MMR genes significantly impair proofreading during DNA replication and result in continuous accumulation of mutations throughout tumour evolution. CMMRD cancers, in contrast to other childhood and adult MMR-proficient cancers exhibit a molecular signature including single nucleotide variants (SNV) present in exponentially higher numbers. Thus, this study was originally designed to test the efficacy of nivolumab in paediatric patients with tumoural bMMRD as a result of underlying CMMRD or Lynch syndrome.

Recent evidence (Shlien, 2015) has shown that, in addition to those associated with CMMRD, a significant proportion of paediatric tumours also show evidence of hypermutation. Data from sequencing of nearly 3000 paediatric tumours (including primary and relapse samples) have indicated that 5.5% have TMB ≥10 mutations per megabase (mut./Mb), with hypermutation seen across a range of diagnoses, including high-grade gliomas, sarcomas, germ cell tumours and

neuroblastoma. A further ~15% of cases have increased TMB in the range 5-10 mut./Mb. The study protocol has therefore been amended to determine whether these hypermutant cancers (regardless of underlying CMMRD status) respond to nivolumab therapy.

1.8 Companion Biomarkers Exploratory Studies

Alongside determination of TMB through cancer gene sequencing panel, the trial will incorporate additional biological studies to further explore predictors of response and the nature of these hypermutant cancers.

A myriad of techniques have been used to analyze and successfully correlate mutation load status and neoantigen expression with immunotherapy success. Studies have used whole exome sequencing (WES) as a method of determining overall mutation burden in treated patients (Snyder, 2014; Snyder, 2015; Le, 2015; Rizvi, 2015). This WES data is then used in combination with HLA halotyping to predict neoantigens. One study also analyzed RNA sequencing data obtained from patients in order to ensure that predicted neoantigens are actually expressed by tumour cells (Le, 2015).

In addition to analyzing tumour characterisitics, studies have also examined immune-related responses to ICIs. RNA sequencing has been used to show increased expression levels of granzyme A and perforin within the the tumour microenvironment (Le, 2015). These toxins are released by cytotoxic T-cells to induce death in target cells. One study used IHC to stain for $CD8^+$ (a marker of cytotoxic T-cells) and showed that MMR proficient tumours contain a greater density of CD8 positive lymphoid cells than MMR deficient tumours (Le, 2015; Westdorp, 2017). Rizvi, *et al.* (2015) artificially synthesized peptide-MHC complexes for candidate neoantigens.

PBMCs were treated with these neoantigens and flow cytometry was used to show that $CD8^+$ T-cells were reactive towards one such neoantigen. They additionally stained for cytokine production and showed that reactive T-cells not only stained positive for CD-8, but also for cytokines associated with activated T-cells (Le, 2015). Our study will use many of these techniques, as well as incorporate novel methods to better characterize those lymphocytes involved in the immune response as well as look at the activation of downstream cell signaling events in these immune cells.

As in previous studies, this trial will incorporate WES and RNA-sequencing of hypermutant tumours in order to determine potential neoantigens, including those actually

expressed in the tumour. Serial patient blood samples, before treatment and at several time points throughout-treatment will be used for the isolation of PBMCs. Flow cytometry will be used to obtain functional profiling on patient lymphocytes (from peripheral blood and from viable tumour cell isolates) by analyzing cell surface markers, as well as markers of T-cell subpopulations (effector vs. memory cells). Enriched populations will be sorted for *in vitro* studies to stain for cytokines, as well as for perforin and granzyme A. Finally, we will perform fluorescent activated flow cytometry (F.A.C.S.) to look at the downstream signaling events involved in the immune system response to nivolumab. Signaling from cytokines, chemokines or receptor engagement activates downstream signaling pathways that are characterized by specific phosphorylation events. Phospho-flow allows single cell analysis of both phosphorylation events as well as cell surface markers simultaneously (Wu, 2010). These tools will allow us to assess changes in mutation burden and the immune response during the course of nivolumab treatment.

Circulating tumour DNA (ctDNA), nucleic acids released by tumours into their surroundings on an ongoing basis, can be found in plasma, urine and cerebral spinal fluid (CSF; Wang, 2015; Khagi, 2017). It is hypothesized that circulating tumour cells (CTC) can be detected from blood. Nucleic acids and CTC exist in bodily fluid in very low copy numbers. Therefore, sensitive techniques need to be performed in order to detect such a low amounts of tumour traces. Several techniques were proved to be sensitive enough for such detection, in particular methods working on the principle of digital droplet PCR. This method is well established in adult oncology as so-called “liquid biopsies” (Khagi, 2017; Wan, 2017). The most frequent approach in adults, consists of tumour mutations detection in blood at the time of tumour recurrence to evaluate clonal evolution and eventually to detect new mutations that were not present in the tumour at the time of diagnosis. ctDNA in serial blood samples will be assessed as a potential surrogate marker of disease burden and response.

2 TRIAL OBJECTIVES

2.1 Primary Objective

To evaluate the objective response rate (ORR) to nivolumab in paediatric patients with refractory or recurrent hypermutated malignancies, including patients with replication repair deficiencies (RRD) such as constitutional mismatch repair deficiency (CMMRD).

2.2 Secondary Objectives

1. To determine the progression free survival (PFS) and overall survival (OS) of paediatric patients with progressive or recurrent hypermutated malignancies including RRD patients such as CMMRD, treated with nivolumab.

2. To evaluate safety and tolerability of nivolumab administered as a single agent at the adult recommended dose of 3 mg/kg every two (2) weeks. To define and describe the toxicities in paediatric patients with progressive or recurrent hypermutated malignancies including RRD patients, such as CMMRD.

2.3 Exploratory/Biology Objectives

1. To explore associations between TMB and response to nivolumab therapy.

2. To discover biomarkers predicting response of hypermutant cancers undergoing PD-1 blockade by investigating tumour neoantigen formation, specific T-cell receptor rearrangements (TCRR) of peripheral lymphocytes and tumour infiltrating lymphocytes (TILs), as well as a detailed characterization and activation of the immune infiltrations including the TILs.

3. To explore the use of minimally invasive methods to monitor and predict response to immune checkpoint inhibition in hypermutant cancers by investigating TCRR, phenotypic profiling of specific immune cells and their activation as a prognostic factor and variances throughout treatment as a response to therapy. As well, to investigate circulating tumour DNA (ctDNA) from serial peripheral blood samples as a surrogate marker of response.

3. STUDY DESIGN

3.1. Study Description

3.1.1 Study Overview

This is an open-label, single arm, multi-center, pilot study of nivolumab in paediatric patients with recurrent or refractory hypermutant malignancies aged 12 months to <25 years of age. Local centres are only obligated to treat/admit patients in accordance their age range capabilities.

The purpose of this study is to assess response of treatment with nivolumab in children with hypermutated cancers, including those with RRD, such as CMMRD syndrome.

This study will be performed in two parts: **Part I – Molecular Profiling** and **Part II – Treatment and Companion Biomarker Studies**.

In order to participate in **Part I**, patient's cancer specimen must undergo a specific gene sequencing panel to determine TMB or else have proof of RRD (as outlined below). Once TMB, or proof of RRD, is established and **Part II** eligibility is confirmed, patients will be stratified into cohorts based on levels of TMB or RRD status (for those patients for whom it is not possible to obtain TMB level) and patients will be enrolled to **Part II**.

Part I - Molecular Profiling

Patients with recurrent or relapse paediatric cancers that are suspected to be hypermutant (see **Part I** Inclusion Criteria in *Section 4.2.1* for exposition) will be consented to **Part I,** submit a specimen (as outlined in the *Lab Manual*) and undergo either the study-specific, next-generation sequencing (NGS) targeted gene panel to determine TMB level or only when sufficient neoplastic specimen is unavailable, provide proof of or tissue for diagnosis of an RRD disorder.

The TMB assay must be performed in a Study Chair or Co-chair specified, CLIA-certified laboratory. Proof of RRD status may be established by assays performed in either a CLIA or ISO 15189-certified laboratory in accordance with local regulations and at the discretion of the Study Chair or Co-chair. Proof of RRD includes functional mutation of polymerase genes (*POLE* and *POLD1*) or an MMR deficiency diagnosed by demonstrating a germline mutation or a loss of

MMR (MLH1, MSH2, MSH6, PMS2, EPCAM or MSH3) protein expression confirmed via negative immunohistochemistry (IHC) staining.

Part II - Treatment and Companion Biomarker Studies

Patients with cancers that have been confirmed as hypermutant based on a report by a specific-TMB assay (acquired via **Part I** participation or previously) or have proof of RRD will be consented and enrolled into to **Part II**.

Cohort stratification of patients enrolled in **Part II** is based on their identified TMB levels or RRD status:

- Cohort A: TMB ≥5 but <10 mutations/Mb (max. 20 patients);
- Cohort B: TMB ≥10 mutations /Mb (max. 30 patients);
- Cohort C: unobtainable TMB in a patient with RRD.

N.B.: Patients stratified to Cohort C will be reallocated to Cohorts A or B if a TMB value subsequently becomes available.

All eligible patients will receive nivolumab intravenously (IV) at a dose of 3mg/kg administered every 14 days (two weeks). Two doses comprise one cycle (28 days or four weeks). Evaluations will be performed according to the schedule provided. Samples to perform 'Companion Biomarkers' research to further our understanding of paediatric hypermutant cancer response to nivolumab will be obtained (see *Lab Manual* for details).

N.B: We strongly recommend that all patients with confirmed or suspected RRD syndromes undergo screening for possible concurrent malignancies (see Section 8.0).

Patients will be monitored for toxicity using standard National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03. Response assessment will use iRECIST criteria for solid tumours (modified for neuroblastoma using the revised INRC), iRANO criteria for CNS malignancies, RECIL 2017 criteria for lymphomas, revised criteria according to Creutzig, et al. (2012) for acute myeloid leukemia (AML; see *Section 10.13*), and criteria as specified in *Section 10.14* for acute lymphoblastic leukemia (ALL).

The Safety visit will be completed when the patient comes off treatment and prior to entering the Follow-Up Period.

3.2 Part II - Treatment and Companion Biomarker Study Duration

Nivolumab will be given in four (4) week cycles (one cycle equals two (2) drug administrations, two (2) weeks apart) for a maximum of up to two (2) years if clinical and radiological benefit is evident. After completion of treatment, patients will enter standard follow up for twelve (12) months. Patients with confirmed disease progression or excessive toxicity will discontinue treatment.

3.3 Independent Review Committee

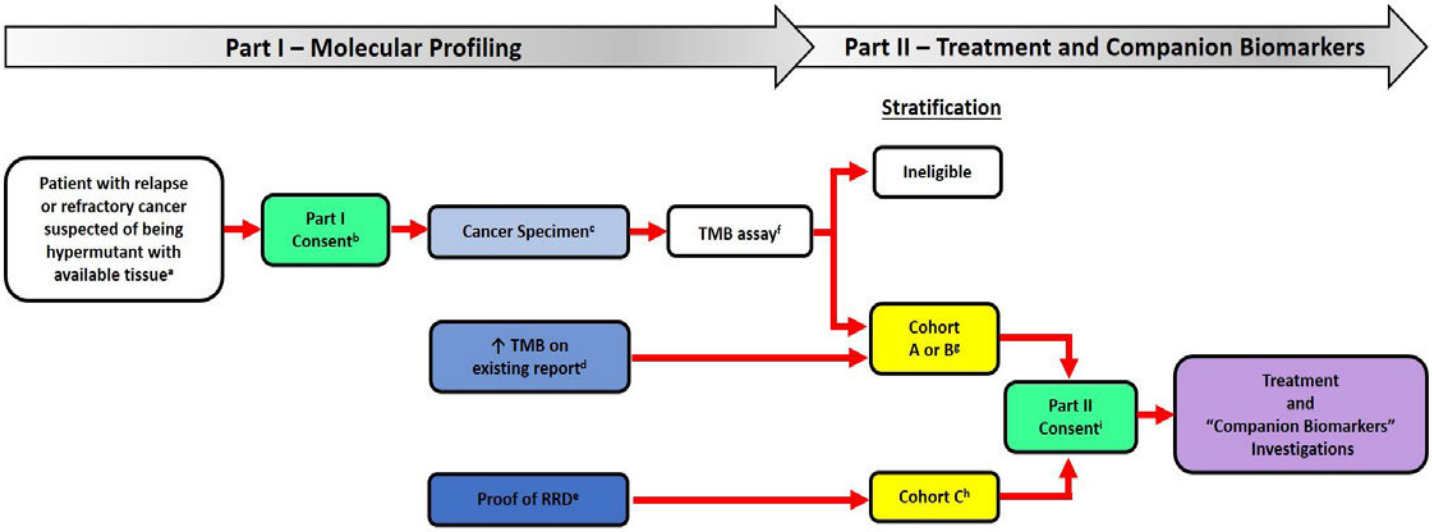
Local ethics board/committee approval of the study must be obtained by a site/institution prior to enrolling and treating patients.

3.4 Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) will be created following the study approval to evaluate the study for safety reasons.

The DSMB will review study data on a bi-annual basis and after the first 10 patients have been accrued. The DSMB may recommend that the trial stops after the inclusion of 10 patients based on toxicity and available efficacy data. A bi-annual careful review of treatment and patient safety will be undertaken by the DSMB and will include review of serious adverse events. The DSMB may recommend that the study stop earlier on the basis of safety concerns.

3.5 Study Flow Diagram



Children and Young Adults (CAYA); TMB = tumour mutation burden; RRD = replication repair deficiency; SickKids = The Hospital for Sick Children.

Figure 1. Study Design. The study is comprised of two parts: **Part I – Molecular Profiling** and **Part II – Treatment and Companion Biomarker Studies**.

a. Recurrent or relapse paediatric cancer patients suspected to be hypermutant include those outlined in *Section 4.2.1*.
b. A separate consent/ assent must be provided for **Parts I** and **II**. The **Part I – Molecular Profiling** portion ensures that eligible patients meet the TMB threshold or RRD status necessary for stratification and progression to **Part II**.
c. Cancer specimen (tumour, bone marrow or whole blood, as relevant) will be processed as outlined in the *Lab Manual.* All assays must be performed in a laboratory with specific certifications as outlined prior. If **Part I** consent is obtained prior to tumour acquisition, and sufficient tissue is available after all diagnostic and trial-related assays, efforts to process extra fresh tumour sample (prior to freezing/ fixation) immediately into viable cells should be made as outlined in the *Lab Manual*. If the patient is eligible, consents and is enrolled in **Part II,** these cells will be used as part of the "Companion Biomarker" research investigations. If the patient is not eligible or does not consent to **Part II**.
d. A report from the specific, CLIA-certified laboratory (outlined in the *Lab Manual*) containing a TMB score may enter the screening process for **Part II** directly.
e. If specimen is not available or a TMB result was not obtained in accordance with study guidelines, a patient with evidence of an RRD disorder will be eligible. Diagnosis of RRD includes proof of either a functional mutation of polymerase genes (*POLE* and *POLD1*) or an MMR deficiency diagnosed by demonstrating a germline mutation or a loss of MMR (MLH1, MSH2, MSH6, PMS2, EPCAM or MSH3) protein expression confirmed via negative immunohistochemistry (IHC) staining performed in an ISO 15189 or CLIA-certified laboratory depending on local regulation (see *Lab Manual*).
f. As determined in specified, CLIA-approved laboratory (see *Lab Manual*).
g. See *Section 3.1.1* for Cohorts A & B details.
h. A patient with proof of diagnosis of RRD may be recruited to Cohort C as outlined in *Section 3.1.1* and the *Lab Manual.* If tumour tissue becomes available while on study, a TMB assay will be performed and these patients may be reallocated to Cohorts A or B. Patients will only remain in Cohort C if impossible to obtain a TMB in perpetuity.
i. A separate consent/ assent must be provided for **Parts I** and **II**. During the **Part II – Treatment and Companion Biomarker Studies** portion, patients enrolled will receive nivolumab (as specified above) and provide samples for accompanying biomarker research (see *Section 2.3, 8.2* and the *Lab Manual*).

4. PATIENT SELECTION

This trial will be conducted in compliance with the protocol, GCP and the applicable local regulatory requirement(s).

4.1 Informed Consent

All patients/Legally Acceptable Representatives (LAR; such as a parent or guardian, as applicable) will be asked to sign informed consent to both **Parts I** and **II** separately as applicable. Assent, when appropriate, will be obtained according to institutional guidelines and ICH GCP (E6), FDA, Declaration of Helsinki and the applicable regulatory requirement(s). Telemedical consent will be accepted, if permitted under local regulations and approved by the local governing REB/IRB for **Part I** only.

The patient may also provide consent/assent for future biomedical research. Additionally, the patient may also provide consent/assent for processing of fresh tumour sample (to viable frozen cells, see *Lab Manual*) if sufficient remaining tumour (after all diagnostic and trial-related assays) is available prior to freezing or fixation for possible use during **Part II** if enrolled.

4.2 Patient Eligibility

4.2.1 Part I - Molecular Profiling Inclusion Criteria

In order to participate in **Part I,** patients must meet the following criteria:

1. **Consent/ Assent:** Patient and/or Legally Acceptable Representative (LAR; such as a parent or guardian, as applicable) must be willing and able to provide written informed consent/assent for the trial as per local requirements.

2. **Age:** patients must be ≥ 12 months and <25 years of age at time of **Part I** enrollment. Local centres are only obligated to treat/ admit patients in accordance their age range capabilities.

3. **Recurrent or relapse paediatric cancer patients suspected to be hypermutant,** including those exhibiting evidence of one or more of the following:

a. high microsatellite instability (MSI-H) in current or previous tumour;
b. a mutation causing loss of mismatch repair gene (*MLH1, MSH2, MSH6, PMS2, EPCAM* or *MSH3*) expression;
c. hypermutation by local sequencing in current or previous tumour;
d. a history of CMMRD, Lynch syndrome, xeroderma pigmentosum (XP), or other established disorder associated with an elevated tumour mutation rate;
e. a functional mutation of polymerase genes (*POLE* or *POLD1*) in current or previous tumour;
f. a functionally impaired RRD pathway by other means;
g. a temozolomide (TMZ) treated current or previous CNS tumour;
h. a predisposing hypermutant cancer signature (i.e. dysregulation of an apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deamination or UV-associated);
i. other factors, which may predicate an elevated mutation burden at the discretion of the Study Chair or Co-Chair.

4. **Diagnosis:** patients must have histologic or cytologic confirmation of malignancy at the time of initial diagnosis or relapse (as specified above). Patients with multiple concurrent and/or sequential neoplasms are eligible, including CNS and haematological malignancies.

5. **Specimen availability:** patients must be able to provide specimen (archival or newly obtained biopsy) of a tumor lesion, appropriately obtained and preserved in a manner compatible for TMB analysis or applicable IHC staining for MMR gene protein expression, if applicable (as described in the *Lab Manual*). Only those with an already ascertained TMB level report from the laboratory specified in the *Lab Manual* or those with proof of RRD as outlined in the *Lab Manual* will be exempt from mandatory tissue submission. If tissue (including archival) is not available, a new tissue specimen may be obtained if deemed clinically appropriate. Any such biopsy will *not* be considered a trial-related procedure.

4.2.2 Part II - Treatment and Companion Biomarker Inclusion Criteria

In order to participate in **Part II**, patients must meet the following criteria:

1. **Consent/ Assent:** Patient and Legally Acceptable Representative (LAR; such as a parent or guardian, as applicable) must be willing and able to provide written informed consent/assent for the trial as per local requirements.

2. **Confirmation of Hypermutation or Proof of RRD:** patient must have completed and verified a sufficient TMB level or have proof of RRD diagnosed in the appropriate lab, as outlined in the *Lab Manual*.

3. **Age:** patients must be ≥ 12 months and < 25 years of age at the time of **Part II** enrollment. Local centres are only obligated to treat/ admit patients in accordance their age range capabilities.

4. **Diagnosis:** patients must have had histologic verification of malignancy at the time of initial diagnosis or at relapse (as specified above). Patients with multiple concurrent and/or sequential neoplasms are eligible, including CNS and haematological malignancies.

5. **Disease status:** patients must have either measurable or evaluable disease in accordance with criteria as outlined in *Section 10*. Tumour lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.

6. **Treatment options:** patient's current disease state must be one for which there is no known curative therapy or therapy proven to prolong survival with an acceptable quality of life. Chemotherapy-naïve patients will be eligible in cases where first-line therapy does not include chemotherapy (e.g. surgery alone for management of ependymoma).

7. **Performance status**: Karnofsky ≥ 50% for patients > 16 years of age or Lansky ≥ 50 for patients ≤ 16 years of age. Patients who are unable to walk because of paralysis, but who are

up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

8. **Previous treatment:** patients must have fully recovered from the acute toxic effects of all prior anti-cancer therapy.
   a. **Myelosuppressive chemotherapy:** at least 21 days after the last dose of myelosuppressive chemotherapy (42 days if prior nitrosourea).
   b. **Hematopoietic growth factors**: at least 14 days after the last dose of a long-acting growth factor (e.g. Neulasta) or 7 days for short-acting growth factor. For agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time during which adverse events are known to occur. The duration of this interval must be discussed with the Study Chair or Co-Chair.
   c. **Biologic (anti-neoplastic agent):** at least 14 days after the last dose of a biologic agent. For agents that have known adverse events occurring beyond 14 days after administration, this period must be extended beyond the time during which adverse events are known to occur. The duration of this interval must be discussed with the Study Chair or Co-Chair.
   d. **Monoclonal antibodies:** at least three (3) half-lives of the antibody after the last dose of a monoclonal antibody.
   e. **Radiation Therapy (XRT):** at least 14 days after local palliative XRT (small port). At least 150 days must have elapsed if prior Total Body Irradiation, craniospinal XRT or if ≥ 50% radiation of pelvis. At least 42 days must have elapsed if other substantial BM radiation.
   f. **Stem Cell Infusion without Total Body Irradiation (TBI):** no evidence of active graft vs. host disease and at least 56 days must have elapsed after transplant or stem cell infusion. Patients with prior allogeneic transplants (including solid organ) are not eligible

9. **Organ Function Requirements:**
   a. **Adequate BM Function Defined as**
      i. Peripheral absolute neutrophil count (ANC) $\geq 0.75 \times 10^9$/L or 750/mm^3.
      ii. Platelet count $\geq 75 \times 10^9$/L or 75,000/mm^3 (transfusion independent, defined as not receiving platelet transfusions for at least 7 days prior to enrollment.

iii. Hemoglobin ≥ 90g/L (transfusion permitted).

iv. Patients with known BM metastatic disease or haematological malignancies will be eligible for study provided they meet haematological criteria. These patients may receive transfusions (e.g. to achieve platelet threshold) provided they are not known to be refractory to platelet transfusions but will not be evaluable for hematologic toxicity.

b. Adequate Renal Function Defined as:

A serum creatinine based on age/gender as provided in *Table 3.*

Table 3. The threshold creatinine values in this Table were derived from the Schwartz formula (Schwartz, 1985) for estimating GFR utilizing child length and stature data published by CDC.

Age	***Maximum Serum Creatinine***			
	Male		**Female**	
	mg/dL	*µmol/L*	*mg/dL*	*µmol/L*
1 to < 2 years	0.6	53	0.6	53
2 to < 6 years	0.8	71	0.8	71
6 to < 10 years	1	88	1	88
10 to < 13 years	1.2	106	1.2	106
13 to < 16 years	1.5	133	1.4	124
≥ 16 years	1.7	150	1.4	124

c. Adequate Liver Function Defined as:

i. Bilirubin (sum of conjugated + unconjugated or total bilirubin) ≤1.5x institutional upper limit of normal (ULN) for age (except for patients with Gilbert's Syndrome, when bilirubin of < 51 µmol/L or 3.0 mg/dL is permitted).

ii. ALT/AST:

1. ≤ 2.5 x institutional ULN for patients without liver metastases.
2. ≤ 5 x institutional ULN for patients with liver metastases.

d. Adequate Pulmonary Function Defined as:

No history of chronic pulmonary disease (such as Cystic Fibrosis) and no evidence of dyspnea at rest, no exercise intolerance due to pulmonary insufficiency and a pulse oximetry > 92% on room air.

e. Adequate Pancreatic Function Defined as:

Serum lipase ≤ ULN. Patients with glucose intolerance should be on a stable regiment and be monitored.

10. For patients with brain tumors, debulking surgery prior to treatment with nivolumab should be considered when appropriate to reduce the risk of pseudoprogression-associated toxicities. Such debulking surgery is not mandatory for trial enrollment. Patients should be recovered from surgery and wait at least 7 days from surgery before first dose.

4.2.3 Part II (ONLY) Exclusion Criteria

1. Pregnancy or Breastfeeding:

Women who are pregnant or breastfeeding and men who are sexually active with women of child bearing potential (WOCBP)* who are not willing to use effective contraception, or to practice abstinence if this is the usual lifestyle and preferred contraception for the patient.**

- Pregnant or breastfeeding women will not be entered on this study due to risks of fetal and teratogenic adverse events as there is no available information yet regarding human fetal or teratogenic toxicities.
- Women of childbearing potential (WOCBP)* must have a negative pregnancy test every 4 weeks. During **Part II** screening, WOCBP must have a negative serum pregnancy test. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab administration.
- WOCBP who are sexually active must be willing to adhere to effective contraception or to practice abstinence if this is the usual lifestyle and preferred contraception for the patient** during treatment and for 5 months after the last dose of nivolumab.
- Men who are sexually active with WOCBP must be willing to adhere to effective contraception** during treatment and for 7 months after the last dose of the study drug.
- Women who are surgically sterile, as well as azoospermic men, do not require contraception.

** "Women of childbearing potential" is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal.*

** List of contraception methods is provided in *Appendix II*.

2. Concomitant Medications

a. **Corticosteroids:** patients requiring systemic steroid therapy or any other form of immunosuppressive therapy within seven (7) days prior to the first dose of trial therapy or while on trial are not eligible. The use of physiologic doses of corticosteroids (up to 5mg/m^2/day prednisone equivalent) is permitted following discussion with the Study Chair or Co-Chair.

   Note: *Use of topical, ocular, intra-articular, intra-nasal or inhaled corticosteroids will not render a patient ineligible. A brief course of corticosteroids for prophylaxis (e.g. contrast dye allergy) or for treatment of non-autoimmune conditions (e.g. delayed-type hypersensitivity reaction caused by contact allergen) is permitted if completed at least 7 days prior to initiation of therapy.*

b. **Investigational Drugs:** patients who are currently receiving another investigational drug are not eligible.

c. **Anti-cancer Agents:** patients who are currently receiving other anti-cancer agents are not eligible.

3. **Patients with a History of Autoimmune Disease:**

   Patients with a history of autoimmune disorder that has required systemic treatment in the previous 2 years are not eligible. Asymptomatic laboratory abnormalities (e.g. ANA, rheumatoid factor, altered thyroid function studies) will not render a patient ineligible in the absence of a diagnosis of an autoimmune disorder. Replacement therapy (e.g. thyroxine, insulin or physiologic corticosteroid replacement therapy) is not considered a form of systemic treatment.

4. **Infection:** Patients who have an uncontrolled infection are not eligible.

5. **HIV and/or Hepatitis B/C patients:** Patients with known HIV/AIDS or acute/chronic Hepatitis B or C are excluded.

6. **Transplant patients:** patients who have received prior allogeneic BM transplants or prior solid organ transplantation are not eligible.

7. **Non-compliance:** patients who in the opinion of the investigator may not be able to comply with the safety monitoring requirements of the study are not eligible.

8. **Previous anti-PD-1 inhibitor and/or anti-PD-L1 inhibitor therapy:** patients who have received prior anti-PD-1 and/or anti-PD-L1 directed therapy (mAb or small molecule) are not eligible.

9. **Live vaccines:** Patients who have received a live vaccine within 30 days of start of study treatment are not eligible.

4.3 Patient Registration Procedures

Consent to the **Part I** assessment must be obtained prior to sending an existing specimen to the Hospital for Sick Children (Toronto, Canada) or to the specific CLIA-certified laboratory agreed by the Study Chair or Co-chair as outlined in the *Lab Manual*. If tissue (including archival) is not available, a new tissue specimen may be obtained if deemed clinically appropriate. Sites will assign each patient with a patient ID study number during **Part I**. Any such biopsy will *not* be considered a trial-related procedure. Potential patients for whom tumour material (either at diagnosis or relapse) has already been sequenced and analyzed in a specific Study Chair or Co-chair approved and CLIA-certified laboratory reporting evidence of hypermutation may consent directly to **Part II**. In the absence of available specimen necessary for participation in the TMB assay, proof of RRD may confer eligibility (as outlined above and in the *Lab Manual*). An explanation of the study and full disclosure of the informed consent document will take place. Patients or Parents/Legal Guardians will be required to sign the appropriate study consent form for **Part I**.

Once the **Part I - Molecular Profiling** assays have been completed and patient is verified as eligible for participation in **Part II - Treatment and Companion Biomarker Studies**, an explanation of the study and discussion of the expected side effects and full disclosure of the informed consent document will take place. Patients or Parents/Legal Guardians will be required to sign the appropriate study consent form for **Part II**.

Only eligible and consented patients will be registered into the study. Registration will be done through *Ozmosis Research Inc*.

Prior to registering a patient, each institution must have submitted all necessary regulatory documentation to *Ozmosis Research Inc*. Access to the eCRFs will only be granted once this documentation has been received. Prior to enrolling a new patient, sites should contact *Ozmosis Research Inc.* to verify study availability.

No patient can receive protocol treatment until eligibility has been confirmed and the Patient Enrollment Form has been submitted to and acknowledged by *Ozmosis Research Inc*. All eligibility criteria must be met at the time of registration. There will be no exceptions. Any questions should be addressed with *Ozmosis Research Inc.* and/or the Study Chair or Co-Chair prior to registration.

The Patient Enrollment Form must be completed, and signed by the investigator prior to enrollment. This form can be faxed to [redacted] or emailed to [redacted] There are four (4) sections to the 'Patient Enrollment Form':

1. **SCREENING PART I** (top section): This section is completed by the site and should be sent to *Ozmosis Research Inc.* at the time of screening into **Part I**. This section must be completed for all **Part I** screened patients, including screen failures.
2. **ENROLLMENT PART I**: This section is completed by the site and should be sent to *Ozmosis Research Inc.* at the time of enrollment into **Part I.**
3. **SCREENING PART II**: This section is completed by the site and should be sent to *Ozmosis Research Inc.* at the time of screening for **Part II**.
4. **ENROLLMENT PART II** (bottom section): This section is completed by the site and should be sent to *Ozmosis Research Inc.* at the time of patient registration/enrollment to **Part II**.

Protocol treatment should begin within 14 days of patient enrollment to **Part II**. All eligible patients enrolled into either part of the study will be entered into a patient registration log at *Ozmosis Research Inc*.

The following information will be required at the time of registration:

- Trial code;
- Treatment centre and investigator;
- Patient's month and year of birth;

- Completed Patient Enrollment Form.

N.B.: *It is the responsibility of the investigator in charge to satisfy him or herself that the patient is indeed eligible before requesting registration.*

5. TREATMENT PROGRAM

5.1 Treatment Plan

All eligible patients will receive nivolumab in the dose of 3 mg/kg every 14 days (2 weeks). Each cycle of therapy will last for 28 days (4 weeks) total, therefore two (2) doses will be administer per cycle, if the patient has not met any of the criteria for removal from therapy. A maximum length of the therapy on this study is 24 months (see *Figure 2*).

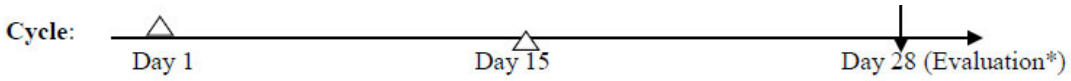


Figure 2. Nivolumab treatment cycle and evaluation plan.

**See Section 8.2 for required disease evaluations. Therapy will be discontinued if there is evidence of confirmed progressive disease (PD) or drug related toxicity that requires removal from therapy.*

Nivolumab will be administered as a 60-minute ± 10-minute infusion. Infusion rate may be slowed as per physician's discretion as clinically indicated (see *Section 6.3*).

The dosing calculations should be based on the actual body weight in kilograms and may be calculated using the weight obtained at the previous dose administration. If the patient's weight on the day and prior to drug administration differs by >10% from the weight used to calculate the dose, the dose should be recalculated. All doses should be rounded to the nearest milligram. There will be no dose modifications allowed.

Pre-medication is not required as infusion reactions are rare, but anaphylactic precautions should be observed during each infusion of nivolumab. If ≥ Grade 2 infusion reaction occurs, the infusion should be stopped and supportive care given as per institutional guidelines. See *Section 6.3* for management and dose modification guidelines for infusion reactions.

During the Cycle 1 Day 1 initial drug administration, investigators are advised to monitor vital signs (see *Section 8.2* for details) and watch for adverse events (AE) such as, fever, chills, shakes, itching, rash, hypertension or hypotension, difficulty breathing and so on beginning at baseline, then every 15 minutes (+/- 5 min.) during administration of the drug. After the completion of the nivolumab infusion, the patient should continue to be monitored every 15 minutes (+/- 5 min.) twice, then every 30 minutes (+/- 5 min.) three (3) times. Following completion of cycle 1

and in patients who have tolerated nivolumab without infusion reactions, frequency of vital sign evaluation following infusion may be reduced at the discretion of, and documented by, the treating physician.

5.2 Criteria for Starting Subsequent Cycles

A cycle, consisting of two (2) doses every 14 days +/- 2 days, may be repeated every 28 days if the patient has not met any of the criteria for removal from therapy.*

Nivolumab administration should be delayed for the following:

1. Any Grade ≥ 2 non-skin, drug-related AE, with the following exceptions:
    - Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay;
    - Any Grade 3 skin, drug-related AE.
2. Any Grade 3 drug-related laboratory abnormality except for the following exceptions:
    - Grade 3 lymphopenia or leukopenia does not require dose delay;
    - If a patient has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity;
    - If a patient has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity;
    - Grade ≥ 3 drug-related amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis does not require dose delay, but requires close follow-up.
3. Any AE, laboratory abnormality or intercurrent illness, which, in the judgment of the investigator, warrants delaying the dose of study medication.

** Patients who require delay of nivolumab should be re-evaluated weekly or more frequently if clinically indicated and resume nivolumab dosing when re-treatment criteria are met (see Section 5.3 for details).*

5.3 Criteria to Resume Treatment

Patients may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Patients may resume treatment in the presence of Grade 2 fatigue;
- Patients who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity;
- Patients with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT or total bilirubin;
- Patients with combined Grade 2 AST/ALT and total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued;
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline (Grade 1) before treatment is resumed. Patients with persistent Grade 1 pneumonitis after completion of a steroid taper over at least 1 month may be eligible for retreatment following discussion with the Study Chair or Co-Chair;
- Patients with drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment following discussion with the Study Chair or Co-Chair.

If the criteria to resume treatment are met, the patient should restart treatment at the next scheduled time point as per protocol. However, if the treatment is delayed past the next scheduled time point as per protocol, this time point should become day one of the subsequent cycle. If treatment is delayed or interrupted for > 6 weeks, the patient must be permanently discontinued from protocol therapy.

6. DOSE MODIFICATION FOR ADVERSE EVENTS

The Study Chair or Co-Chair must be notified of any dosage modification or use of myeloid growth factor.

6.1 Dose Modifications for Haematological Toxicity

Patients who experience Grade 4 thrombocytopenia (platelet count <25 x 10^9/L or 25,000/mm^3) or Grade 4 neutropenia lasting at least five (5) days will be removed from protocol therapy. Patients with Grade 3 or 4 febrile neutropenia can remain on the study; however, dose should be held until toxicities return to baseline or ≤ grade 1.

6.2 Dose Modifications for Non-Haematological Toxicity

Patients who have any Grade 3 or Grade 4 non-hematological toxicity attributable to the investigational drug with the specific exclusion of the following, will be removed from the study:

- Grade 3 ALT that returns to levels that meet initial eligibility criteria or baseline within seven (7) days and does not require systemic immunosuppression;
- Grade 3 liver enzyme elevation, including AST/GGT that returns to baseline within 7 days and does not require systemic immunosuppression;
- Grade 3 or 4 serum electrolyte or mineral abnormalities responsive to supplementation;
- Grade 3 or 4 amylase or lipase abnormalities that are not associated with diabetes mellitus (DM), associated liver or gall bladder inflammation or clinical manifestations of pancreatitis;
- isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset;
- Grade 3 rash/oral lesions that resolves to Grade ≤ 1 within seven (7) days;
- Grade 3 or 4 seizures (multiple seizures despite medical intervention or life-threatening seizures/status epilepticus) that may occur in the context of pseudo-progression;
- fever greater than 40°C of ≤24 hr. duration;
- Grade 3 fatigue that resolves to Grade ≤ 2 within 7 days;
- Grade 3 creatinine increase that resolves to Grade ≤ 1 or baseline within seven (7) days.

Patients who experience the following non-hematological toxicities attributable to protocol therapy will also be removed from the study:

- Grade 2 fever that does not resolve to Grade ≤ 1 within seven (7) days;
- Grade 2 uveitis, eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 prior to next scheduled dose;
- Grade 2 non-hematological toxicity requiring systemic immunosuppressive therapy. This includes, but is not limited to, autoimmunity of the lung, heart, kidney, bowel, CNS, pituitary or eye;
- Grade 2 endocrine toxicity requiring hormone replacement, with the exception of Grade 2 hypothyroidism, thyroiditis and thyroid dysfunction adequately managed with thyroid hormone replacement;
- Grade 2 adrenal insufficiency;
- Grade 3 colitis or Grade 3 diarrhea attributable to protocol therapy of more the seven (7) days of duration.

Any non-hematological toxicity requiring greater than seven (7) days delay in therapy will be criteria for removal from the study. Other non-hematological toxicities attributable to the investigational drug will be dose adjusted as outlined below (see *Section 6.3 – 6.12*).

6.3 Dose Modifications for Infusion-Related Reactions

For patients who have allergic or acute infusion reactions to nivolumab therapy, modifications based on grade should be as follows:

Grade (CTCAE v.4.03)	Action
Grade 1	• Monitor patient until recovery from symptoms (fever, chills, shakes, itching, rash, hypertension or hypotension, or difficulty in breathing during and immediately after administration of nivolumab); infusion rate may be slowed. • If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor patient closely. • The following prophylactic premedication is recommended for future infusions: diphenhydramine 1 mg/kg with max 50 mg (or equivalent) and/or acetaminophen (paracetamol) 10-15 mg/kg (max 1000 mg) at least 30 minutes before additional nivolumab administrations, slowing infusion rate as above.
Grade 2	• Stop infusion, begin an IV infusion of normal saline, and treat the patient with diphenhydramine 1 mg/kg with max 50 mg IV (or equivalent) and/or acetaminophen (paracetamol) 10-15 mg/kg (max 1000 mg); remain at bedside and monitor patient until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. • If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor patient closely.

	• If symptoms recur, then no further nivolumab will be administered at that visit. Administer diphenhydramine 1 mg/kg with max 50 mg IV (or equivalent), and remain at bedside and monitor the patient until resolution of symptoms. The following prophylactic premedication is recommended for future infusions: diphenhydramine 1 mg/kg with max 50 mg IV (or equivalent) and acetaminophen (paracetamol) (10-15 mg/kg, max 1000 mg) should be administered at least 30 minutes before additional nivolumab administrations. If clinically indicated, corticosteroids (recommended dose: 1-2 mg/kg/day methyl-prednisolone IV or equivalent) may be used.
Grade 3 or 4	• Immediately discontinue infusion of nivolumab. • Begin an IV infusion of normal saline, and treat the patient as per institutional guidelines for the treatment of anaphylaxis. • Patient should be monitored until the investigator is comfortable that the symptoms will not recur. • Nivolumab will be permanently discontinued. • Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor patient until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids). • Please note that late occurring events including isolated fever and fatigue may represent the presentation of systemic inflammation. Please evaluate accordingly.

6.4 Dose Modifications for Skin Rash and Oral Lesions

Skin Rash and Oral Lesions	Management/Next Dose of Nivolumab
≤ Grade 1	• No change in dose*
Grade 2	• Continue protocol therapy.* • Topical steroids do not require protocol therapydiscontinuation. If prolonged symptoms require systemic corticosteroids, decisions regarding whether protocol therapymay be reinstituted following weaning of immunosuppression must be made in consultation with Study Chair or Co-Chair.
Grade 3	• Hold* until ≤ Grade 1; if resolves within seven (7) days, then resume at same dose level. • Topical steroids do not require protocol therapydiscontinuation. If prolonged symptoms require systemic corticosteroids, decisions regarding whether protocol therapymay be reinstituted following weaning of immunosuppression must be made in consultation with Study Chair or Co-Chair.
Grade 4	• Discontinue therapy, systemic corticosteroids indicated.
Patients with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphigoid. 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: See Skin AE Management Algorithm (*Section 6.4.1*).	

6.4.1 Recommended Skin Adverse Event Management Algorithm

Toxicity (CTCAE v.4.03)	Management	Follow-up
Grade 1-2 **Rash:** Covering ≤ 30% BSA*	• Symptomatic therapy (e.g. antihistamines, topical steroids); • Continue protocol therapyas per protocol.	**If persists >1-2 weeks or recurs:** • Consider skin biopsy; • Hold protocol therapy; • Consider 0.5-1.0 mg/kg/day Methylprednisolone IV or oral equivalent; • Once improving, taper steroids over at least 1 month; • Consider prophylactic antibiotics for opportunistic infections • Then resume nivolumab therapy per protocol. **If worsens:** • Treat as Grade 3-4.
Grade 3-4 **Rash:** Covering > 30% BSA; life threatening consequences*^	• Hold or discontinue therapy as per protocol' • Consider skin biopsy; • Dermatology consult; • 1.0 – 2.0 mg/kg/day methylprednisolone IV or IV equivalent.	**If improves to Grade 1:** • Taper steroids over at least 1 month; • Add prophylactic antibiotics for opportunistic infections • Resume nivolumab therapy as per protocol.

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 considered when switching to the equivalent dose of oral corticosteroids.

**Refer o NCI CTCAE v.4.03 for term specific grading criteria.*

^If Stevens-Johnson syndrome (SJS)/ toxic epidermal necrolysis (TEN) is suspected, withhold nivolumab therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue nivolumab therapy.

6.5 Dose Modifications for Hepatic/Pancreatic Adverse Events

Liver Function Elevation	Management/Next Dose of Nivolumab
Grade 1	• Continue protocol therapy.
Grade 2	• Hold until laboratory values return to baseline and management with corticosteroids, if needed, is completed.
Grade 3 – 4	• Off protocol therapy.
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 for Hepatic AE Management Algorithm (*Section 6.5.1*).	

Pancreatitis; enzyme elevations CTCAE v.4.03	Management/Next Dose of Nivolumab
Grade 1	• Continue protocol therapy.
Grade 2 Amylase elevation or radiological findings only.	• Continue protocol therapy.
Grade 2 Pancreatitis and/ or lipase elevation or radiological findings only.	• Hold until baseline; Resume at same dose level if asymptomatic.
Grade 3 Severe pain; Vomiting;	• Hold protocol therapy until recovery ≤ grade 1 unless exception in *Section 6.2* is met.

Medical intervention indicated (e.g. analgesia, nutritional support above baseline).	
Grade 4 Life threatening consequences; Urgent intervention indicated.	• Hold off protocol therapy until recovery to baseline or ≤ Grade 1.
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 below (see *Section 6.5.1*).	

6.5.1 Recommended Hepatic Adverse Event Management Algorithm

Consider imaging for obstruction.

Toxicity **(CTCAE v.4.03)**	**Management**	**Follow-up**
Grade 1 AST or ALT > ULN to 3.0x ULN and/ or T. bilirubin > ULN to 1.5x ULN.	• Continue protocol therapy	• Continue routine LFT monitoring according to the required clinical, laboratory evaluations in *Section 8.2*. **If worsens:** • Treat as Grade 2 or 3-4.
Grade 2 AST or ALT > 3.0 to ≤ 5x ULN and/ or T. bilirubin > 1.5 to ≤ 3 x ULN.	• Delay nivolumab per protocol • Increase LFT monitoring to every 3 days	**If returns to baseline:** • Continue routine LFT monitoring per the required clinical, laboratory evaluations in *Section 8.2*. • Resume protocol therapy

		If elevations persist >5-7 days or worsen: • 0.5-1mg/kg/day methylprednisolone IV or oral equivalent • When LFT returns to Grade 1 or baseline, taper steroids over at least 1 month • Upon improvement, nivolumab may be resumed after corticosteroid taper, if needed. • Consider prophylactic antibiotics for opportunistic infections • If worsening or no improvement occurs despite initiation of corticosteroids, corticosteroid dose should be increased to 1 to 2 mg/kg/day methylprednisolone equivalents and nivolumab must be permanently discontinued.
Grade 3 or 4 AST or ALT > 5 x ULN or T. bili. > 3 x ULN.	• Discontinue protocol therapy* • Increase LFT monitoring to every 1-2 days • 1.0-2.0mg/kg/day methylprednisolone IV or oral equivalent** • Add prophylactic antibiotics for opportunistic infections • Consult gastroenterologist	**If improves to Grade 2:** • Taper steroids over at least 1 month. **If does not improve in > 3-5 days, worsens or rebounds:** • Add mycophenolate mofetil 1 g BID • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

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 considered when switching to the equivalent dose of oral corticosteroids.

**Nivolumab therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN or T. bilirubin ≤ 5 x ULN.*

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

6.6 Dose Modifications for Gastrointestinal Adverse Events

Diarrhoea/ Colitis	Management/Next Dose of Nivolumab
Grade 1	• Continue protocol therapy and treat symptomatically.
Grade 2	• May observe and treat symptomatically for seven (7) days. If persists greater than seven (7) days, then off protocol therapy.
Grade 3	• Off protocol therapy.
Grade 4	• Off protocol therapy.
Patients who require steroids should be taken off study treatment. *Evaluation for all patients for additional causes includes C. diff., acute, self-limited infectious, foodborne illness, ischemic bowel, diverticulitis, and IBD.*	
Recommended management: See GI AE management Algorithm below (see *Section 6.6.1*).	

Other GI Nausea/ Vomiting	Management/Next Dose of Nivolumab
Grade 1	• No change in dose.
Grade 2	• Hold pending evaluation for gastritis duodenitis and other immune adverse events or other causes. Resume at same dose level if resolution to ≤ Grade 1 within seven (7) days.
Grade 3	• Hold pending evaluation until ≤ Grade 1. Resume at same dose level. If symptoms do not resolve within seven (7) days with symptomatic treatment patients should go off protocol therapy
Grade 4	• Off protocol therapy
Patients with grade 2 or 3 N-V should be evaluated for upper GI inflammation and other immune related events. Recommended Gastrointestinal Adverse Event Management Algorithm	

6.6.1 Recommended GI Adverse Event Management Algorithm

Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Toxicity (CTCAE v.4.03)	Management	Follow-up
Grade 1 **Diarrhea**: < 4 stools/ day over baseline. **Colitis:** asymptomatic.	• Continue protocol therapy • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately. **If worsens:** • Treat as Grade 2 or 3/4
Grade 2 **Diarrhea:** 4-6 stools/ day over baseline; IV fluids indicated <24hrs; not interfering with ADL. **Colitis:** abdominal pain; blood in stool.	• Nivolumab should be withheld per protocol. • Symptomatic treatment	**If improves to Grade 1** • Resume protocol therapy **If persists > 5-7 days or recurs:** • 0.5-1.0mg/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 • Resume nivolumab per protocol. **If worsens or persists > 3-5 days with oral steroids:** • Treat as Grade 3 or 4 and nivolumab must be permanently discontinued.
Grade 3 **Diarrhea:** ≥ 7 stools/ day over baseline; incontinence; IV fluid ≥ 24 hours; interfering with ADL **Colitis:** severe abdominal pain, medical intervention indicated, peritoneal signs	• Hold protocol therapy • Corticosteroids at dose of 1-2 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy • Nivolumab should be withheld and corticosteroids initiated at	**If improves:** • Continue steroids until grade 1, then taper over at least 1 month. • Upon improvement, nivolumab may be resumed after corticosteroid taper. If worsening or no improvement occurs despite initiation of corticosteroids, nivolumab must be permanently discontinued.

	a dose of 1-2 mg/kg/day methylprednisolone equivalents.	**If persists > 3-5 days or recurs after improvement:** • Consider adding Infliximab 5 mg/kg (if no contraindication). **Note:** *Infliximab should not be used in cases of perforation or sepsis.*
Grade 4 **Diarrhea or Colitis:** life-threatening, perforation.	• Nivolumab must be permanently discontinued, and corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents	**If improves:** • Continue steroids until grade 1, then taper over at least 1 month **If persists > 3-5 days or recurs after improvement:** • Consider adding 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 considered when switching to the equivalent dose of oral corticosteroids.

6.7 Dose Modifications for Pneumonitis

Pneumonitis	Management/Next Dose of Nivolumab
Grade 1	• Asymptomatic patients with radiographic changes should be managed with dose delay and consultation with respirology and infectious disease (ID). Nivolumab can be resumed at the same dose after the resolution of the radiographic changes.
Grade 2	• Hold dose until symptoms resolve, radiographic abnormalities improve to baseline and management with corticosteroids is completed. Resume no change in dose after pulmonary and/or ID consultation if lymphocytic pneumonitis is excluded.
Grade 3	• Off protocol therapy

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 below (see *Section 6.7.1*).	

6.7.1 Recommended Pulmonary Adverse Event Management Algorithm

Evaluate with imaging and pulmonary consultation. Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue nivolumab therapy. Evaluate with imaging and pulmonary consultation.

Toxicity (CTCAE v.4.03)	Management	Follow-up
Grade 1 Pneumonitis: Radiographic changes only.	• Consider delay of protocol therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and ID consults	• Re-image with CXR every 3 weeks **If worsens:** • Treat as Grade 2 or 3-4
Grade 2 Pneumonitis: Mild to moderate new symptoms.	• Hold infusion • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1 mg/kg/day methylprednisolone IV or oral equivalent • Consider bronchoscopy, lung biopsy	• Re-image every 1-3 days **If improves:** • When symptoms return to near baseline, taper steroids over at least 1 month and then resume nivolumab therapy per protocol; • Consider prophylactic antibiotics. **If not improving after 2 weeks or worsening:** • Treat as Grade 3 or 4
Grade 3 or 4 Pneumonitis: Severe new symptoms; new/	• Discontinue protocol therapy; • Hospitalize; • Pulmonary and ID consults;	**If improves to baseline:** • Taper steroids over at least 6 weeks.

worsening hypoxia; life-threatening.	• 2-4 mg/kg/day methylprednisolone IV or IV equivalent; • Add prophylactic antibiotics for opportunistic infections; • Consider bronchoscopy, lung biopsy.	**If not improving after 48 hours or worsening:** • Add additional immunosuppression (e.g. infliximab, cyclophosphamide, IVIG, or mycophenolate mofetil).

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 considered when switching to the equivalent dose of oral corticosteroids.

6.8 Dose Modifications for Fatigue

Fatigue	Management/Next Dose of Nivolumab
Grade 1	• No change in dose.
Grade 2	• No change in dose
Grade 3	• Hold until ≤ Grade 2. If resolves within 7 days, 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.	

6.9 Dose Modifications for Neurologic Adverse Events

Neurologic events	Management/Next Dose of Nivolumab
≤ Grade 1	• Continue protocol therapy.*
Grade 2	• Hold until resolution to baseline.* Resume with no change in dose.*
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 taken off protocol therapy.*
Recommended management: See Neurologic Adverse Event Management Algorithm below (see *Section 6.9.1*).

6.9.1 Recommended Neurologic Adverse Event Management Algorithm

Toxicity (CTCAE v.4.03)	Management	Follow-up
Grade 1 **Neurological Toxicity:** Asymptomatic or mild symptoms; Intervention not indicated.	• Continue protocol therapy	• Continue to monitor the patient. **If worsens:** • Treat as Grade 2 or 3-4.
Grade 2 **Neurological Toxicity:** Moderate symptoms; limiting instrumental ADL.	• Delay infusion per protocol; • Treat symptoms per local guidelines; • Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent.	**If improves to baseline:** • Within 7 days resume protocol therapy, if persists >7 days discontinue protocol therapy. **If worsens:** • Treat as Grade 3-4.
Grade 3-4 **Neurological Toxicity:** Severe symptoms; limiting self-care ADL; life threatening.	• Discontinue protocol therapy; • Obtain neurology consult; • Treat symptoms per local guidelines • 1-2 mg/kg/day methylprednisolone IV or IV equivalent; • Add prophylactic antibiotics for opportunistic infections.	**If improves to Grade 2:** • Taper steroids over at least 1 month. **If worsens or atypical presentation:** • Consider IVIG or other immunosuppressive therapies per local guidelines.

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 considered when switching to the equivalent dose of oral corticosteroids.

6.9.2 Special Considerations for Corticosteroid Administration in Patients with CNS Tumour Pseudo-progression.

In patients under treatment for brain tumours, worsening of pre-existing neurological focal deficits, suggesting tumour progression or recurrence, can be accompanied by neuroradiological appearances of oedema and/or a contrast enhancing lesion within the tumour bed. However, this radiological pattern is not necessarily associated with disease progression and can be related to immunological changes associated with treatment. This section discusses the management of patients who show clinical and/or radiological manifestations that can relate to this phenomenon of pseudo-progression.

For symptomatic patients, dexamethasone is the most commonly used corticosteroid in this context (Kall, 2004; Dietrich, 2011). The aim of this treatment is to attenuate clinical manifestations (in particular associated sign and symptoms of increased cranial pressure. Several clinical studies have shown that dexamethasone can inhibit maturation of dendritic cells and subsequently their potential for antigen presentation, dexamethasone can also impair natural-killer-cell activity. Therefore, dexamethasone doses and duration of therapy should be limited to the minimum amount needed to control neurologic symptoms. All CNS disease progression will be assessed using iRANO criteria (see *Section 10.8*), which accounts for CNS tumour pseudo-progression.

It is unclear the extent to which pseudo-progression may occur in non-CNS conditions. For this reason, investigators are encouraged to continue the administration of nivolumab while waiting for a repeat tumour assessment, at least 4 weeks apart, in the event of radiological evidence of progressive disease but stable clinical status. Administration of corticosteroids in case of pseudo-progression in non-CNS conditions should be discussed with the Study Chair or Co-chair. All solid tumour progression will be assessed using iRECIST criteria (see *Section 10.2*), which accounts for immunotherapeutic effects during treatment with ICIs, such as nivolumab.

6.10 Immunotherapy Continuation Pending Confirmation of Progression

A decision of whether a patient should continue immunotherapy pending confirmation of radiographic disease progression should be established based on perceived benefits and risks as per the response criteria outlined in *Section 10*.

Continuation of immunotherapy might be considered pending follow-up imaging as long as patients are deriving apparent clinical benefit with minimal and acceptable toxic effects.

Immunotherapy should be interrupted for patients who require treatment with corticosteroids for evolving symptoms associated with cerebral oedema or who have more than mild treatment-related toxic effects such as at least grade 2 immune-related adverse events. Resumption of immunotherapy may be considered when symptoms resolve, steroids are reduced and the gadolinium enhancing tumour burden is classified as stable disease, partial response, or complete response on a follow-up scan, or when relevant treatment-related toxic effects have resolved to grade 1 or less or pre-treatment baseline. Decision to continue/discontinue immunotherapy should be made after discussion with the Study Chair or Co-chair.

6.11 Dose Modifications for Endocrine Adverse Events

Endocrine Hypophysitis; Adrenal Insufficiency	**Management/Next Dose of Nivolumab**
≤ Grade 1	• Continue protocol therapy.
Grade 2	• Off protocol therapy, unless exception in *Section 6.2* is met (Grade 2 hypothyroidism, thyroiditis and thyroid dysfunction adequately managed with thyroid hormone replacement).
Grade 3	• Off protocol therapy.
Grade 4	• Off protocol therapy.
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.	
Recommended management: See Endocrine AE Management Algorithm (see *Section 6.11.1*).	

6.11.1 Recommended Endocrine Adverse Event Management Algorithm

Consider visual field testing, endocrinology consultation, and imaging if clinically indicated.

Toxicity (CTCAE v.4.03)	Management	Follow-up
Asymptomatic TSH elevation	• Continue protocol therapy • If TSH < 0.5 x LLN, or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: include fT4 at subsequent cycles as clinically indicated; • Consider endocrinology consult.	• N/A
Symptomatic Endocrinopathy	• Evaluate endocrine function; • Consider pituitary scan. **Symptomatic with abnormal lab/pituitary scan:** • Hold nivolumab infusion; • 1-2 mg/kg/day methylprednisolone IV or 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 and consult endocrinology.	**If improves (with or without hormone replacement):** • Taper steroids over at least 1 month; • Consider prophylactic antibiotics for opportunistic infections; • Resume nivolumab therapy as 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)	• Discontinue protocol therapy; • 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.	• N/A

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 considered when switching to the equivalent dose of oral corticosteroids.

** For symptomatic **hypothyroidism**, nivolumab should be withheld, and thyroid hormone replacement should be initiated as needed. For symptomatic **hyperthyroidism**, nivolumab should be withheld and methimazole should be initiated as needed. Corticosteroids at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents should also be considered if acute inflammation of the thyroid is suspected. Upon improvement, nivolumab may be resumed after corticosteroid taper, if needed. Monitoring of thyroid function should continue to ensure appropriate hormone replacement is utilized.*

*For symptomatic **adrenal insufficiency**, nivolumab should be withheld, and physiologic corticosteroid replacement should be initiated as needed. Monitoring of adrenal function and hormone levels should continue to ensure appropriate corticosteroid replacement is utilized.*

*For symptomatic **hypophysitis**, Nivolumab should be withheld, and hormone replacement should be initiated as needed. Corticosteroids at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents should also be considered if acute inflammation of the pituitary gland is suspected. Upon improvement, Nivolumab may be resumed after corticosteroid taper, if needed. Monitoring of pituitary function and hormone levels should continue to ensure appropriate hormone replacement is utilized.*

*For symptomatic **diabetes**, nivolumab should be withheld, and insulin replacement should be initiated as needed. Monitoring of blood sugar should continue to ensure appropriate insulin replacement is used.*

6.12 Dose Modification for Fever

Fever (CTCAE v.4.03)	Management/Next Dose of Nivolumab
Grade 1	• Continue protocol therapy
Grade 2	• Hold until ≤ Grade 1. If resolves to ≤ Grade 1 within seven (7) days, resume at same dose level. If fever does not resolve to ≤ Grade 1 within seven (7) days, discontinue protocol therapy.
Grade 3	• Hold until ≤ Grade 1. If resolves to ≤ Grade 1 within 24 hours, resume at same dose level. If fever does not resolve to ≤ Grade 1 within 24 hours, discontinue protocol therapy.
Grade 4	• Off protocol therapy
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	

6.13 Dose Modifications for Renal Adverse Events

Refer to algorithm below for dose modifications for renal adverse events and recommended management guidelines.

6.13.1 Recommended Renal Adverse Event Management Algorithm

Toxicity (CTCAE v.4)	Management	Follow-up
Grade 1 Creatinine > ULN and > than baseline but ≤ 1.5 x baseline.	• Continue protocol therapy; • Monitor creatinine weekly	**If returns to baseline:** Resume routine creatinine monitoring per protocol. **If worsens:** • Treat as Grade 2 or 3/4
Grade 2 or 3 Creatinine > 1.5 x baseline to ≤ 6x ULN	• Delay nivolumab per protocol; • Monitor creatinine every 2-3 days; • 0.5-1.0 mg/kg/day methylprednisolone IV or oral equivalent; • Consider renal biopsy and consult nephrology.	**If returns to Grade 1 or baseline:** • Taper steroids over at least 1 month • Consider prophylactic antibiotics for opportunistic infections; • Resume nivolumab as per protocol • Routine creatinine monitoring as per protocol. **If elevations persists > 7 days or worsen:** • Treat as Grade 4.
Grade 4 Creatinine > 6x ULN	• Discontinue protocol therapy; • Monitor creatinine daily; • 1.0 - 2.0 mg/kg/day methylprednisolone IV or IV equivalent; • Consult nephrologist; • Consider renal biopsy.	**If returns to Grade 1:** • 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 considered when switching to the equivalent dose of oral corticosteroids.

7. SUPPORTIVE CARE AND OTHER CONCOMITANT THERAPY

7.1 Concurrent Anti-Cancer Therapy

Concurrent anti-cancer therapy, including chemotherapy, radiation therapy, immunotherapy, or biologic therapy may NOT be administered to patients receiving study drug. If these treatments are administered the patient will be removed from protocol therapy.

7.2 Investigational Agents

No other investigational agents may be given while the patient is on study.

7.3 Supportive Care

Appropriate antibiotics, blood products, anti-emetics, fluids, electrolytes and general supportive care are to be used as necessary. Specific supportive care measures for management of autoimmune reactions are detailed below (see throughout *Sections 7.4-7.8* and all of *Section 7.9*):

7.4 Skin Related Toxicity

For skin-related Grade 3 autoimmune toxicity lasting > 7 days or Grade 4 autoimmune toxicity, including severe generalized pruritus or rash, symptomatic treatment will be given, and patients will be removed from protocol therapy. Therapy will be as clinically indicated and may include local skin care, antihistamines, or corticosteroids (which can be local/topical or systemic). The use of topical corticosteroids for grades 1 – 3 dermatitis will be allowed, and will not require patients to be removed from study. In the case of late-occurring hypersensitivity symptoms (e.g., appearance within one week after treatment of a localized or generalized pruritus), symptomatic treatment may be given (e.g., oral antihistamine, topical steroids for the skin).

A dermatologist should evaluate persistent (lasting greater than seven (7) days) and/or severe rashes or pruritus. Patients should be advised to seek medical evaluation if they notice new-onset rash. Early consultation with a dermatology specialist and a biopsy should be considered if there is uncertainty as to the cause of the rash, or if there is any unusual appearance or clinical feature associated with it. Other drugs that may cause rash should be considered in the differential and, if possible, discontinued. A biopsy should be performed if appropriate and photos should be obtained.

If symptoms or signs of SJS or TEN appear, nivolumab should be withheld and the patient should be referred for specialized care for assessment and treatment. If the patient has confirmed SJS or TEN, permanent discontinuation of nivolumab is mandatory.

Please refer to *Sections 6.4 and 6.4.1* for dose modification and management of the skin toxicities.

7.5 Pneumonitis

Early recognition and treatment of pneumonitis is critical to its management. Patients should be advised to seek medical evaluation promptly if they develop new-onset dyspnea, cough, or fever or if they have worsening of these baseline symptoms. It is important that an evaluation/work-up distinguishes between non-drug-related causes (e.g., infection or progression of disease) and a possible drug-related pulmonary toxicity as the management of these events can be quite different. For symptomatic nivolumab-related pneumonitis, the principal treatment is corticosteroids. Refer to *Section 6.7.1* for recommended pulmonary adverse event management algorithm.

Grade 1 asymptomatic patients with radiological changes (e.g., focal ground glass opacities and patchy infiltrates) may be managed with nivolumab dose delay. Patients with Grade 2 pneumonitis or with symptoms of dyspnea, cough, or fever should be managed with dose delay, and treatment with corticosteroids should be considered. Nivolumab can be resumed at the same dose after the resolution of the symptoms and radiographic changes, cessation of corticosteroid therapy and after pulmonary and/or ID consultation if lymphocytic pneumonitis is excluded. Note, that in cases where nivolumab treatment was restarted, recurrence of pneumonitis was reported infrequently. All patients with Grade 3-4 pneumonitis (with more extensive radiographic findings, and hypoxia) should permanently discontinue nivolumab and treat with high-dose corticosteroids. Patients with more severe cases of pneumonitis, who did not initially respond to corticosteroids, can be treated with addition of immunosuppressive therapy (infliximab, cyclophosphamide, IVIG, or mycophenolate mofetil) as per discretion of the treating physician.

7.6 Ocular Toxicity

Patients who report any new visual symptom, ocular findings on exam, or change in vision should be immediately referred to an ophthalmologist. Ophthalmologic evaluation should include

but will not be limited to examination of the conjunctiva, anterior and posterior chambers and retina, normal and dilated slit-lamp examination. The patient will be treated as deemed appropriate by the ophthalmologist, including peri-ocular steroid injections or steroid eye drops if necessary to manage low-grade events. High-grade events should be managed with systemic corticosteroids. Complaints of double vision should also prompt medical evaluation. In addition to ocular inflammatory events, a work-up should also consider pituitary inflammation as a cause. Vogt-Koyanagi-Harada syndrome (VKH) is a T-cell mediated autoimmune attack on melanocytes that should be ruled out. VKH manifests as a multi-system disorder characterized by granulomatous panuveitis with exudative retinal detachments, often associated with neurologic and cutaneous manifestations. Based on the severity of such a syndrome, nivolumab treatment should be withheld or discontinued, and corticosteroids administered accordingly.

7.7 Gastrointestinal Toxicity

Early recognition and treatment of diarrhea and colitis are critical to their management. Patients should be advised to seek medical evaluation if they develop new-onset diarrhea, blood in stool, or severe abdominal pain or if they have worsening of baseline diarrhea. As GI symptoms are common in patients with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes (e.g., infection or progression of disease) and a possible drug-related AE as the management can be quite different. Any patient experiencing diarrhea (which may be defined as watery stool, or increase in the frequency stools above Grade 1 with urgency or nocturnal bowel movement, or melena or hematochezia) should be further evaluated for etiology that should include a search for an infectious etiology, *C. Difficile* colitis and other alternative infections as clinically indicated. Consideration should be given to discontinuing medications known to exacerbate colitis.

It is recommended that colitis or enterocolitis of Grade 1 be evaluated as above for other non-immune mediated causes, then monitored closely and treated symptomatically without steroids, including a trial of loperamide may be used. For Grade $\geq$ 2 colitis or enterocolitis, recommendations include endoscopy and/or abdominal CT imaging.

Even if colonoscopy does not reveal gross findings of colitis, biopsies should be performed, and strong consideration should be given to upper endoscopy and biopsies. Patients with gross or

biopsy proven colitis or enteritis should receive IV steroids (recommend 1 mg/kg methylprednisone daily for seven (7) days) followed by a minimum 30-day taper. In patients, with Grade 3 or Grade 4 enterocolitis that does not respond to high dose steroids after seven (7) days, further therapies should be administered as clinically indicated in consultation with gastroenterology subspecialists. Caution should be taken in the use of narcotics in patients with diarrhea, colitis, or abdominal pain as pain medicines may mask the signs of colonic perforation. Consultation with a gastroenterologist should be sought for all moderate- and high-grade cases of GI AEs.

7.8 Hepatotoxicity

Concern for immune-mediated liver toxicity may be elicited following LFT elevation of three-fold over baseline and/or right upper quadrant abdominal pain or unexplained nausea or vomiting. Patients should be advised to seek medical evaluation if they notice jaundice (yellow appearance of skin or sclera) or if they develop bruising, bleeding, or right-sided abdominal pain. Physicians should monitor LFTs prior to each nivolumab treatment. As LFT abnormalities are common in patients with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes as management may be quite different. Other aetiologies for transaminitis should be considered and evaluated and may include, but are not limited to neoplastic, concurrent medications, viral hepatitis, and other toxic aetiologies. Evaluation for autoimmune aetiologies may be evaluated by ANA, pANC, and anti-smooth muscle antibody tests, as well as hepatology consultation with possible biopsy. Higher-grade hepatic AEs, including drug induced liver illness may be managed with corticosteroids (with or without mycophenolate mofetil).

7.9 Pancreatic Toxicity

Pancreatitis has rarely been associated with checkpoint inhibitors and should be considered in cases of abdominal pain associated with elevations of amylase and lipase. Asymptomatic elevations in lipase and amylase have been reported in monotherapy trials. Very few patients reported associated symptoms (e.g., abdominal pain) or radiographic findings (e.g., stranding) consistent with pancreatitis. Thus, there does not seem to be clinical significance to the elevated laboratory values. As lipase/amylase abnormalities are not uncommon in patients with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes (e.g.,

progression of disease, concomitant medications, or alcohol) and a possible drug-related cause as the management can be quite different. The recommended management of nivolumab-related elevated lipase/amylase values centers around close observation. Physicians should ensure that patients have no associated symptoms consistent with pancreatitis, such as abdominal pain.

Treatment of pancreatitis should be supportive and may include consultation with gastroenterology subspecialists. Asymptomatic elevations should be monitored approximately on a weekly basis. For sustained asymptomatic Grade 4 elevations and elevated pancreatic enzymes with symptoms consistent with pancreatitis, nivolumab should be discontinued per protocol instructions, and a gastroenterologist should be consulted.

7.10 Endocrinopathies

Patients experiencing symptoms such as fatigue, myalgia, impotence, mental status changes, constipation, or other symptoms thought to be associated with endocrine abnormalities should be evaluated for thyroid, pituitary, or adrenal endocrinopathies and an endocrinologist should be consulted. It is possible that events may occur within weeks of beginning treatment, but also after many months (while still on treatment). More than one endocrine organ may be involved and may need to be evaluated. Patients should be advised to seek medical evaluation if they notice new-onset fatigue, light-headedness, or difficulty with vision or if baseline fatigue worsens. As fatigue is common in patients with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes, such as progression of disease, anemia, concomitant medications, or depression) and a possible drug-related AE as the management can be quite different.

Patients with Grade 2 hypothyroidism should be evaluated by an endocrinologist for further management. Patients with Grade 2 hypothyroidism adequately managed with thyroid hormone replacement may continue protocol therapy. Patients with Grade 3 or greater hypothyroidism will be removed from the study. These patients should be managed according to *Section 6.11* and evaluation by an endocrinologist is recommended for further management. Patients who enter the study on thyroid replacement should have their medication adjusted to maintain TSH in the normal range.

7.11 Neurologic Toxicity

Neurologic symptoms can manifest as central abnormalities (e.g., aseptic meningitis, encephalopathy, or encephalitis) or peripheral sensory/motor neuropathies (e.g., Guillain-Barre Syndrome, myasthenia gravis complicated with sepsis and fatality). Early recognition and treatment of adverse neurologic reactions is critical to its management. Patients should be advised to seek medical evaluation if they notice impairment in motor function (e.g., weakness), changes in sensation (e.g., numbness), or symptoms suggestive of possible central nervous system abnormalities such as new headache or mental status changes. As neurologic symptoms can be common in patients with cancer, it is important that an evaluation/work-up distinguishes between non-drug-related causes (e.g., progression of disease, concomitant medications, or infection) and a possible drug-related events as the management can be quite different. The principal treatments for neurologic toxicity are dose delay, corticosteroids, and IV immunoglobulin as outlined in the safety algorithm (see *Section 6.9* and sub-sections). For high-grade related neurological symptoms, nivolumab should be discontinued.

7.12 Renal Toxicity

Some elevated creatinine and biopsy-confirmed tubulointerstitial nephritis and allergic nephritis have been infrequently observed following treatment with nivolumab. Events were managed with corticosteroids and, in all cases, renal function partially or fully improved.

Physicians should monitor creatinine regularly. As creatinine abnormalities are common in patients with cancer and other comorbidities, it is important that an evaluation/work-up distinguishes between non-drug-related causes (e.g., dehydration, concomitant medications, hypotension, or progression of disease). Corticosteroids and biopsy should be considered as outlined in *Section 6.13* and *6.13.1*.

7.13 Autoimmune or Immune System Disorders Effecting Other Organ Systems

Patients experiencing symptoms that may be associated with autoimmune or immune mediated adverse events possibly, probably or definitely related to protocol therapyshould be evaluated and monitored closely. These may include but are not limited to pneumonitis, sarcoid-

like granuloma and neurologic events including hypophysitis, encephalitis, aseptic meningitis, and cranial neuropathy especially cranial nerve seven (CNVII). Consideration should be given to subspecialty consultation particularly if systemic immune suppression is considered.

7.14 Other Immune-Mediated Adverse Events

For suspected immune-related adverse reactions, adequate evaluation should be performed to confirm etiology or exclude other causes. Based on the severity of the adverse reaction, nivolumab should be withheld or discontinued, and corticosteroids administered accordingly. The Study Chair or Co-chair should be consulted. Upon improvement, nivolumab may be resumed after corticosteroid taper. If there is recurrence of any Grade 3 or 4 immune-related adverse reactions or life-threatening immune-related adverse reactions, nivolumab must be permanently discontinued.

Rare cases of myotoxicity (myositis, polymyositis, polymyalgia rheumatica, myocarditis, and rhabdomyolysis) outcome, have been reported with nivolumab. If a patient develops signs and symptoms of myotoxicity, close monitoring should be implemented, and the patient should be referred to a specialist for assessment and treatment without delay. Based on the severity of myotoxicity, nivolumab should be withheld or discontinued after discussion with the Study Chair or Co-Chair, and appropriate treatment instituted. For Grade 3 or 4 myotoxicity, nivolumab should be permanently discontinued.

7.15 Growth Factors

Growth factors that support platelet or white cell number or function can only be administered for culture proven bacteremia or invasive fungal infection. Patients MUST NOT receive prophylactic myeloid growth factor in the first cycle of therapy. The Study Chair or Co-Chair should be notified before growth factors are initiated.

8. EVALUATION SCHEDULE AND DATA TO BE COLLECTED

All clinical and laboratory studies to determine eligibility must be performed within 14 days prior to enrollment in either **Part I, Part II,** or both unless otherwise indicated. Laboratory values used to for inclusion or exclusion criteria purposes (see *Section 4.2* and sub-sections) must be no older than 14 days at the start of therapy. Additional laboratory tests do not need to be repeated if therapy starts within seven (7) days of obtaining lab values for inclusion/exclusion. If a post-enrollment lab value is outside the limits of acceptance, or too old, they must be re-checked within 48 hours prior to initiating therapy; this includes, CBC with differential, bilirubin, ALT/AST and serum creatinine. If the re-check values continue to be outside the limits of acceptance, the patient may not receive protocol therapy. If the laboratory values return to the eligible limits, the patient is permitted to re-enroll if **all** other eligibility criteria are met, including exclusion criteria for concomitant medications and ICIs. In this case, all eligibility laboratory values will have to be repeated. Imaging studies must be obtained within 14 days prior to start of protocol therapy (and the tumour imaging must be repeated, if necessary).

Note: *Screening for concurrent malignancies is strongly recommended in patients with confirmed or suspected CMMRD and should be arranged prior to initiation of treatment (see Table 4; Westdorp, 2017). The effectiveness of hematological screening is questionable since non-Hodgkin lymphomas and acute lymphoid leukemia are rapidly growing tumors and surveillance may not improve the outcome for patients with these malignancies (Vasen, 2014; Westdorp, 2017).*

Table 4. Recommended surveillance protocol for patients with CMMRD (Tabori, 2017).

Examination	Start age	Frequency	Tumours	Comment
MRI brain	At diagnosis	Q 6 months	CNS tumours	Should not be replaced with WBMRI
WBMRI	6 years	Q annually	All tumours	Should not replace dedicated CNS imaging
CBC	1 year	Q 6 months	Leukemia	May be considered
Abdominal U/S	1 year	Q 6 months	Lymphoma	May be considered Can be alternated with WBMRI
Upper gastrointestinal endoscopy; VCE, ileocolonoscopy	4 to 6 years	Q annually	GI tumours	Upper and lower endoscopy, to increase in frequency when polyps are found
Gynecological exam, transvaginal U/S, pipelle curettage, UA, dipstick	20 years	Q annually	GU tumours	As per Lynch syndrome guidelines

Abbreviations: WBMRI = whole body MRI; U/S = ultrasound; VCE = visual capsule endoscopy; GI = gastrointestinal; UA = urine analysis; GU = genitourinary

8.1 Study Outline: Part I – Molecular Profiling

Part I Procedures	**Pre-Screening Assessment**	**Screening**	**Molecular Profiling Assay**	**Use of Profiling Assay Results**
Window	Prior to screening	Post-informed consent	~2 weeks	Post-assay results & prior to **Part II**
Administrative procedures				
Part I informed consent/assent[1]	X			
Suspicion of hypermutation[2]	X			
Eligibility assessment[3]		X		
Medical History		X		
Stratification[4]				X
Relay assay results[5]				X
Laboratory investigations				
Provide specimen[6]		X		
Provide slides for IHC (optional)[7]		X		
TMB assay[8]			X	
IHC[9]			X	
Recommended CMMRD work-up (optional)[10]				X

[1] A separate informed consent must be obtained for **Part I** and **Part II** (when eligible). All eligible patients must undergo study **Part I**, except for those with a TMB assay report from a previously obtained specific CLIA-certified laboratory already (see *Lab Manual*).

[2] See *Section 4.2.1* for a detailed description of eligible recurrent or relapse paediatric cancer patients suspected to be hypermutant.

[3] See *Section 4.2.1* for a list of ***Part I**: Molecular Profiling* inclusion criteria.

[4] Patients with applicable TMB levels will be stratified in to cohorts as follows: A) TMB ≥5 but <10 mut./Mb; B) TMB ≥10 mut. /Mb; C) unknown TMB in patient/unobtainable tissue for a patient with RRD (see *Section 4.2* for details).

[5] The results of the TMB assay obtained from an individual's participation in **Part I** of the study may be disclosed with his/her consent to his/her health care providers for the purpose of obtaining appropriate medical care, as well, this information will be relayed back to the patient, their parents/ legal guardian, their attending local physician and the clinical trial team. Patients with eligible results will be asked to sign a different **Part II**-specific informed consent prior to **Part II** screening.

[6] Neoplastic specimen will be necessary for enrollment to **Part I** (see *Lab Manual* for details). If remaining tissue exists, it may be retained for possible use in **Part II** companion biomarker studies, if the patient consents to **Part II** and for the tissue use specifically. If the patient does not qualify for enrollment in **Part II** or does not wish to participate in the tumour tissue portion of the companion biomarker studies for **Part II**, remaining whole tissue will be returned to the local physician upon request. Tissue will not be biopsied/obtained for the sole purpose of the study.

[7 & 9] Recommended for all patients with suspected or confirmed RRD only and/or no available tissue available for TMB assay; not mandatory as specified in *Section 3.5* and *Lab Manual* for details. Patients may optionally provide tissue for IHC assessment of MMR gene protein expression for diagnostic purposes relevant to the study at the discretion of the Study Chair or Co-chair, Patients with confirmed or suspected CMMRD should undergo regular tumour surveillance according to published guidelines.

[8] TMB analysis must be performed in a Study Chair or Co-chair approved, CLIA-certified laboratory

[10] *Optional* Patients with confirmed or suspected CMMRD should undergo regular tumour surveillance according to published guidelines. See *Section 8.0: Table 4* for recommended surveillance to be performed prior to enrollment in **Part II**.

8.2 Study Calendar: Part II – Treatment and Companion Biomarker Studies

PART II	**Pre-Treatment**	**Cycle 1**				**Subsequent cycles**			**Post- treatment**	
	Screening	**D1**	**D8**	**D15**	**D22**	**D1**	**D15**	**End of Tx visit**	**Safety Visit[20]**	**Follow-up[21]**
Window / **Procedures**	-14d	n/a	±2d	±2d[18]	±2d	±2d[18]	±2d[18]	n/a	± 7d	± 14d
Administrative procedures										
Part II Informed consent/assent[1]	X									
Inclusion/exclusion criteria	X									
Medical History	X									
Nivolumab administration[2]		X		X		X	X			
AE evaluation		Continuously throughout								
Concomitant meds		Continuously throughout								
Clinical Assessment[22]										
Physical exam	X	X	X	X	X	X	X	X	X	X
Vital signs[3]	X	X[3]	X	X[3]	X	X	X[3]	X	X	X
Neurological exam[4]	X	X[17]				X			X	X
Height and weight[5]	X	Weight only		Weight only		X	Weight only	X	X	X
Performance status	X					X			X	X
Evaluation of Tanner stage[6]	X									
Evaluation of menstrual status[7]	X									
Laboratory/Imaging investigations[22]										
Pregnancy test[8]	X	X				X				
CBC with differential[9]	X	X[17]	X	X	X	X	X		X	X
Biochemistry[10]	X	X[17]	X	X	X	X	X		X	
Amylase, lipase, CRP	X					X			X	
TSH[11]	X					X			X	
Disease assessment[12]	X					(X)[12]		X[12]		X[12]
PET-CT or full body MRI (including head)[13]	X									
Colonoscopy[13]	X									
Companion Biomarkers										
Tumour Tissue[14]	X[14]					(X)[14]				
Blood for DNA		X[19]								
Blood for RNA		X[19]								
Blood for lymphoblast cell line establishment		X[19]								
Blood for circulating tumour DNA, processed immediately[15]		X[19]				X		X		X
Blood extracted for PBMCs, processed immediately[16]		X[19]		X		X		X		X

[1] A separate informed consent must be obtained for **Part I** and **Part II** (when eligible). All eligible patients must undergo study **Part I**, except for those whom possess a TMB assay report from the specific CLIA-certified laboratory (see *Lab Manual*) and directly begin screening for **Part II**.

[2] All eligible patients will receive nivolumab at the dose of 3 mg/kg once every 14 days ± 2 days; on approximately days 1 (D1) and 15 (D15) of each cycle. Two doses comprise one cycle. Nivolumab will be administered as a 60-min +/- 10 min infusion. Refer to *Section 5.1* and *9.8* for dosing and administration details, respectively. The dosing calculations should be based on the actual body weight in kilograms and may be calculated using the weight obtained at the previous dose administration. If the patient's weight on the day and prior to drug administration differs by >10% from the weight used to calculate the dose, the dose should be recalculated. All doses should be rounded to the nearest milligram. There will be no dose modifications allowed. Any dose delays or omissions should be discussed with the Study Chair or Co-chair prior to the scheduled administration date. An exception made during an emergent circumstance should be discussed with the Study Chair or Co-chair as soon as possible.

[3] Vital signs (HR, RR, BP, temperature, and pulse oximetry) and assessment of AEs (e.g. rash) are to be performed pre-dose and every 15 ± 5 minutes during administration of study drug. At the completion of infusion, vital signs are to be performed every 15 ± 5 min twice then every 30 ± 5 min x 3 times. Following completion of cycle 1 and in patients who have tolerated nivolumab without infusion reactions, frequency of vital signs evaluation following infusion may be reduced at the discretion of, and documented by, the treating physician.

[4] Neurological exams will be required only for patients with brain tumours or when CNS-specific concerns arise.

[5] The dosing calculations should be based on the actual body weight in kilograms and may be calculated using the weight obtained at the previous dose administration. If the patient's weight on the day and prior to drug administration differs by >10% from the weight used to calculate the dose, the dose should be recalculated. All doses should be rounded to the nearest milligram. There will be no dose modifications allowed.

[6] Refer to *Section 21* (*Appendix III*) for Tanner Staging.

[7] Female patients only.

[8] Women of childbearing potential (WOCBP; and those with Tanner stage of ≥ 3) must have a negative pregnancy test every 4 weeks. During the **Part II** screening window (-14 days), WOCBP must have a negative serum pregnancy test. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab administration and must be willing to adhere to effective contraception during treatment and for 5 months after the last dose of nivolumab. Males who are sexually active with women of childbearing potential must be willing to adhere to effective contraception during and for 7 months after the last dose of nivolumab. Abstinence is an acceptable method of birth control. Additional pregnancy tests (serum or urine) should be obtained during treatment in accordance with institutional guidelines.

[9] If patients have Grade 4 neutropenia then CBCs should be checked twice weekly or as clinically indicated until recovery to Grade 3 or until meeting the criteria for removal from the study.

[10] Biochemistry includes: sodium, potassium, calcium, chloride, phosphate, magnesium, serum creatinine, BUN (urea), bilirubin (conjugated/unconjugated or total), ALT and AST.

[11] Free T4 should also be measured for patients with an abnormal TSH level. Guidance on the management of patients who develop hypothyroidism is included in *Section 7.10*. TSH will be assessed at end of each cycle, within 3 days prior to the next cycle.

[12] During the study, disease evaluations will be done at end of cycle 1 (window of -7 days) and every two (2) months (window of -7 days) as per *Section 10* applicable response evaluation criteria. Tumour disease assessments will also be done at end of treatment visit (window of +/-7 days) and once every three (3) months (window of +/-14 days) during follow-up for 1 year. Tumour disease assessments during follow-up may be performed at referring hospital. Evaluations required will depend on patient diagnosis. For patients with solid tumours, CT or MR imaging of appropriate sites is mandatory. For patients with leukaemia, BM evaluation is adequate.

[13] Recommended for patients with confirmed or suspected CMMRD only; not mandatory. Regardless of study participation, patients with confirmed or suspected CMMRD should undergo regular tumour surveillance according to published guidelines (see *Section 8, Table 4*, Tabori, 2017).

[14] Tissue provided in **Part I** may be used for *Companion Biomarker* studies with patient consent. Whenever possible, additional samples will be obtained for biological analyses in the event that a patient requires surgery/ a biopsy after starting treatment and cancerous tissue is removed. Tumour will be only biopsied/ surgically removed for medical/ diagnostic purposes and not specifically for the purposes of the study. When possible (with patient consent), please contact Study Chair or Co-Chair as well as refer to *Lab Manual* prior to surgery/ biopsy acquisition while on study to facilitate time and preservative-sensitive sample processing.

[15] Blood for circulating tumour DNA (ctDNA) is required at baseline, at the end of Cycle 1, but prior to C2D1 dose (window -7 days from C1D28), every two (2) months at the end of the cycle prior to the subsequent CXD1 dose (window -7 days from CXD28 of the previous cycle) during the study, at the end of study visit and every 3 months subsequently (window ± 14 days) during follow up for 1 year (i.e. at the times of disease re-evaluation). To be extracted and processed immediately on same day (as per *Lab Manual*). All *Companion Biomarker* assays must be performed approximately 4 weeks after CXD1, irrespective of any dose omissions or delays. The results of these assays should reflect the effect of the two former doses, not the status at CXD1. All attempts to compile blood draws to visit dates prior to drug administration should be made.

[16] Blood extracted PBMCs for T-cell functional studies are required at baseline, on Cycle 1, Day 15 (C1D15; window –7 days), at the end of Cycle 1, but prior to C2D1 dose (window -7 days from C1D28), every month at the end of the cycle, prior to the subsequent dose

(window -7 days from CXD28 of the previous dose) during the study, at the end of study visit and every 3 months (window ±14 days) during follow up for 1 year. To be extracted and processed immediately on same day (as per *Lab Manual*). All *Companion Biomarker* assays must be performed approximately 4 weeks after CXD1 (excluding the C1D15 blood sample), irrespective of any dose omissions or delays. The results of these assays should reflect the effect of the two former doses, not the status at CXD1. All attempts to compile blood draws to visit dates prior to drug administration should be made

[17] These assessments for C1D1 pre-dose do not need to be repeated if these were done for baseline within seven (7) days prior to C1D1.

[18] Assessments are to be done on days CXD1 ±2 days or CXD15 ±2 days on the day of and prior to nivolumab administration.

[19] Samples may be taken up to 7 days prior to C1D1 dosing.

[20] 30 days after last dose.

[21] Every 3 months for 1 year.

[22] Unlike the *Companion Biomarkers*, if the CXD1 of cycle drug administration is delayed or omitted, all *Clinical Assessments* and *Laboratory/ Imaging Investigations* (and their respective windows) should adjust to the new CXD1, reflecting the patient's status prior to the upcoming drug administration. Please see respective footnotes above for details for changes to the *Companion Biomarker* assays.

8.3 Radiological Studies

Patients, who respond (complete remission [CR] or partial remission [PR]) to therapy, have pseudo-progression or have long-term stable disease (SD; ≥ six [6] cycles) on protocol therapy will be centrally reviewed at The Hospital for Sick Children. CRO will notify The Hospital for Sick Children of any patient requiring central review. The Hospital for Sick Children will then request that the treating institution forward the requested images for central review. The central image evaluation results will be entered for data analysis.

The images are to be sent (preferably CD) to The Hospital for Sick Children.

8.4 Biological and Companion Biomarker Studies

Please refer to the *Lab Manual* for the instructions on tissue and blood collection and shipment arrangements. All eligible patients will be asked to consent to parallel biological and companion biomarker studies to further advance our knowledge of paediatric patients with hypermutant cancers being treated with nivolumab. With patient consent, available data from locally REB approved biobanks may be added and used in conjunction to data obtained in this clinical trial to enhance our understanding of the mechanisms at play. All patient information will be de-identified in accordance with REB approval and patient consent, and any information obtained as part of the study will not be shared/ divulged reciprocally.

8.4.1 Tumour Tissue

In order to be included in **Part I** of the study, patients must have at minimum neoplastic specimen available as specified in the *Lab Manual*. With patient consent, said specimens will be assayed using NGS in a specific CLIA–certified laboratory approved by the Study Chair or Co-Chair to assess TMB for eligibility purposes. If this tissue is not available, but a TMB report from the specific CLIA–certified laboratory approved by the Study Chair or Co-Chair is already available, this information can be used for eligibility purposes. Whenever possible, both a fresh, formalin-fixed, paraffin-embedded tumour tissue sample and a fresh frozen tumour tissue sample are preferred to be used for TMB, other *Companion Biomarkers* studies and future biomedical research outlined above. Fresh frozen tissue is highly recommended for affiliated biomarker study once on trial. Please refer to *Lab Manual* for the detailed instructions for submission of the tissues.

In addition, all patients are also highly encouraged to submit paraffin embedded tumour sample to assess for MMR protein expression loss by IHC in a CLIA-certified environment for genes including, but not limited to MLH1, MSH2, MSH6, and PMS2. These results may also be used for inclusion to **Part II,** Cohort C (see above for details). As part of the *Companion Biomarker* studies in **Part II,** immune-related markers including but not limited to CD3, CD4, CD8, CD68, PD-1, and PD-L1 expression will also be assessed by IHC.

In the event a patient requires a biopsy or surgery and tumour tissue is removed while on study, tissue will be requested for biological analyses.

8.4.2 Blood Tissue

All patients are required to submit blood sample for DNA and RNA gene sequencing to assess for RRD syndromes as well as other biomarker assays.

All patients are required to submit blood sample to establish a lymphoblast cell line.

All patients are required to submit serial blood samples at baseline and during treatment to assess for the T-cell fraction enrichment (from PBMCs) in response to nivolumab, and circulating tumour DNA (from plasma) all extracted and processed immediately same day. Please refer to *Lab Manual* for the detailed instructions for submission of the tissues.

9. EXPERIMENTAL AGENT

9.1 Nivolumab

BMS-936558, MDX1106, ONO-4538, anti-PD-1, NSC#748726, Opdivo ®.

9.2 Structure and Molecular Weight

Nivolumab is a soluble protein consisting of 4 polypeptide chains, which include 2 identical heavy chains consisting of 440 amino acids and 2 identical light chains. Molecular weight is 146 221 Daltons.

9.3 Supply

Nivolumab will be supplied by Bristol-Myers Squibb (BMS) and distributed directly by BMS or via an approved supplier.

9.4 Formulation

The agent is a clear to opalescent, colorless to pale yellow liquid, with light (few) particulates. It is available in a 100 mg/10 mL vial containing a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/mL in sodium citrate, sodium chloride, mannitol, diethylenetriamine pentacetic acid (pentetic acid) and polysorbate 80 (Tween® 80) to a pH of 6.0. A small amount of overfill (0.7 mL) is included with each vial to account for VNS (vial, needle, syringe) loss. The 10-mL type I flint glass vials are stoppered with butyl rubber stoppers and sealed with aluminum seals.

9.5 Storage

Nivolumab vials for injection must be stored as per conditions outlined in Investigator Brochure.

9.6 Solution Preparation

Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to a final concentration of 1 – 10 mg/mL. Vial

contents from different lots should not be mixed in the same infusion. **Please refer to the current Investigator Brochure for details.**

9.7 Stability

The administration of undiluted and diluted nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution of nivolumab injection prepared for dosing may be stored for 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 under room light. The maximum 8-hour period under room temperature and room light conditions for nivolumab injection in IV bag includes the product administration period (60 minutes). Vials of nivolumab for injection do not contain preservatives or bacteriostatic agents and should be prepared as soon as possible prior to administration using aseptic technique. **Please refer to the current Investigator Brochure for details.**

9.8 Administration

Nivolumab should be administered as an IV infusion over 60 minutes +/- 10 min through a 0.2 to 1.2 micron pore size, low-protein binding polyethersulfone membrane in-line filter. Nivolumab is not to be administered as an IV push or bolus injection.

No incompatibilities between nivolumab injection and polyvinyl chloride (PVC), non-PVC/non-DEHP (di[2-ethylhexyl]phthalate) IV components, or glass bottles have been observed.

9.9 Agent Ordering and Agent Accountability

BMS will be providing, packaged and labeled nivolumab complying with applicable local laws and regulations and made available directly to the sites participating in the study. Each batch of nivolumab will be delivered along with complete information regarding manufacture and expiration dates.

9.10 Accountability and Destruction of Agent

The Principal Investigator (or an authorized designee) at each participating institution must maintain a careful record of the inventory of the Investigational medicinal product received using

the Drug Accountability Form. The study drug may be destroyed as per site's destruction policies and documentation of study drug destruction will be submitted to CRO, providing the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
- Written procedures for on-site disposal are available and followed. A copy of the procedures must be provided to CRO upon request.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the containers. The method of disposal, i.e., incinerator, licensed sanitary landfill, or licensed waste disposal vendor must be documented.
- Accountability and disposal records are complete, up-to-date, and available for CRO to review throughout the clinical trial period. If conditions for destruction cannot be met, please contact CRO. CRO to discuss with BMS.

It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

Study drug may be returned to BMS if requested by the Study Chair or Co-Chair.

10. RESPONSE EVALUATION CRITERIA

10.1 Common Terminology Criteria for Adverse Events (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. A copy of the CTCAE version 4.03 can be downloaded from the CTEP website (http://ctep.cancer.gov).

10.2 Response Criteria for Patients with Solid Tumours

See the table in *Section 8* and *8.2* for the schedule of tumour evaluations. In addition to the scheduled scans, a confirmatory scan should be obtained 28 days following initial documentation of objective response.

Response and progression will be evaluated in this study using the modified Response Evaluation Criteria in Solid Tumours (RECIST 1.1) for immune-based therapeutics guidelines deemed iRECIST (Seymour, 2017). Immunotherapeutics may result in infiltration of immune cells leading to transient increase in the size in malignant lesions, or undetectable lesions becoming detectable ('pseudo-progression'). The criteria are identical to those of RECIST 1.1 (Eisenhaur, 2009) in many respects but have been adapted to account for instances where an increase in tumour burden, or the appearance of new lesions, does not reflect true tumour progression.

RECIST 1.1 will continue to be used to define whether tumour lesions, including lymph nodes, are measurable or non-measurable, as well as for the management of bone lesions, cystic lesions, and lesions with previous local treatment and the method of measurement (Eisenhaur, 2009). The principles used to establish objective tumour response are largely unchanged from RECIST 1.1. The major change from RECIST 1.1 is the concept of resetting the bar if progression is followed at the next assessment by tumour shrinkage (Seymour, 2017).

10.2.1 Definitions

10.2.1.1 iRECIST Definition

Responses assigned using iRECIST have a prefix of "i", which indicates immune response assigned using iRECIST. For example, "immune" complete response (iCR) or partial response (iPR), and unconfirmed progressive disease (iUPD) or confirmed progressive disease (iCPD) to differentiate them from responses assigned using RECIST 1.1. Similar nomenclature is used for stable disease (iSD). New lesions are assessed and sub-categorized into those that qualify as target lesions (new lesion, target) or non-target lesions (new lesion, non-target).

10.2.1.2 Evaluation for Objective Response

Patients who exhibit objective disease progression prior to the end of cycle 1 will be considered evaluable for response. For all other patients, only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response, whether it be unequivocal or iUPD , but not iCPD (before iCR, iPR or iSD). Patients who have lesions present at baseline that are evaluable, but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or progression of the lesions (unequivocal or iUPD, but not iCPD, before iCR, iPR or iSD).

10.2.2 Disease Parameters

10.2.2.1 Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray, as ≥ 10 mm with CT scan or ≥10 mm with calipers by clinical exam for a maximum of five lesions (two per organ). All other disease is considered non-target (must be ≥10 mm in short axis for nodal disease). All tumour measurements must be recorded in millimeters (or decimal fractions of centimeters). New lesions (as per iRECIST) are assessed as per RECIST 1.1 criteria, but are recorded separately on the case report form (and not included in the sum of lesions for target lesions identified at baseline).

10.2.2.2 Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

10.2.2.3 Non-measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: *Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.*

10.2.3 Assessment of Target Lesions

All measurable lesions up to a maximum of two (2) lesions per organ and five (5) lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which case the next largest lesion that can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum of diameters (iSOD). If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumour regression in the measurable dimension of the disease.

If iUPD criteria were met on the basis of progression in the target or non-target disease, or the appearance of new lesions, then RECIST 1.1-assigned progression in another lesion category in the confirmatory scan also confirms iCPD. Each time point response is based on the assessment of target lesions, non-target lesions, and new lesions. For time point response (as per iRECIST criteria), the management of lymph nodes, lesions that become too small to measure, lesions that split or coalesce, and the definition of complete response, partial response, stable disease, and progressive disease are clearly outlined throughout *Section 10.2* (Seymour, 2017).

For target lesions, iCR, iPR, and iSD can all be assigned after iUPD has been documented, as long as iCPD was not confirmed. iUPD is defined by RECIST 1.1 criteria for progressive disease and can be assigned multiple times as long as iCPD is not confirmed at the next assessment. Progression is confirmed in the target lesion category if the next imaging assessment after iUPD (4–8 weeks later) confirms a further increase in sum of measures of target disease from iUPD, with an increase of at least 5 mm. However, the criteria for iCPD (after iUPD) are not considered to have been met if CR, PR or SD criteria (compared with baseline and as defined by RECIST 1.1) are met at the next assessment after iUPD (Seymour, 2017). The status is reset (unlike RECIST 1.1, in which any progression precludes later complete response, partial response, or stable disease). iCR, iPR, or iSD should then be assigned; and if no change is detected, then the time point response is iUPD (Seymour, 2017).

10.2.4 Assessment of Non-Target Lesions

All other lesions (or sites of disease) including any measurable lesions over and above the five (5) target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

10.2.5 Assessment of New Malignant Lesions

The assessment of non-target lesions at each time point follows similar principles. iUPD (but not iCPD) can have been documented before iCR or when the criteria for neither CR nor PD have been met (referred to as non-iCPD/non-iUPD) and can be assigned several times, as long as iCPD was not confirmed. iUPD is defined by RECIST 1.1 criteria; however, iUPD can be assigned

multiple times as long as iCPD is not confirmed at the next assessment. PD in the non-target lesion category is confirmed if subsequent imaging, done four to eight weeks after iUPD, shows a further increase from iUPD.

The criteria for iCPD are not judged to have been met if RECIST 1.1 criteria for complete response or non-iCR/non-iUPD are met after a previous iUPD. The status is reset (unlike RECIST 1.1) and iCR, or non-iCR/non-iUPD is assigned; if no change is detected, the time point response is iUPD (Seymour, 2017). Changes in the largest diameter (unidimensional measurement) of the tumour lesions, but the shortest diameter of malignant lymph nodes are used in the RECIST v.1.1 criteria. In view of the potential for patients treated with immunotherapies to show early signs of disease progression, but ultimately to benefit from treatment, patients with evidence of progressive disease will also be evaluated using iRECIST criteria and may continue on study provided criteria outlined below are met.

RECIST 1.1 defines the appearance of new malignant lesions as denoting true disease progression, providing that other lesions (artefacts or benign intercurrent disease) are appropriately assessed and discounted if not malignant. If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up assessment will clarify whether it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan (Eisenhaur, 2009).

Aspects of new lesion assessment unique to iRECIST, include if a new lesion is identified (thus meeting the criteria for iUPD) and the patient is clinically stable, treatment should be continued. New lesions should be assessed and categorised as measurable or non-measurable using the RECIST 1.1 principles. a maximum of five lesions, no more than two per site, at least 10 mm in long axis (or 15 mm in short axis for nodal lesions), and recorded as New Lesions-Target (NLT) and New Lesion-Non-Target (NLNT) to allow clear differentiation from baseline target and non-target lesions. New lesions may either meet the criteria of NLT or NLNT to drive iUPD (or iCPD). However, the measurements of target lesions should **not** be included in the sum of measures of original target lesions identified at baseline. Rather, these measurements will be collected on a separate table in the case record form.

Results in UPD but iCPD is only assigned on the basis of this category if at next assessment additional new lesions appear or an increase in size of new lesions is seen (≥ 5 mm for sum of new

lesion target or any increase in new lesion non-target). The appearance of new lesions when none have previously been recorded, can also confirm iCPD.

Other measurable and non-measurable lesions are recorded as new lesion non-target. New lesions do not need to meet the criteria for new lesion target to result in iUPD (or iCPD); new lesion non-target can also drive iUPD or iCPD. PD is confirmed (iCPD) in the new lesion category if the next imaging assessment, done at four to eight weeks after iUPD, confirms additional new lesions or a further increase in new lesion size from iUPD (sum of measures increase in new lesion target ≥5 mm, any increase for new lesion non-target in the absolute value of the sum of NLT or an increase (but not necessarily unequivocal increase) in the size of NLNT lesions or the appearance of additional new lesions.

10.2.6 Protocol Exceptions for Delayed Response ('Pseudo-progression')

Patients in whom the magnitude of increase in tumour size is >20% but <40%, may remain on study for up to 12 weeks after start of protocol therapy if the following criteria are met:

- Lesions <10 mm will not be considered new lesions; new lesions ≥10 mm of longest diameter must be included in the total tumour burden;
- In the judgment of the treating clinician, the patient does not show evidence for rapid disease progression or the patient has shown evidence for clinical benefit;
- There is no decrease in performance status;
- The patient is tolerating the study drug;
- Continued treatment with nivolumab will not delay an imminent intervention required to prevent serious complications (e.g. CNS metastases, which require radiation therapy or surgery).

10.2.7 Confirming Progression

For patients who remain on study despite increase in tumour size >20%, imaging to include target lesions must occur every cycle if clinically indicated, or if 'pseudo-progression' appears based on inflammatory response, and the same radiographic and clinical criteria must be met in order to remain on study. If tumour size subsequently diminishes to <20% increase from baseline, the patient may be followed according to the standard protocol guidelines which will involve less

frequent imaging. The decision to continue treatment beyond radiographic evidence for disease progression should be discussed with the Study Chair or Co-Chair and documented in the study record.

Unlike RECIST 1.1, iRECIST requires the confirmation of progression. iCPD is confirmed if further increase in tumour burden, compared to the last assessment, is seen as evidenced by one or more of the following:

- Continued increase in tumour burden (from iUPD) where RECIST 1.1 definitions of progression had been met (from nadir) in target, non-target disease or new lesions;
- Progression in target disease worsens with an increase of at least 5 mm in the absolute value of the sum;
- Continued unequivocal progression in non-target disease with an increase in tumour burden;
- Increase in size of previously identified new lesion(s) (an increase of at least 5mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions;
- RECIST 1.1 criteria are met in lesions types (target or non-target or new lesions) where progression was not previously identified, including the appearance of additional new lesions;
- Clinical stability is considered when deciding whether treatment is continued after iUPD.

If iUPD is not confirmed at the next assessment, then the appropriate response will be assigned (iUPD if the criteria are still met, but no worsening, or iSD, iPR or iCR if those criteria are met compared to baseline). The prior documentation of iUPD does not preclude assigning iCR, iPR, or iSD in subsequent time-point assessments or as best overall response (BOR) providing that iCPD is not documented at the next assessment after iUPD. Tumour disease evaluation will be by objective response rate (ORR = complete response [CR] and partial response [PR]). Patients with response of 'stable disease' (SD), iCR, iPR, or iSD will also be reported as part of the final analysis of clinical benefit, but will not contribute to the primary efficacy outcome measure (Seymour, 2017).

Treatment beyond initial iUPD is permitted in patients who are clinically stable to continue on treatment until the next assessment (≥four [4] weeks later); this next imaging assessment should

be no longer than eight (8) weeks later, to ensure that patients remain fit for salvage therapies All decisions regarding continuation or discontinuation of therapy should be made by the patient, the local PI and either the Study Chair or Co-Chair. An assignment of clinical stability requires that no worsening of performance status has occurred, that no clinically relevant increases in disease-related symptoms such as pain or dyspnoea occur that are thought to be associated with disease progression, and that no requirement for intensified management of disease-related symptoms exists, including increased analgesia, radiotherapy, or other palliative care.

Patients who have iUPD and are not clinically stable should be designated as not clinically stable in the case report form. This designation will allow the best overall response to be calculated and the date of iUPD to be used in estimates of PFS.

10.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. A collection of scans (but not independent review) is mandatory per study protocol and confirmation of progression is required.

10.3.1 Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

10.3.2 Chest X-Ray

Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

10.3.3 Conventional CT and MRI

This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Ideally, the same type of scanner should be used, and the image acquisition protocol should be followed as closely as possible to prior scans.

10.3.4 PET-CT

At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

10.3.5 Tumour Markers

Tumour markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

10.3.6 Cytology, Histology

These techniques can be used to differentiate between partial responses ([i]PR) and complete responses ([i]CR) in rare cases (e.g., residual lesions in tumour types, such as germ cell tumours, where known residual benign tumours can remain).

Cytology should be obtained if an effusion appears or worsens during treatment when the measurable tumour has met criteria for response or stable disease.

10.3.7 FDG-PET

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

A. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
B. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

Note: *A 'positive' FDG-PET scan lesion means one that is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.*

10.4 Response Criteria for Patients with Solid Tumour and Measurable Disease

10.4.1 Time Point and Best Overall Response

The time point response is calculated using the response assigned for each category of lesion (as for RECIST 1.1), but takes into account the last time point response. The algorithm for patients with no previous iUPD is identical to RECIST 1.1 (Eisenhaur, 2009). For patients with iUPD at the last time point response, the next time point response is dependent on the status of all lesions, including target, non-target, new lesion target, and new lesion non-target; on whether any increase in size has occurred (either a further increase in size or a sufficient increase to assign a new iUPD if the criteria were not previously met); or the appearance of additional new lesions.

For iRECIST, the best overall response (iBOR) is the best time point response recorded from the start of the study treatment until the end of treatment, considering any requirement for confirmation. iUPD will not override a subsequent best overall response of iSD, iPR, or iCR, meaning that iPR or iSD can be assigned (time point response or iBOR) even if new lesions have not regressed, or if unequivocal progression (non-target lesions) remains unchanged, providing that the criteria for iCPD are not met. The duration of iCR and iPR is from the time point when the criteria for iCR or iPR are first met, whereas the duration of iSD is still calculated from baseline.

Assessments that are not done or are not evaluable should be disregarded. For example, an iUPD followed by an assessment that was not done or not evaluable, and then another unconfirmed progressive disease, would be indicative of iCPD.

Assessments done after protocol therapyis discontinued can be considered in identification of iBOR. All imaging done during the follow-up period should continue to be recorded on the case report form ad used to assess response until subsequent therapies are initiated, as the protocol and informed consent document permit.

10.4.2 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target and non-target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm. If immunocytology is available, no disease must be detected by that methodology. Normalization of urinary catecholamines or other tumour markers if elevated at study enrollment (for patients with neuroblastoma).

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

Unconfirmed progressive disease (iUPD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. iUPD must be assessed between four to eight weeks to confirm progression and become iCPD.

Confirmed progressive disease (iCPD): iUPD must be assessed between four to eight weeks to confirm progression and be deemed as iCPD. There must be at least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

Progressive Disease (PD): New Lesion if the next imaging assessment, conducted at least four (4) weeks, but not more than eight (8) weeks after iUPD confirms further progression from iUPD with either an increase of at least 5 mm in the absolute value of the sum of NLT or an increase (but not necessarily unequivocal increase) in the size of NLNT lesions OR the appearance of additional new lesions.

Note: *In presence of SD or PR in target disease but unequivocal progression in non-target or non-measurable disease, the patient has PD if there is an overall level of substantial worsening in non-target disease such that the overall tumour burden has increased sufficiently to merit discontinuation of therapy.*

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for iUPD or PD, taking as reference the smallest sum diameters while on study.

10.4.3 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumour marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: *If tumour markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.*

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Best Overall Response (iBOR): Each patient will be classified according to his "best response" for the purposes of analysis of treatment effect. Best response is determined as outlined in *Section 10.4.1* and *11* from a sequence of overall response assessments.

10.5 Response Criteria for Patients with Solid Tumours and Evaluable Disease

Evaluable Disease: The presence of at least one lesion, with no lesion that can be accurately measured in at least one dimension. Such lesions may be evaluable by nuclear medicine techniques, immunocytochemistry techniques, tumour markers or other reliable measures.

Complete Response (CR): Disappearance of all evaluable disease.

Partial Response (PR): Partial responses cannot be determined in patients with evaluable disease

Stable Disease (SD): That which does not qualify as Complete Response (CR), Partial Response (PR), or Progressive Disease.

Unconfirmed progressive disease (iUPD): The appearance of one or more new lesions or evidence of laboratory, clinical, or radiographic progression. iUPD must be assessed between four to eight weeks to confirm progression and become iCPD.

Confirmed progressive disease (iCPD): iUPD must be assessed between four to eight weeks to confirm progression and be deemed as iCPD.

Progressive Disease (PD): The appearance of one or more new lesions or evidence of laboratory, clinical, or radiographic progression if the next imaging assessment, conducted at least four (4) weeks, but not more than eight (8) weeks after iUPD confirms further progression from iUPD with either an increase of at least 5 mm in the absolute value of the sum of NLT or an increase (but not necessarily unequivocal increase) in the size of NLNT lesions OR the appearance of additional new lesions.

Best Overall Response (iBOR): Each patient will be classified according to his "best response" for the purposes of analysis of treatment effect. Best response is determined as outlined in *Section 10.4.1* and *11* from a sequence of overall response assessments.

10.6 Response Criteria for Neuroblastoma Patients

Response criteria follow the revised INRC criteria with special consideration for possible pseudo-progression (Park, 2017). Assessment will include anatomic imaging for primary and metastatic soft tissue disease, nuclear medicine imaging using ^{123}I-MIBG or FDG-PET for assessment of soft tissue and bone disease and BM aspirates/ trephine biopsies are recommended for assessment of marrow disease at the discretion of the local treating physician (Park, 2017). Tissue biopsies may be used as an adjunct to verify the presence of viable neuroblastoma or ganglioneuroblastoma that is evaluable for response. Urine catecholamine levels will not be used to evaluate response because of a lack of standardization in specimen collection and analysis and the influence of diet on results (Park, 2017).

10.6.1 Neuroblastoma Patients with Primary and Metastatic Soft Tissue Disease

To assess primary and metastatic soft tissue tumor response in most patients. MIBG uptake (or FDG for tumors that are not MIBG-avid) will be used to determine which metastatic soft tissue lesions considered measurable by iRECIST will be deemed target lesions for assessment of response.

MIBG Positive Lesions: Patients who have a positive MIBG scan at the start of therapy will be evaluable for MIBG response. The use of ^{123}I-MIBG imaging is recommended for all scans. If the patient has only one MIBG positive lesion and that lesion was radiated, a biopsy must be done at least 28 days after radiation was completed and must show viable neuroblastoma.

MIBG Negative Lesions: Patients whose tumors do not concentrate MIBG, FDG-PET is an alternative modality for tumor detection, although FDG is less specific than MIBG because of uptake of FDG in inflammatory lesions, as well as normal and cytokine-stimulated BM (Park, 2017; Tsai, 2017). Less specific for neuroblastoma, a tissue biopsy of at least one of the lesions may be required to confirm that FDG-avid, MIBG-non-avid lesions are histologically confirmed to be neuroblastoma and/or ganglioneuroblastoma. Increased FDG avidity in lymphoid tissue associated with response to ICIs and may give the initial impression of disease progression (Tsai, 2016). Increased FDG uptake in benign lymphoid tissue seen on PET/CT may be a surrogate

marker of immune activation and treatment response, although more prospective studies are necessary (Cho, 2017).

The following terms will be used to define neuroblastoma primary and metastatic soft tissue lesions:

Target lesions (TL): Disease sites (non-lymphoid soft tissue or lymph node) that meet the criteria of measurable size ≥ 10 mm in the longest dimension or ≥ 15 mm in short axis, respectively.

- Either uptake on MIBG or FDG for MIBG-non-avid tumours (with recommended biopsy) or biopsy positive for neuroblastoma or ganglioneuroblastoma.

Non-Target Lesions (NTL): Lesions that are active tumour sites but do not meet TL criteria.

- Non-target soft tissue lesions will include leptomeningeal, cerebrospinal fluid, ascites, or pleural effusion tumours and lesions smaller than 10 mm that are considered likely to be active tumor based on clinical correlation;
- Small soft tissue lesions and lymph nodes that measure shorter than 15 mm on short axis will be considered non-target lesions if they are biopsied and proven to consist of viable tumor;
- Non–lymph node soft tissue lesions at least 10 mm in diameter and lymph nodes larger than 15 mm on short axis that are not MIBG or FDG avid and do not contain viable tumor (if biopsied) will not be considered either target or non-target lesions.

Discrete Lymph Node: Single lymph node that can be discretely identified (i.e. cervical node); measured by short axis.

Sum of Diameters: Sum of the shortest axis of discrete lymph nodes added to the sum of the longest diameter on non-lymph node soft tissue metastases;

- Conglomerate masses of non-discrete lymph nodes will be measured using longest diameter.

10.6.2 Primary Tumour

The following criteria will be used to report anatomic and MIBG (or FDG-PET in non-MIBG-avid lesions) imaging response by the treating institution:

Complete Response (CR): Less than 10 mm residual soft tissue at primary site.

- Complete resolution of all MIBG or FDG-PET uptake at primary site.

Partial Response (PR): Greater than 30% decrease in the longest diameter of the primary site.

- MIBG or FDG-PET uptake at primary site stable, improved or resolved.

Stable Disease (SD): Neither sufficient shrinkage for PR nor sufficient increase for PD at the primary site.

Unconfirmed progressive disease (iUPD): The increase in uptake intensity/ appearance of one or more lesions or evidence of laboratory, clinical, or radiographic progression. iUPD must be assessed between four to eight weeks to confirm progression and become iCPD.

Confirmed progressive disease (iCPD): iUPD must be assessed between four to eight weeks to confirm progression and be deemed as iCPD

Progressive Disease (PD): Development of new MIBG positive lesions.

- Greater than 20% increase in the longest diameter taking as a reference the smallest sum on study (including baseline0
- Minimum absolute increase of 5 mm in longest dimension
- Mass does not meet PD measurement criteria, but has fluctuating MIBG avidity will not be considered PD.
- The response of MIBG lesions will be assessed on central review using the Curie scale (Ascierto, 2011).
- Central review responses will be used to assess efficacy for study endpoint. As such the iUPD nomenclature does not apply.

Note: *This scoring should also be done by the treating institution for end of course response assessments.*

10.6.3 Metastatic Soft Tissue and Bone Disease

^{123}I-MIBG uptake will be used for evaluation of response at osteomedullary lesions (Park, 2017). For patients whose tumors do not concentrate MIBG, FDG-PET or PET/CT scan will be used for tumor detection in bone. Anatomic imaging will not be used to evaluate osteomedullary lesions, because these lesions may not shrink in size using CT/MRI even in the absence of residual viable tumor. Additionally, osseous lesions without a soft tissue mass are considered non-

measurable by standard RECIST criteria. The measurable extramedullary soft tissue components of bone lesions will be assessed using the same criteria used for other soft tissue sites (see *Section 10.5.1*).

The body is divided into 9 anatomic sectors for osteomedullary lesions, with a 10th general sector allocated for any extra-osseous lesion visible on MIBG scan. In each region, the lesions are scored as follows. The absolute extension score is graded as by adding the score of all segments (*Figure 3)*:

0 = no site per segment

1 = 1 site per segment

2 = more than one site per segment

3 = massive involvement (>50% of the segment)

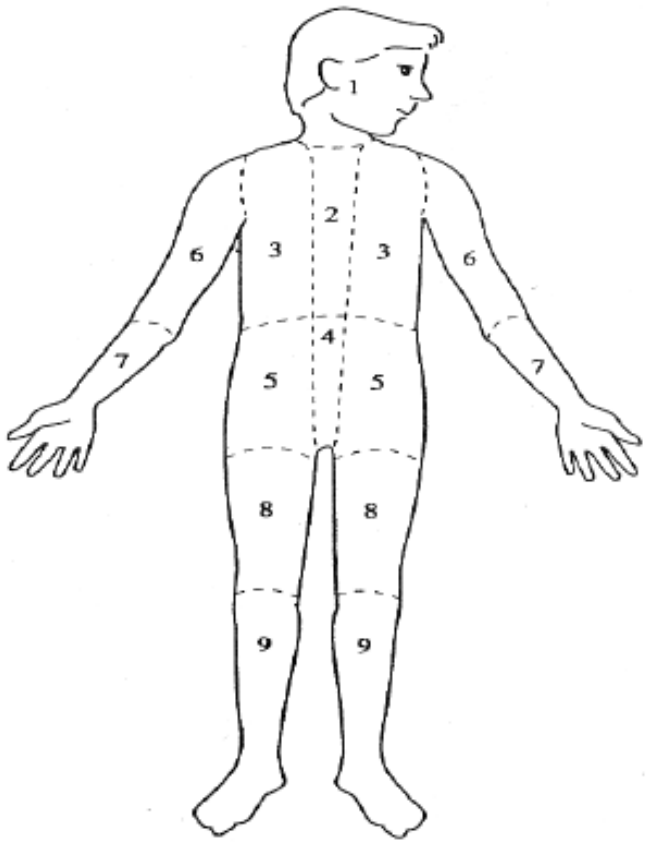


Figure 3. Absolute Score anatomic sectors diagram for osteomedullary lesions, with a 10th general sector allocated for any extra-osseous lesion visible on MIBG scan.

The relative score is calculated by dividing the absolute score at each time point by the corresponding pre-treatment absolute score. The relative score of each patient is calculated at each response assessment compared to baseline and classified as below (Park, 2017):

Complete Response (CR): All areas of uptake on MIBG scan completely resolved, defined as:

- Non-primary target and non-target lesions measure < 10mm;
- Lymph nodes identified as target lesions decrease to a short axis < 10 mm;
- MIBG uptake or FDG-PET uptake (for MIBG-non-avid lesions) of non-primary lesions resolves completely.

Partial Response (PR): ≥ 30% decrease in sum of diameters (see above for definition) of non-primary target lesions compared with baseline, and all of the following:

- NTL may be stable or smaller in size;
- No new lesions;
- ≥ 50% reduction in MIBG absolute bone score (relative MIBG bone score ≥ 0.1 to ≤ 0.5; see *Figure 3*) ≥ 50% reduction in the number of FDG-PET-avid bone lesions.
    - For patients with soft tissue metastatic disease, resolution of MIBG and/or FDG-PET uptake at the soft tissue sites is not required, but all size reduction criteria must be fulfilled;

Stable Disease (SD): Neither sufficient shrinkage for PR nor sufficient increase for PD of non-primary lesions.

Progressive disease (PD): Any of the following:

- Any new soft tissue lesion detected by CT/MRI that is also MIBG avid or FDG-PET avid;
- Any new soft tissue lesion seen on anatomic imaging that is biopsied and confirmed to be neuroblastoma or ganglioneuroblastoma;
- Any new bone site that is MIBG avid;
- A new bone site that is FDG-PET avid (for MIBG-non-avid tumours) and has CT/MRI findings consistent with tumour or has been confirmed histologically to be neuroblastoma or ganglioneuroblastoma;
- >20% increase in longest diameter taking as reference the smallest sum on study (this includes the baseline sum) and minimum absolute increase of 5 mm in sum of diameters of target soft tissue lesions;

- Relative MIBG score ≥ 1.2.
- The response of MIBG lesions will be assessed on central review using the Curie scale (Ascierto, 2011).
- Central review responses will be used to assess efficacy for study endpoint. As such the iUPD nomenclature does not apply.

Note: *This scoring should also be done by the treating institution for end of course response assessments*.

10.7 Response Criteria for Neuroblastoma Patients with Metastatic Bone Marrow Involvement

Assessment of BM involvement is achieved via evaluation of bilateral aspirates and bilateral trephine biopsies, a total of four (4) sampled sites. Assessment of BM for neuroblastoma cells must use morphologic criteria in conjunction with appropriate antibodies to confirm the identity of neuroblastoma cells by immunocytology (if available) and/or IHC (Park, 2017). Only BM samples of suitable quality should be investigated, as specified by Burchill, *et al*. (2016). Exact quantification of BM involvement at all sites should be reported; the percentage of tumor infiltration of BM space assessed by histologic evaluation divided by the number of hematopoietic or mononuclear cells evaluated to obtain a percentage of involvement (methodology described by Burchill, *et al.* (2016). The BM sample with the highest percentage of tumor infiltration is used in the response algorithm. BM must be obtained within 28 days prior to study enrollment with tumour cells seen on routine morphology. Response will be compared with baseline disease evaluations before enrollment in **Part II**.

Neuroblastoma infiltration in the marrow can be heterogeneously distributed throughout the skeleton (Park, 2017). Due to the clinical impact of this heterogeneity, detection of more than 0% to ≤ 5% tumor infiltration in BM will represent a new category of minimal disease. See below for BM metastasis response definitions determined by cytology/ histology:

Complete Response (CR): BM with no tumour infiltration on reassessment, independent of baseline tumour involvement.

Progressive disease (PD): Any of the following, detected in 2 consecutive BM biopsies or aspirations done at least 21 days apart:

- BM without tumour infiltration that becomes > 5 % tumour infiltration on reassessment;
- BM with tumour infiltration that increases by > 2x and has > 20 % tumour infiltration on reassessment.

Minimal Disease (MD): Any of the following:

- BM with ≤ 5% tumour infiltration and remains > 0 to ≤ 5% tumour infiltration on reassessment;
- BM with no tumour infiltration that has ≤ 5% tumour infiltration on reassessment;
- BM with > 20% tumour infiltration that has > 0 to ≤ 5% tumour infiltration on reassessment.

Stable Disease (SD): BM with tumour infiltration that remains positive with > 5% tumour infiltration on reassessment but does not meet CR, MD, or PD criteria.

Note: In the case of discrepant results between aspirations/ biopsies from two or more sites taken at the same time, the highest infiltration result should be reported.

10.7.1 Best Overall Response (iBOR)

The best overall response is the best response recorded from the start of the treatment until disease progression/ recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria. See *Tables 6-9* for details. All iUPD require re-evaluation four to eight weeks later to confirm disease progression (iCPD).

Best overall response will be determined by combining response of the individual components (i.e., soft tissue, bone, and BM disease). All components must be evaluated and of sufficient quality to fully assess overall response. An overall CR requires that all involved components have a CR. An overall PR includes a PR of all soft tissue and bone sites or non-involvement in one of these components, but allows residual MD in the bone marrow. Minor response (MR) requires PR or CR in at least one component, SD for at least one component, and no evidence of progressive disease in any component. PD in any one component defines overall PD.

Best Overall Response (BOR): Each patient will be classified according to his "best response" for the purposes of analysis of treatment effect. Best response is determined from the sequence of the overall response assessments as described below.

Table 5. Evaluation of Best Overall Response (iBOR) for Patients with Measurable Disease (i.e., Target Disease).

Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥ 28 days Confirmation
CR	Non-CR/Non-PD	No	PR	≥ 28 days Confirmation
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once ≥ 28 days from baseline
iUPD	Any	Yes or No	iCPD/PD	no prior SD, PR or CR
Any	iUPD**	Yes or No	iCPD/PD	
Any	Any	Yes	PD	

** See iRECIST manuscript for further details on what is evidence of a new lesion (Seymour, 2017).*
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.*
Note*: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.*

Table 6. Evaluation of Best Overall Response for Patients with Non-Measurable Disease (i.e., Non-Target Disease).

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* *'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised*		

Table 7. Sequences of overall response assessments with corresponding best response.

1st Assessment	2nd Assessment	Best Response
IUPD	iCPD	PD
Stable, PR, CR	iUPD	Requires reassessment 4 - 8 weeks.
Stable	Stable	Stable
Stable	PR, CR	Stable
Stable	Not done	Not RECIST classifiable
PR	PR	PR
PR	CR	PR
PR, CR	Not done	Not RECIST classifiable
CR	CR	CR

Table 8. Overall Response for Patients with Neuroblastoma and Measurable Disease.

CT/MRI	MIBG	BM	Catecholamine	Overall
iUPD	Any	Any	Any	
Any	iUPD	Any	Any	PD
Any	Any	Any	Any	PD
Any	Any	iUPD	Any	PD
SD	CR/PR/SD	Non-PD	Any	SD
PR	CR/PR	Non-PD	Any	PR
CR/PR	PR	Non-PD	Any	PR
CR	CR	Non-PD	Elevated	PR
CR	CR	CR	Normal	CR

Table 9. Overall Response Evaluation for Neuroblastoma Patients and MIBG Positive Disease.

MIBG	CT/MRI	BM	Catechol	Overall
iUPD	Any	Any	Any	PD
Any	New Lesion	Any	Any	PD
Any	Any	Any	Any	PD
Any	Any	iUPD	Any	PD
SD	No New Lesion	Non-PD	Any	SD
PR	No New Lesion	Non-PD	Any	PR
CR	No New Lesion	Non-PD	Elevated	PR
CR	No New Lesion	CR	Normal	CR

- *Only if patients are enrolled without disease measurable by CT/MRI, any new or newly identified lesion by CT/MRI that occurs during therapy would be considered progressive disease and not unconfirmed immune-progressive disease (iUPD) requiring reassessment to be diagnosed as iCPD.*

10.7.2 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The Duration of Overall Complete Response (CR): measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of Stable Disease (SD): Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

10.8 Response Criteria for CNS Tumours

10.8.1 Special Considerations for Tumour "Pseudo-progression" Phenomena

In Neuro-Oncology, progressive imaging findings in patients receiving immunotherapy pose challenges in interpretation of those radiological findings and may result in premature discontinuation of potentially beneficial therapy (Okada, 2015).

Correct interpretation of progressive imaging findings after administration of immunotherapy is essential because early progressive radiographic changes do not always preclude subsequent therapeutic benefit.

Two main explanations exist for a possible disconnect between early worsened imaging findings and subsequent therapeutic benefit. First, effective immune responses might need time to evolve, and early imaging might reflect true progressive disease, including the development of new lesions. Nonetheless, once induced, an effective anti-tumour immune response might subsequently lead to clinical benefit. Second, because the mode of action might include an inflammatory response in areas of macroscopic and microscopic infiltrative tumour, localized inflammatory responses might mimic radiological features of tumour progression with increased enhancement and oedema (Bouffet, 2016).

For cases of pseudo-progression, histopathology typically shows remarkable immune-cell infiltration, such as $CD8^+$ T lymphocytes, but not mitotically active tumour cells. Further radiological confirmation to define progressive disease is an important, novel aspect of immune-related response criteria, together with the need of follow-up imaging to confirm a radiographic response (Okada, 2015). For example, the temporal window for TMZ/RT→TMZ pseudo-progression generally peaks within three months, the time frame for immunotherapy-associated pseudo-progression remains to be defined and may differ by class of ICI (Okada, 2015). The current RANO guidelines, do not permit treatment continuation beyond actual tumor progression because subsequent therapeutic benefit supporting this practice has not been documented for oncology treatments other than immunotherapies, but the iRANO do.

Neuro-oncology criteria that are based on guidance for determination of tumor progression outlined by the immune-related response criteria (irRC) and the response assessment in neuro-oncology (RANO) working group, deemed iRANO have developed and as such, for CNS tumours treated with ICI, iRANO response criteria guidelines will be used (Wen, 2010; van den bent, 2011; Okado, 2015; Lin, 2015) are used to evaluate tumour response.

10.8.2 Selection of Target and Non-Target Lesions

For most CNS tumours, only one lesion/mass is present and therefore is considered a "target" for measurement/follow up to assess for tumour progression/response. If multiple measurable lesions are present, up to five (5) should be selected as "target" lesions. Target lesions should be selected on the basis of size and suitability for accurate repeated measurements. All other lesions will be followed as non-target lesions. The lower size limit of the target lesion(s) should be at least twice the thickness of the slices showing the tumour to decrease the partial volume effect (e.g., 8 mm lesion for a 4-mm slice).

Any change in size of non-target lesions should be noted, though does not need to be measured.

10.9 Response Criteria for Target Lesions

Response criteria are assessed based on the product of the longest diameter and its longest perpendicular diameter. Development of new disease or progression in any established lesions is

considered PD, regardless of response in other lesions – e.g., when multiple lesions show opposite responses, the progressive disease takes precedence.

For patients with early progressive imaging findings (within six (6) months from initiation of the immunotherapy), including patients who develop new lesions but who do not have substantial neurological decline, confirmation of radiographic progression by follow-up imaging should be sought three (3) months after initial radiographic evidence of progressive disease to decrease the likelihood of prematurely declaring progressive disease in patients with pseudo-progression or delayed response.

Imaging within the 3-month follow-up can be done as medically appropriate at the discretion of the treating physician.

Patients with confirmation of further radiographic progression based on a comparison with the scan that first showed evidence of disease progression (new reference scan) should be classified as having progressive disease with the date of disease progression back-dated to the first date that the patient met criteria for radiographic progression. In those patients, non-responsiveness to treatment should be assumed and therapy discontinued.

Patients who develop substantial new or worsened neurological deficits not due to comorbid events or a change in co-administered medication at any time within the 3-month follow-up window should be designated as non-responsive to treatment and should discontinue immunotherapy. For these patients, the date of actual tumour progression should also be back-dated to the date when radiographic progressive disease was initially identified.

If follow-up imaging does not confirm further disease progression compared with the scan of the tumour that first showed initial progressive changes, but instead there is stabilization or reduction in tumour burden, treatment should be continued or resumed in the absence of increased corticosteroid dosing.

Patients who develop worsening radiographic findings compared with the pre-treatment baseline scan more than 6 months from starting immunotherapy are expected to have a low likelihood of ultimately deriving clinical benefit and should be regarded as non-responsive to treatment with a recommendation to discontinue therapy.

10.9.1 New Lesions

Appearance of new lesions is a criterion that defines PD according to original RANO guidelines; however, transient appearance of new enhancing lesions at either local or distant sites may occur among neuro-oncologic patients receiving immunotherapies (Bouffet, 2016). These may be cases of pseudo-progression, which are confirmed histopathology revealing remarkable immune cell infiltration. In such situations, careful radiologic and clinical assessments are warranted. In some cases, such new enhancing lesions may reflect immune responses directed against infiltrative brain tumor cells (see *Table 10*).

Table 10. iRANO response criteria for target CNS lesions (Okada, 2015).

Complete Response (CR)	Disappearance of all enhancing disease and/or non-target lesions for ≥4 weeks; no new lesions; stable or improved T2/FLAIR; no more than physiological steroids; clinically stable or improved.
Partial Response (PR)	≥50% decrease in the sum of biperpendicular diameters of enhancing disease for ≥4 weeks; no new lesions; stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved. • If Brain Metastases, ≥ 30% decrease in the sum of the longest diameter of target lesions for ≥ 4 weeks as well.
Minor Response (MR)	For LGG, 25-49% decrease in the sum of biperpendicular diameters of T2/ FLAIR disease for ≥ 4 weeks; no new lesions; clinically stable or improved.
Stable Disease (SD)	Does not qualify for CR, PR, or PD; no new lesions; stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved.
Progressive Disease (PD)	Confirmation of progression on follow-up imaging ≥ 3 months after initial radiographic progression, indicated below: • ≥25% decrease in the sum of biperpendicular diameters of enhancing disease; • or new lesions; • or substantial worsened T2/FLAIR;

	• or substantial clinical decline. • If Brain Metastases, ≥ 20% decrease in the sum of the longest diameter of target lesions or unequivocal progression of enhancing non-target lesions as well. • Confirmation of pseudo-progression (vs. true PD) requires no new or significantly worsen neurological deficits not due to co-morbid event or concurrent medication ≤ 6 months from initiation of nivolumab. The lesions are added to the total lesion area for follow up (see *Table 11).*

Table 11. Key considerations of iRANO response criteria.

	iRANO (if ≤ 6 months after start of immunotherapy).	iRANO (if > 6 months after start of immunotherapy).
Is a repeat scan required to confirm radiographic PD for patients without clinical decline?	Yes	No
Minimal time interval for confirmation of progression for patients without significant clinical decline?	≥ 3 months	N/A
Is further immunotherapy treatment allowed after initial radiographic PD (if clinically stable) pending progression confirmation?	Yes	N/A
Does a new lesion define PD?	Yes	Yes

10.9.2 Confirmation of Radiographic Progression

Traditional imaging criteria to define PD may be less reliable and could lead to premature discontinuation of potentially beneficial therapy. Early increases in lesion size or new lesions do not define PD unless further progressive changes are confirmed upon follow-up imaging, provided that patients are not experiencing clinical decline. Particularly for indications such as glioblastoma, where effective therapeutic interventions are limited, and durable responses are elusive, continuation of immunotherapies beyond initial progression may lessen the likelihood of prematurely discontinuing potentially effective therapy (Okada, 2015; Bouffet, 2016).

Spider-plots describing changes in tumor volume over time for solid tumor patients undergoing immune checkpoint blockade demonstrate that early progressive radiographic findings typically stabilize or improve within three months for the majority of patients who ultimately derive clinical benefit (Topalian, 2012; Hamid, 2013; Brahmer, 2012). Based on these observations, among patients with early progressive imaging findings including the development of new lesions who are not experiencing significant neurologic decline, confirmation of radiographic progression via follow-up imaging should be sought no sooner than three months after initial radiographic evidence of PD is detected, to decrease the likelihood of prematurely declaring PD in patients with pseudo-progression or delayed response. Imaging within the three (3)- months follow-up period can be performed as medically appropriate at the discretion of the treating clinician. Among those with confirmation of further radiographic progression based on comparison to the scan which first revealed evidence of progression, or who exhibit significant clinical decline at any time, should be classified as PD with the date of disease progression back-dated to the first date that the patient met criteria for radiographic progression. Such patients should be discontinued from their current immunotherapy regimen.

If follow-up imaging does not confirm further progression compared to the scan which first revealed initial progressive changes, but instead reveals stabilization or reduction in tumor burden, in the absence of increased corticosteroid dosing, treatment should be continued or resumed (see *Figure 4*).

Patients with significant neurologic decline, regardless of imaging findings, are deemed to have PD, providing their decline is not attributable to co-morbid events such as seizures or changes in medication, notably decreased corticosteroid dosing. For such patients, radiographic

confirmation of PD is neither necessary nor appropriate and their date of PD is the date they developed significant neurologic decline attributable to underlying tumor.

Anecdotal reports of glioma patients treated with tumor vaccination and ICI therapy have described pseudo-progressive radiographic findings that also typically manifest within six months of treatment initiation (Hoos, 2010; Sampson, 2010; Pollack, 2014).

Conversely, there is no evidence that patients develop delayed clinical benefit or radiographic response if they develop progressive radiographic findings more than six (6) months after initiating immunotherapy.

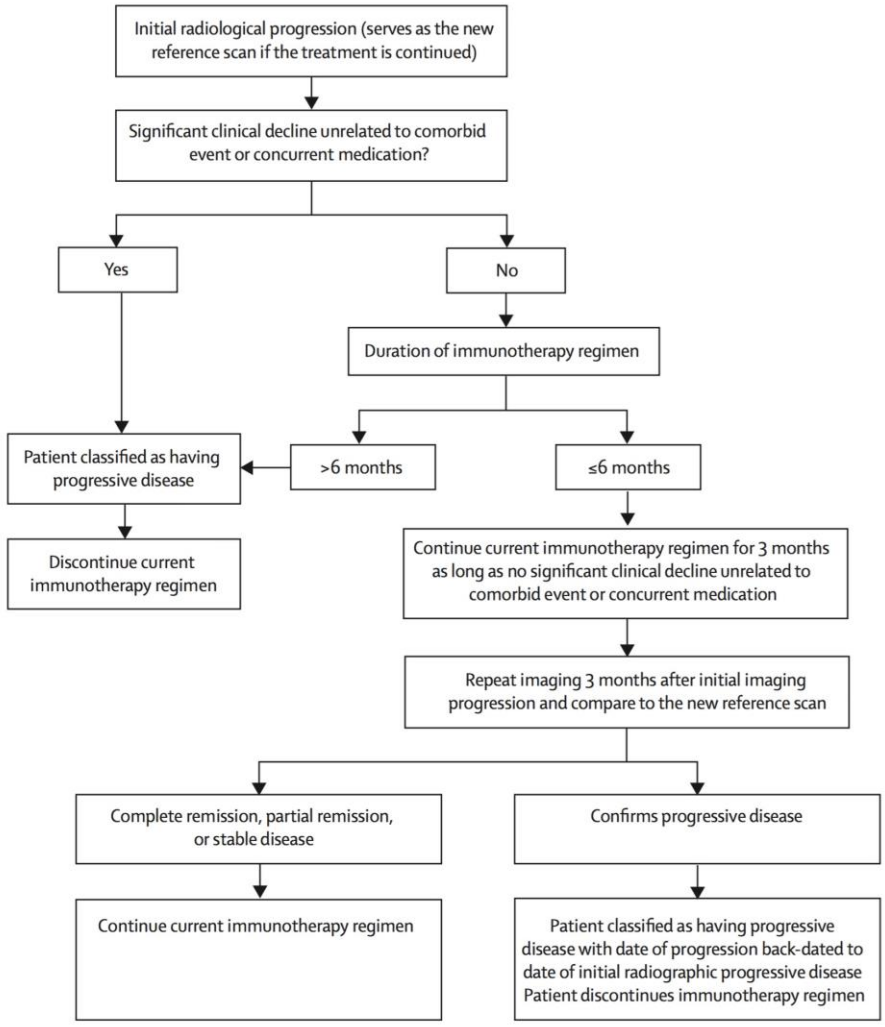


Figure 4. iRANO treatment algorithm for the assessment of progressive imaging findings in patients with neuro-oncological malignancies undergoing immunotherapy (Okada, 2015).

10.9.3 Non-Target Lesions

10.9.3.1 Tissue Acquisition to Aid Response Assessment

In uncertain cases in which acquisition of tumor histopathology via biopsy or resection is considered feasible, pathological assessment may be considered to clarify the etiology of progressive imaging findings. Discussion with the Study Chair or Co-chair prior to surgery is strongly encouraged if timing permits. If pathology confirms a predominance of recurrent tumor, the etiology should be considered to be true progression. For cases where no evidence of viable tumor is detected, or where a prominence of gliosis/inflammation with limited viable tumor is observed, the etiology should be considered consistent with treatment effect, such patients should be classified as stable and allowed to continue therapy.

10.9.3.2 Response Criteria for Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions.

Incomplete Response/Stable Disease (IR/SD): The persistence of one or more non-target lesions.

Progressive Disease (PD): The appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions confirmed on follow-up imaging ≥ 3 months after initial radiographic progression with requires no new or significantly worsen neurological deficits not due to co-morbid event or concurrent medication ≤ 6 months from initiation of nivolumab.

10.10 Response Criteria for Tumour Markers (if available)

Tumour markers will be classified simply as being at normal levels or at abnormally high levels.

10.11 Response Evaluation for Patients Receiving Therapeutic Corticosteroids

During the course of treatment, if pseudo-progression occurs, higher doses of corticosteroids may be necessary to control symptoms, and this may impact on disease response evaluation. Patients who require increased corticosteroids within two weeks of MRI assessment

relative to the dose taken at the time of the prior assessment, cannot be classified as CR, PR, or SD and should be classified as non-evaluable at that time point. Conversely, patients who decrease corticosteroids within two weeks of MRI assessment, relative to the dose taken at the time of the prior assessment, cannot be classified as PD and should be classified as non-evaluable.

10.12 Overall Response Assessment

The overall response assessment takes into account response in both target and non-target lesions, the appearance of new lesions and normalization of markers (where applicable), according to the criteria described in the table below. The overall response assessment is shown in the last column, and depends on the assessments of target, non-target, marker and new lesions in the preceding columns (see *Table 12*).

Table 12. Overall response for CNS lesions.

Target Lesions	Non-Target Lesions	Markers	New Lesions	Overall Response
CR	CR	Normal	No	CR
CR	IR/CD	Normal	No	PR
CR	Non-PD	Abnormal	No	PR
PR	Non-PD	Any	No	PR
CD	Non-PD	Any	No	CD
PD	Any	Any	Yes or No	PD
Any	PD	Any	Yes or No	PD
Any	Any	Any	Yes	PD

10.13 Response Criteria for Patients with AML

The AML response criteria are derived according to Creutzig, *et al*. (2004, 2012) with some revisions from the AML International Working Group (IWG) Criteria (Cheson, 2003).

The following definitions will be used:

Complete remission (CR): All of the following must be achieved:

- BM blasts <5% (M1 BM);
- Absence of circulating blasts or extramedullary disease;
- Absolute neutrophil count (ANC) ≥1.0 x 10^9/L;
- Platelet count ≥80 x 10^9/L.

Occasionally, a rare peripheral blood blast may be identified during marrow regeneration; however, the marrow must be M1 status and with no Auer rods. Flow cytometry may also be useful to distinguish between leukemia and a regenerating BM. There is no requirement for BM cellularity.

Morphological CR with partial recovery of platelet count (CRp): All of the following must be achieved:

- BM blasts <5% (M1 BM) with no Auer rods;
- Absence of circulating blasts or extramedullary disease;
- Recovery of ANC ≥1.0 x 10^9/L;
- Platelet transfusion independence (defined as, no platelet transfusions for 1 week).

Morphological CR with incomplete count recovery (CRi): All of the following must be achieved:

- BM blasts <5% (M1 BM) with no Auer rods;
- Absence of circulating blasts or extramedullary disease;
- All CR criteria except for residual neutropenia (<1.0 x 10^9/L) or thrombocytopenia (<80 x 10^9/L) without platelet transfusion independence (defined as, no platelet transfusions for 1 week).

Cytogenetic complete remission (CRc):

- The patient must also have attained and be assigned morphologic response of CR or CRp or CRi as defined above.
- Must have a revision to a normal karyotype with a minimum analysis of 20 metaphases.

Partial Response (PR): A decrease of at least 50% blasts percentage (to M2: 5% to 25%) in the BM aspirate. BM must have adequate cellularity (e.g., > 15%) to determine response. PR status will not be included in calculation of response to the regimen.

A repeat BM aspiration after several weeks may be required to distinguish between a PR and increased blasts caused by BM regeneration. A value of < 5% blasts may also be considered a PR if Auer rods are present.

Treatment Failure (TF):

- Failure to achieve CR or CRi
    - Any M2 (5-25% blasts) or M3 (5-25% blasts) BM aspirate that does not qualify for PR status;
    - M1 (5% blasts) BM aspirate with evidence of circulating blasts or extramedullary disease;

Relapse: Morphologic relapse after CR/CRp/CRi is achieved and documented, defined as:

- A reappearance of leukemic blasts in the peripheral blood or ≥ 5% blasts (M1) in BM not attributable to any other cause (such as, BM regeneration after consolidation therapy).
- If there are no circulating blasts, but the BM contains 5% to 25% blasts (M2)
    - A repeat BM performed at least a week later is necessary to distinguish relapse from BM regeneration.
- The reappearance or development of cytologically proven extramedullary disease;
    - Molecular and/or genetic relapse is characterized by reappearance of a cytogenetics or molecular abnormality.

Should flow cytometry analyses suggest relapse (by the reappearance of a similar immunophenotype to the original leukemia) in the presence of < 5% blasts, or ≥ 5% blasts in a regenerating marrow, a repeat BM aspirate performed at least a week later is necessary to confirm relapse by morphologic methods. In such instances, the date of recurrence is defined as the first date that ≥5% blasts were observed (in BM).

Unevaluable: Aplastic or severely hypo-cellular marrow with any blast percentage. In this instance, marrow evaluation should be repeated weekly until response determination can be made through at least Day 49.

10.14 Response Criteria for the Patients with ALL

Complete Remission (CR): All of the following must be achieved:

- BM blasts <5% (M1 BM and adequate marrow cellularity);
- Absence of circulating blasts or extramedullary disease;
- Absolute neutrophil count (ANC) ≥0.75 x 10^9/L
- Platelet count ≥75 x 10^9/L

Qualifying marrow and peripheral counts should be performed within 1 week of each other.

Morphological CR with partial recovery of platelet count (CRp): All of the following must be achieved:

- BM blasts <5% (M1 BM);
- Absence of circulating blasts or extramedullary disease;
- Recovery of ANC ≥750/mcL
- Insufficient recovery of platelets (< 75 000/mcl).

Morphological CR with incomplete count recovery (CRi): All of the following must be achieved:

- BM blasts <5% (M1 BM);
- Absence of circulating blasts or extramedullary disease;
- No recovery of ANCs (ANC < 750/mcl)
- Insufficient recovery of platelets (< 75 000/mcl).

Partial Remission (PR): All of the following must be achieved:

- Complete disappearance of circulating blasts and achievement of M2 BM status (5-25% blasts), without new sites of extramedullary disease;
- Recovery of ANCs (ANC > 750 mcL).

Partial Remission – Cytolytic (PRCL): Complete disappearance of circulating blasts and achievement of at least 50% reduction from baseline in BM blast count.

Stable Disease (SD): The patient does not satisfy the criteria for PD, or has recovery of ANC > 750 mcL and fails to qualify for CR, CRp, or PR.

Progressive Disease (PD): An increase of at least 25% in the absolute number of circulating leukemic cells, measured pre-therapy or during the first 14 days following start of therapy; or other laboratory or clinical evidence of PD, with or without recovery of ANC or platelets.

10.15 Response Criteria for Patients with Lymphoma

The lymphoma response criteria are derived from the International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017) (Younes, 2017).

10.15.1 Assessment of Tumour Burden

Assessment of tumor burden in lymphoma should use the sum of longest diameters (SLD) of a maximum of three (3) target lesions in patients with disseminated disease (Younes, 2017). Target lesions should be selected from those with the largest size that can be reproducibly measured and preferably representing multiple sites and/organs. In most cases, lymph nodes can be considered target lesions if the lymph node longest diameter measures 15 mm or greater. Between ten (10) and 14mm is considered abnormal and should not be selected as target lesions and those less than ten (10) mm are considered normal (Schwartz 2009; Eisenhaur, 2009). Certain anatomical sites (such as, the inguinal, axillary, and portocaval), contain normal lymph nodes that may exist in a narrow, elongated form, and such nodes should not be selected as target lesions if alternatives are available. Extra-nodal lesions are selected as target lesions, if they have a soft tissue component, make the size threshold and have easy reproducibility for repeat measurements. All other lesions should be identified as non-target lesions, recorded at baseline (without measurements) and reported as either present, absent, or clear progression.

10.15.2 Imaging and Baseline Scans

Whenever possible, the same imaging modality should be used at baseline and subsequently. CT scan imaging (with oral and intravenous contrasts) remains the gold standard for determining tumor measurements. In certain situations, where minimizing exposure to ionizing radiation is desirable, or where CT provides suboptimal assessment (such as primary bone lymphoma), standard MRI can be used to determine baseline and subsequent tumor measurements. [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) is recommended in the initial staging work up all FDG-PET avid lymphomas (Cheson, 2014; Barrington, 2014). In certain cases, measurements may be performed on the CT-component of a combined PET/CT images, provided it has adequate resolution. Patients with diffuse large B-cell lymphoma with a

negative FDG-PET uptake in the BM (BM), do not rule out BM involvement, especially discordant histology; however, a positive FDG-PET uptake in the BM may obviate the need for a BM biopsy (Adams, 2014).

In patients with newly diagnosed lymphoma, a BM biopsy is recommended at baseline to determine the stage of disease and is mandatory for previously untreated patients with indolent B-cell lymphoma, mantle cell lymphoma, and T-cell lymphoma at the discretion of the local treating physician. Patients with Hodgkin lymphoma without FDG uptake in the BM or presence of B-symptoms do not need a BM biopsy at baseline, as BM in this situation is extremely unlikely to modify stage. Additional BM biopsies should be performed as part of usual clinical practice at the discretion of the local physician (Younes, 2017).

10.15.3 Response Assessment of Lymphoma

See the *Table 13* for the schedule of tumour evaluations. Response and progression of lymphomas will be evaluated in this study using the International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017; Younes, 2017).

Table 13. RECIL 2017: Response categories based on assessment of target lesions (Younes, 2017). Percent change in the sum of diameters of target lesions from nadir.

	% change in SLD of Target Lesions from Baseline	FDG-PET	BM Involvement	New Lesions
Complete Response (CR)	• Complete disappearance of all target lesions and all nodes with long axis <10 mm; • ≥30% decrease in the sum of the longest diameters of target lesions (PR) with normalization of FDG-PET.	• Normalization of FDG-PET (Deauville score 1-3).	Not involved	No
Partial Response (PR)	• ≥30% decrease in the sum of the longest diameters of target lesions but not a CR.	• Positive (Deauville score 4-5).	Any	No
Minor Response (MR)	• ≥10% decrease in the sum of the longest diameters of	Any	Any	No

	target lesions but not a PR (<30%).			
Stable Disease (SD)	• <10% decrease or ≤20% increase in the sum of the longest diameters of target lesions.	Any	Any	No
Progression of Disease (PD)	• >20% increase in the sum of the longest diameters of target lesions; • For small lymph nodes measuring <15 mm post therapy, a minimum absolute increase of 5 mm and the long diameter should exceed 15 mm; • Appearance of a new lesion.	Any	Any	Yes or No

This revised definition of PD will eliminate the false interpretation of disease progression due to treatment-related inflammatory flares/ pseudo-progression reported with ICIs. The previous Lugano Criteria definition of PD is in conflict with clinical practice as it calls for stopping or changing therapy when a single lymph node increases in size from 1.0 to 1.6 cm, even though the overall sum of products may have shown significant reduction. (Witzig, 2009; Chanan-Khan, 2011). An increase in the size of previously involved small lymph nodes by >20% while other lesions are decreasing may be a pseudo-progression (especially at the beginning of treatment) and should not be designated a PD, unless there is continued increase in size on subsequent imaging studies (Witzig, 2009; Park, 2010; Armand, 2016, Zinzani, 2015, Younes, 2016). Patients should be allowed to remain on trial until the response or lack thereof is clarified on subsequent appropriate imaging, upon discussion with the investigators and patient and the Study Chair or Co-Chair. Confirmation of PD requires two consecutive scans at least four (4) weeks apart, and inclusion of new lesion measurements to the total tumor burden (Cheson, 2016; Younes, 2017).

Any mixed response will be called an MR, as long as the SLD is consistent with the aforementioned definition; however, MR will be called SD as long they fulfill all the criteria for SD in *Table 13.* Whenever possible, questionable small FDG-PET avid lesions should be confirmed by a histologic or cytologic analysis. Appearance of a new FDG-PET avid lesion that is smaller than the thresholds mentioned in *Table 13* should be closely monitored, and whenever possible, a biopsy should be performed to determine its nature.

To clarify, patients who achieve a CR (normalization of all lymph node measurements and disappearance of extra-nodal lesions), at least one previously involved lymph node should increase in size to measure 15mm in the long diameter, with a minimum absolute increase of at least 5mm from nadir to be considered for PD. Accordingly, an increase in a lymph node longest diameter from 8 to 13mm is not considered a PD, even though there is 38% increase in the measurement, since the lesion did not exceed 15 mm. Similarly, a change from 12 to 16mm does not qualify as a PD even though the new measurement exceeds 15 mm, since the absolute increase was <5 mm.

10.15.4 Small Responsive Lymph Nodes

In cases where baseline tumor burden is low, and only a few lesions measuring around two (2) cm in longest diameter, treatment effect may shrink the long axis of a target lymph node to a normal value of <10 mm. Although the lymph node is now within normal size range (consistent with CR), the percentage of diameter reduction may be <30% (less than a PR). In these cases, a "normalized" diameter of "0, or resolved" should be used to calculate the SLD, to ensure accurate response designation (see sample calculation in *Table 14*).

Table 14. Sample calculating sum of diameters to include small responsive lymph nodes using normalized diameters (Younes, 2017).

Target Lesions	Lesion 1	Lesion 2	Lesion 3	SOD	% change from baseline	Response designation
Baseline (cm)	1.6	1.7	2.0	5.3	N/A	N/A
Nadir actual (cm)	0.9	1.4	1.8	4.1	23	MR
Nadir normalized (cm)	0; resolved	1.4	1.8	3.2	40	PR (or CR if PET –ve)

10.15.5 Appearance of a New Extra-nodal Lesion

With the use of PET imaging, a new small PET avid lesion may appear during or after therapy. A minimum of 1 cm in largest diameter of new extra-nodal lesions is required to assign PD directly. New smaller but suspicious lesion should be designated as equivocal, and if later confirmed (by CT or biopsy) as being due to lymphoma, the documented date of progression should be the date of when it was first identified as equivocal.

10.15.6 Measurement of Splitting Lesions

Frequently, effective therapy may convert a large confluent mass to several smaller constituent lymph nodes. In this case, the measurement of each lymph node should be carried out and entered in the calculation of sum of diameters. However, to avoid an overall increase in the number of target lesions, sub-designations of A, B, C, etc. for any target lesion that has undergone splitting should be created.

10.15.7 Spleen Measurement

The spleen vertical length can be calculated by multiplying the number of spleen slices in transverse CT views by the thickness of each slice, or by measuring splenic coronal diameter on a PET maximum intensity projection image.

10.15.8 Integrating Target and Non-Target Lesions

In case of disseminated disease, the status of non-target lesions should be taken into account before formulating the final response status, as outline in *Table 15.*

Table 15. Response designation incorporating bet response of target lesions and non-target lesions (Younes, 2017).

Target Lesion	Non-target Lesion	New Lesion	Response Designation
CR	CR	No	CR
CR	PR,MR, or SD	No	PR (by CT with normalization of FDG-PET is CR)
CR	UE	No	UE
PR	UE	No	UE
PR	CR	No	PR
PR	PR,MR, or SD	No	PR
MR	UE	No	UE
MR	CR	No	MR
MR	PR,MR, or SD	No	MR
SD	UE	No	UE
SD	CR, PR, or MR	No	SD
SD	SD	No	SD
PD	Any	Yes/No	PD
Any	PD	Yes/No	PD
Any	Any	Yes	PD
CR	No	No	CR
PR	No	No	PR
MR	No	No	MR
SD	No	No	SD

10.16 Response Criteria for Patients with More than One Tumour

In the rare event that a patient has more than one tumour (e.g. glioblastoma and colon cancer), the evaluation will take into account the combination of responses as follows: response = either of CR + CR, CR + PR, CR + SD, PR + SD, while the absence of response or progression will include all other situations.

11. STATISTICAL CONSIDERATIONS

This is a pilot study for which the initial aim was to accrue 20 paediatric patients with bMMRD tumours. Following amendment, the study inclusion criteria will be expanded to include those with hypermutant tumours (as determined by estimation of TMB by targeted gene sequencing), as well as those with CMMRD, including bMMRD tumours, and consequently the total enrollment will be increased to 50 patients.

The primary objective is to determine the ORR as well as to estimate the efficacy of nivolumab in patients with hypermutant tumours. The study is predicated on the hypothesis that patients with hypermutated tumours will derive greater benefit from nivolumab than those without; however, the relevant threshold of TMB is not yet established. Consequently, in this pilot study, patients will be recruited into two cohorts depending on TMB. This will provide the opportunity to treat patients with a relatively low TMB (≥5 to <10/Mb, estimated ~15% of tumors) to assess for responses to nivolumab, while a separate cohort for patients with TMB (≥10/Mb, estimated ~5% of tumors) will ensure that overall study enrollment is not dominated by patients with lower TMB.

A Simon two-stage design will be used within each cohort.

For cohort A (TMB (≥5 to <10/Mb), the null hypothesis that the true response rate is only 10% will be tested against a one-sided alternative. In the first stage, 10 patients will be accrued. If there are 1 or fewer responses in these 10 patients, the cohort will be closed. Otherwise, 10 additional patients will be accrued for a total of 20. The null hypothesis will be rejected if 5 or more responses are observed in 20 patients. This design yields a type I error rate of 0.05 and power of 85% when the true response rate is 35%. Accrual to this cohort will stop if evidence accumulates that the efficacy is lower than the specified acceptable levels. If five or more (≥5) patients experience objective responses (CR+PR), then we will conclude that Nivolumab is sufficiently active in patients with TMB ≥5 to <10/Mb to warrant recommendation for continued investigation.

For Cohort B (TMB ≥10/Mb), in the first stage, 10 patients will be accrued. If there are 1 or fewer responses in these 10 patients, the cohort will be closed. Otherwise, 20 additional patients will be accrued for a total of 30. The null hypothesis will be rejected if 6 or more responses are observed in 30 patients. This design yields a type I error rate of 0.05 and power of 80% when the true response rate is 30%. Accrual to this cohort will stop if evidence accumulates that the efficacy is lower than the specified acceptable levels. If six or more (≥6) patients experience objective

responses (CR+PR), then we will conclude that Nivolumab is sufficiently active in patients with TMB ≥10/Mb to warrant recommendation for continued investigation.

Patients already recruited to study at the time of amendment will be retrospectively allocated to the appropriate cohort based on TMB. It is anticipated that most of these patients with CMMRD will have TMB ≥10/Mb and will therefore fall into cohort B. Any patients with CMMRD for whom TMB is not available will be analyzed separately in Cohort C.

In order to make decisions about further exploring the use of nivolumab in this patient population, a likelihood Bayesian analysis will also be used to provide estimates of likely effect sizes based on results from this pilot study. Posterior probability distributions for response rate will be plotted, using non-informative priors.

For example, in Cohort A, a response in 5 of 20 patients (estimate response rate 0.25), indicates a probability that the true RR is >0.2 of 0.77; while there is only a 0.01 probability that the true RR is <0.1. Similarly, for Cohort B, a response in 6 of 30 patients (estimated RR 0.2), indicates a probability that the true RR is >0.2 of 0.57; while there is a 0.03 probability that the true RR is <0.1.

Patients who are evaluable for objective response are those who meet the definition as per *Section 10.2.1.2*.

All enrolled patients who received at least one dose of any study drug will be evaluable for safety. A safety evaluation will be performed by the Safety Committee after Patient 1 Cycle 1, Patient 4 Cycle 1, Patient 7 Cycle 1, and Patient 10 Cycle 1. Enrollment will be held after each time point until a safety evaluation of adverse events and serious adverse events confirms it is safe to resume enrollment.

Any deviation from this statistics section of the protocol along with the accounting for missing, unused and spurious data will be described in the final report.

12. SAFETY AND REPORTING PROCEDURES

12.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product during the course of a study and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the medicinal product.

Disease signs, symptoms, and/or laboratory abnormalities already existing prior to the use of the product are not considered AEs after administration of the study product unless they reoccur after the subject has recovered from the pre-existing condition or they represent an exacerbation in intensity or frequency.

A laboratory test abnormality considered clinically relevant (e.g. causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations) or judged relevant by the Investigator should be reported as an adverse event.

An abnormal laboratory value is considered to be an AE if the abnormality:

- results in discontinuation from the study;
- requires treatment, modification/ interruption of investigational product dose, or any other therapeutic intervention;
- or is judged to be of significant clinical importance.

Regardless of severity grade, only laboratory abnormalities that fulfill a seriousness criterion need to be documented as a serious adverse event. If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page/screen of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE. If possible, the laboratory abnormality should be recorded as a medical term and not simply as an abnormal laboratory result (e.g., record thrombocytopenia rather than decreased platelets).

12.2 Adverse Event Documentation

Adverse events will use the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE). This study will utilize the CTCAE Version 4.03 for adverse event reporting.

All AEs must be recorded on the electronic case report forms (eCRFs). Documentation must be supported by an entry in the patient's file. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product as judged by the Investigator, action taken and outcome.

12.3 Serious Events

A Serious Adverse Event or Reaction is any AE occurring at any dose that:

- results in death;
- is life-threatening;
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- in a congenital anomaly / birth defect;
- is an important medical event that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above (example: intensive treatment in an emergency room or at home for bronchospasm, convulsions that do not result in hospitalization). Medical and scientific judgment should be exercised in deciding whether some events should be considered as serious because their quick reporting to the Sponsor may be of interest for the overall conduct of the study;
- elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice (suspicious for Drug-Induced Liver Injury [DILI] defined by an elevated ALT or AST (> 3 x baseline value), in combination with either an elevated total bilirubin (> 2 x ULN) or clinical jaundice in the absence of cholestasis or other causes of hyperbilirubinemia);
- Suspected transmission of an infectious agent by the study drug.

Life-threatening: The term "life-threatening" in the definition of "serious" refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event that hypothetically might have caused death if it were more severe.

Hospitalization: Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following exceptions is met:

- The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study or for prophylactic insertion of a gastric feeding tube).

OR

- The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care).

OR

- The admission results in a hospital stay of less than 12 hours

However, it should be noted that invasive treatment during any hospitalization may fulfill the criteria of 'medically important' and as such may be reportable as a serious adverse event dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.

Disability means a substantial disruption of a person's ability to conduct normal life's functions.

Important Medical Event: Any adverse event may be considered serious because it may jeopardize the subject and may require intervention to prevent another serious condition.

Any Death (regardless of cause) that occurs from the time of administration of the first dose of protocol therapy until 30 days after the final administration of the study drug, and any death occurring after this time that is judged at least possibly related to prior treatment with the study drug, will be promptly reported.

Overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

All SAEs must be recorded on eCRFs. All SAEs, whether **related or not related** to study drug, that are thought to be associated with protocol-specified procedures must be collected and reported using SAE Form to Ozmosis within 24 hours of becoming aware of the event.

Pregnancies occurring in study subjects/sexual partner(s) will be treated as per standard procedure for SAEs. Pregnancies occurring in study subjects or their sexual partner(s) after study drug treatment should be reported separately on Pregnancy Report Form and the subject must discontinue the protocol treatment.

12.4 Reporting Serious Adverse Events

All serious adverse events must be reported by using the SAE form and must be submitted to *Ozmosis Research Inc*.

Serious Adverse Event Reporting Instructions

All serious adverse events must be reported as follows:

Within 24 hours: Report initial information by fax or email to:

Ozmosis Research Inc.
[redacted]

The initial information should always contain:

- Name of Reporter/Investigator
- Subject Identification
- Adverse Event Term
- Study Drug Dose and Start/Stop Dates

On the next working day: Fax completed trial-specific Serious Adverse Event form.

12.5 Procedures for Expedited Reporting

12.5.1 Responsibility for Reporting Serious Adverse Events to Regulatory Authorities

SAEs will be submitted to Regulatory Authorities according to the applicable guidelines and regulations within that country. The Hospital for Sick Children will provide expedited reports of SAEs to Health Canada (including the 7-day notification for death and life-threatening events), i.e. events which are BOTH serious AND unexpected, AND which are thought to be possibly related to protocol treatment (or for which a causal relationship with protocol treatment cannot be ruled out).

The CRO responsible for monitoring sites outside of Canada will be responsible to submitting SAEs to that local Regulatory Authority as per local guidelines and regulations.

12.5.2 Responsibility for Reporting Serious Adverse Events to Drug Provider

Ozmosis Research Inc. will be responsible for submitting SAE reports (Initial and/or Follow-up reports) to BMS. The SAE must be reported to BMS by Ozmosis Research Inc. within 24 hours of receipt of the SAE report from the sites. The foregoing is applicable to all SAEs, irrespective of causality.

12.5.3 Reporting Serious Adverse Events to Local Research Ethics Boards

Ozmosis Research Inc. will notify all Investigators of all SAEs that are reportable to Regulatory Authorities from this trial or from other clinical trials as reported to BMS. This includes all serious events that are unexpected and related to protocol treatment. Investigators must notify their ethics board/committee according to institutional requirements and file the report with their Investigator Site File. Documentation that SAEs have been reported to ethics board/committee must be kept on file at the CRO as applicable.

Documentation can be any of the following:

- a letter from the REB acknowledging receipt;
- a stamp from the REB, signed and dated by REB chair, acknowledging receipt;

- a letter demonstrating the SAE was sent to the board.

All expedited serious adverse events occurring within a centre should also be reported to local ethics board/committees.

12.6 Follow up on Adverse and Serious Adverse Events

For the SAEs that have been deemed by the investigator as unrelated to protocol treatment, the SAE reporting period begins on the first date of treatment and ends on the date of Safety Visit or 100 days after discontinuation of the study drug, whichever is later. For the SAEs that have been deemed by the investigator as at least possibly related to protocol treatment, the SAE must be reported even if this occurs after the date of scheduled Safety Visit or past 30 days after discontinuation of the study drug.

The investigator shall provide follow-up information in a new follow-up SAE form. All SAEs must be followed until resolved, become chronic, or stable unless the patient is lost to follow up. Resolution status of such an event should be documented on the eCRF.

The eCRF should capture all AEs occurring from cycle 1 day 1 till the date of Safety Visit or 100 days after discontinuation of the study drug, whichever is later.
In addition, any known untoward event of any severity that occurs subsequent to the AE reporting period that the Investigator assesses as at least possibly related to the protocol therapy (i.e., the relationship cannot be ruled out) should also be reported as an AE.

12.7 Relationships

For all AEs, relationship to study drug will be reported on the appropriate AE eCRF page. The Investigator must judge whether the study drug caused or contributed to the AE in which case it is considered to be an adverse drug reaction (ADR), and report it as either:

1. Related (definitely, probably or possibly)
    - There is a reasonable possibility that the study drug caused or contributed to the AE.

- This conclusion may be supported by the following observations, though these are not required for the determination of relatedness.

- There is a plausible time sequence between onset of the AE and study drug administration.
- There is a plausible biological mechanism through which study drug may have caused or contributed to the AE.

2. Not related (unlikely, unrelated)

- It is highly unlikely or impossible that the study drug caused or contributed to the AE.
    - This conclusion may be supported by the following observations, though these are not required for a determination of not related:
- Another cause of the AE is evident and most plausible.
- The temporal sequence is inconsistent between the onset of the AE and study drug administration; a causal relationship is considered biologically implausible.

13. PROCEDURES FOR DISCONTINUATION OF A PATIENT FROM INVESTIGATIONAL PRODUCT

13.1 Procedures for Withdrawal from Study

Patient / Legally Acceptable Representative (LAR; such as a parent or guardian, as applicable) are at any time free to withdraw from study (study medication and assessments), without prejudice to further treatment (withdrawal of consent). Such patients and/or their legal guardians will always be asked about the reason(s) and the presence of any adverse events. If possible, they will be seen and assessed by an investigator. Adverse events will be followed up.

Withdrawn patients who meet the criteria to be evaluable for objective response or non-target response as per *Section 10.2.1.2* will not be replaced. Patients who withdraw from the study prior to meeting the criteria to be evaluable in *Section 10.2.1.2* will be replaced on study.

If a patient does not return for a scheduled visit, every effort should be made to contact the patient and/or their legal guardians. In any circumstance, every effort should be made to document patient outcome and reason for withdrawal, if possible. The investigator should request the patient return for a final visit, if applicable and follow-up with the patient regarding any unresolved adverse events.

If the patient and/or their legal guardians withdraw from the trial and also withdraw consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The Study Chair or Co-Chair may retain and continue to use any data collected before such withdrawal of consent.

13.2 Premature Withdrawal/Discontinuation Criteria

1. Clinical (including physical examination or serum tumour markers) or radiographic evidence of progressive disease > 12 weeks after start of protocol therapy.
2. Adverse Events requiring removal from protocol therapy (See throughout *Section 7*).
3. Patient and/or legal guardian's choice to withdraw from treatment (follow-up permitted by patient and/or legal guardians).
4. Patient and/or legal guardian choice to withdraw consent to study (cessation of follow-up).

5. Non-compliance that in the opinion of the investigator does not allow for ongoing participation.
6. Need for anti-cancer therapy not specified in the protocol.
7. Patient lost to follow-up.
8. Physician determines it is not in the patient's best interest.
9. Repeated eligibility laboratory studies (CBC with differential, bilirubin, ALT (SGPT) or serum creatinine) are outside the parameters required for eligibility prior to the start of protocol therapy (See *Section 8.2*).
10. Study is terminated by Sponsor.
11. Pregnancy.

Patients who are removed from protocol therapy should continue to have the required observations until the originally planned end of the cycle or until all adverse events have resolved whichever happens later. The only exception is with documentation of the patient's withdrawal of consent. The reason for study removal and the date the patient was removed must be documented in the electronic Case Report Form.

13.3 Off Study Criteria

1. 30 days after the last dose of the investigational drug.
2. Death.
3. Lost to follow-up.
4. Withdrawal of consent for any further required observations or data submission.
5. Enrollment onto another therapeutic (anti-cancer) study.

14. ETHICS

14.1 Informed Consents

Patient / Legally Acceptable Representative (LAR; such as a parent or guardian, as applicable) consent/assent must be obtained according to local Institutional and/or University Human Experimentation Committee requirements prior to any study-specific procedures. It will be the responsibility of the local participating investigator to obtain the necessary clearance, and to indicate in writing to the CRO that such clearance has been obtained, before the trial can commence at that centre. Sample consent forms (in the language of majority for the applicable country and/ or other languages deemed necessary by the local PI) for the trial will be provided. A copy of the initial full board ethics approval and approved consent form must be sent to the CRO.

14.2 Ethics Board Approval

Each participating centre will have on file with the CRO, a list indicating the composition of its ethics board/committee consistent with applicable regulatory guidelines. This list will be updated as appropriate.

For Canadian sites, a Health Canada, REB Attestation Form must be completed and signed by the REB representative. Alternatively, an attestation may be included in the signed local ethics approval document. This documentation must be received by the CRO before the centre can be locally activated.

Initial approval: All study sites are required to obtain full board local ethics approval of the protocol and consent form by the appropriate ethics board/committee prior to commencement of the clinical trial at each site.

Continuing approval: Annual (or as required by the ethics board/committee) re-approval may be required for as long as patients are being followed on protocol. It will be investigator's responsibility to apply for and obtain the re-approval.

Amendment: All protocol amendments will be confirmed in writing and submitted, as appropriate, for review by the ethics board/committees and health authorities. Amendments will be reviewed and approved by applicable regulatory authorities prior to central implementation of the amendment, and by ethics board/committees prior to local implementation, EXCEPT when the

amendment eliminates an immediate hazard to clinical trial patients or when the change(s) involves only logistical or administrative aspects of the trial.

Ethics board/Committees Refusals: If an ethics board/committee refuses to approve this protocol (or an amendment/revision to this protocol), the CRO must be notified immediately of the date of refusal and the reason(s) for the refusal. Notification will then be made to Health Canada.

Serious Adverse Events, Safety Updates and Investigator Brochure Updates: During the course of the study serious adverse events, safety updates or investigator brochure updates may be sent to you for reporting to your ethics board/committee. If/when this occurs, documentation of ethics board/committee submission of this information must be forwarded to the CRO.

15. DOCUMENTATION, RECORD ACCESS AND MAINTENANCE OF STUDY RECORDS

15.1 Documentation of Patients Participation

A statement acknowledging the participation of a patient in this clinical trial must be documented in the patient's medical records. Sites will file the patient's signed ICF.

15.2 Regulatory Requirements

All regulatory documents as required by each country will be required to be completed and submitted to the CRO.

15.3 Patient Confidentiality and Access to Source Data

The results of the TMB assay and/ or diagnostics assays for RRD syndromes obtained during **Part I** may be disclosed with his/her consent to his/her health care providers for the purpose of obtaining appropriate medical care. This information will be relayed back to the patient, their parents/ legal guardian, their attending local physician and the clinical trial team prior to consent and enrollment in **Part II (Treatment and Companion Biomarker Studies).** All information about the patient will remain confidential to all other personnel. Any research information obtained about the patient will be kept entirely confidential. Once deemed ineligible or enrolled in **Part II**, all patient information obtained will be de-identified. A patient will not be identified by name, only by his/her unique patient ID study number. The patient's name or any identifying information will not appear in any reports published as a result of this study.

However, information obtained from individual patient's participation in the study may be disclosed with his/her consent to the health care providers for the purpose of obtaining appropriate medical care. The patient's medical records/charts, tests will be made available to Ozmosis Research Inc., The Hospital for Sick Children, its potential eventual partners, the Canadian HPFB/TPD, the REB/IRB and any other regulatory authorities. This is for the purpose of verifying information obtained for this study. Confidentiality will be maintained throughout the study within the limits of the law.

A patient's name will not be given to anyone except the researchers conducting the study, who have pledged an oath of confidentiality. All identifying information will be kept

confidentially, under the supervision of the study Principal Investigator and will not be transferred outside of the local site hospital and The Hospital for Sick Children.

A patient may take away his/her permission to collect, use and share information about him/her at any time. If this situation occurs, the patient will not be able to remain in the study. No new information that identifies the patient will be gathered after that date. However, the information about the patient that has already been gathered and transferred may still be used and given to others as described above in order to preserve the scientific integrity and quality of the study.

15.4 Confidentiality of the Study

Data generated as a result of this study are to be available for inspection on request by local health authority auditors, the Sponsor's Study Monitors and other personnel (as appropriate) and by the ethics board/committee. The Investigator shall permit the Sponsor, authorized agents of the Sponsor, the CRO and regulatory agency employees to enter and inspect any site where the drug or records pertaining to the drug are held, and to inspect all source documents. The protocol and other study documents contain confidential information and should not be shared or distributed without the prior written permission of the Study Chair or Co-chair.

15.5 Registration of Clinical Trial

Prior to the first patient being registered/enrolled into this study, the Sponsor will be responsible for ensuring that the clinical trial is registered and maintain up to date registration appropriately to remain eligible for publication in any major peer-reviewed journal, adhering to the guidelines put forth by the International Committee of Medical Journal Editors (ICMJE).

15.6 Data Reporting

The data will be collected using electronic CRFs (eCRFs) and analyzed after entry into the Study Database.

15.7 Case Report Forms

The CRFs for the study will be electronic and will be provided by the CRO. Please see the study specific eCRF completion guidelines which have been provided to your site by the CRO. The timelines and details for submission of CRFs are included in these guidelines.

15.8 Maintenance of Study Records

To enable evaluations and/or audits from Regulatory Authorities, the CRO or the Sponsor, the Investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records, CRFs and hospital records), all original signed informed consent forms, source documents, and detailed records of treatment disposition.

Each site will retain study records for the duration as required by the country's regulations or as specified in the Clinical Trial Agreement, whichever is longer.

If the investigator relocates, retires, or for any reason withdraws from the study, then the Sponsor should be prospectively notified. The study records must be transferred to an acceptable designee, such as another investigator, another institution, or to the Sponsor. The investigator must obtain the Sponsor's written permission before disposing of any records.

16. QUALITY ASSURANCE AND QUALITY CONTROL

Each site is responsible for conducting the trial at their site in accordance with International Conference on Harmonization Good Clinical Practice (GCP). As per the Guidelines of Good Clinical Practice (CPMP/ICH/135/95), the Sponsor will be responsible for implementing and maintaining quality assurance and quality control systems.

16.1 On Site Monitoring/Auditing

The CRO will organize on-site monitoring of this study to be conducted at each site depending on accrual and as per the monitoring plan.

Sites may be patient to an inspection by the regulatory authorities of the countries where this trial has been submitted to. Other audits may be conducted by the study Sponsor and/or its representatives, the CRO and/or the company supplying the drug for the study.

17. ADMINISTRATIVE PROCEDURES

17.1 Amendments to the Protocol

Modifications of the signed protocol are only possible by approved protocol amendments authorized by the Sponsor. All protocol amendments will be approved by the ethics board/committee prior to implementation. The Investigator must not implement any deviation from, or change to the protocol, except where it is necessary to eliminate an immediate hazard to trial patient or when the change(s) involves only logistical or administrative aspects of the trial.

17.2 Protocol Deviations/Violations

All violations or deviations are to be reported to the site's ethics board/committee as per ethics board/committee guidelines). All ethics board/committee correspondence is to be forwarded to the CRO. The site must notify the CRO and/or Study Chair or Co-Chair immediately of any protocol violations.

17.3 Premature Discontinuation of the Study

The Sponsor reserves the right to discontinue the trial for any reason but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigators must contact all participating patients immediately after notification. Standard therapy and follow-up for patients will be assured and, where required by the applicable regulatory requirement(s), the relevant regulatory authority(ies) will be informed.

The ethics board/committees will be informed promptly and provided with a detailed written explanation for the termination or suspension. As directed by the Study Chair or Co-Chair, all study materials must be collected and all eCRFs completed to the greatest extent possible.

18. LEGAL ASPECTS

18.1 Publication Policies and Disclosure of Data

For publications, authorship will be determined by the Study Chair and Co-Chair. Additional authors will be those who have made the most significant contribution to the overall success of the study. This contribution will be assessed, in part but not entirely, in terms of patients enrolled and will be reviewed at the time of publication and/ or end of the trial by the Study Chair or Co-Chair.

19. APPENDIX I: PERFORMANCE SCALES

Karnofsky		Lansky	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease.	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly.
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

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20. APPENDEX II: CONTRACEPTION METHODS

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION

1. Male condoms with spermicide
2. Hormonal methods of contraception including combined oral contraceptive pills, vaginal ring, injectables, implants and intrauterine devices (IUDs) such as Mirena used by WOCBP patient or male patient's WOCBP partner; Female partners of male patients participating in the study may use hormone based contraceptives as one of the acceptable methods of contraception since they will not be receiving study drug
3. Non-hormonal IUDs, such as ParaGard
4. Tubal ligation
5. Vasectomy
6. Complete Abstinence*

**Complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Patients who choose complete abstinence are not required to use a second method of contraception. Acceptable alternate methods of highly effective contraception must be discussed in the event that the patient chooses to forego complete abstinence.*

21. APPENDIX III: TANNER STAGING

Male puberty stage:

Stages Description:

Stage 1 Preadolescent. Testes, scrotum, and penis are about the same size and proportion as those in early childhood.

Stage 2 Scrotum and testes have enlarged, and there is a change in the texture of scrotal skin and some reddening of scrotal skin.

Stage 3 Growth of the penis has occurred, at first mainly in length but with some increase in breadth. There has been further growth of the testes and the scrotum.

Stage 4 The penis is further enlarged in length and breadth, with development of glans. The testes and the scrotum are further enlarged. There is also further darkening of scrotal skin.

Stage 5 Genitalia are adult in size shape. No further enlargement takes place after stage 5 is reached.

Female puberty stage:

Stages Description:

Stage 1 Preadolescent; only papillae are elevated.

Stage 2 Breast bud and papilla are elevated, and a small amount is present; areola diameter is enlarged.

Stage 3 Further enlargement of breast mound, increased palpable glandular tissue.

Stage 4 Areola and papilla are elevated to form a second mound above the level of the rest of the breast.

Stage 5 Adult mature breast; recession of areola to the mound of breast tissue, rounding of the breast mound, and projection of only the papilla are evident.

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