

**PBTC-045:** A Safety and Preliminary Efficacy trial of MK-3475 (pembrolizumab; anti-PD-1) in children with recurrent, progressive or refractory high-grade gliomas (HGG), DIPGs and hypermutated brain tumors

### **Abstract and Schema**

This is a two phase safety and preliminary efficacy study. The first phase is a safety study to assess the dose limiting toxicities and define a recommended phase II dose (RP2D) for MK3475 (pembrolizumab). In the safety phase of the study, a cohort of up to 6 eligible patients per stratum will be enrolled initially at the starting dose level (2mg/kg q3 weeks) which is the recommended dose in adults. The first two courses (first 6 weeks) will be used to as the safety evaluation period. Once a dose level is determined to be safe in a given stratum in the first 6 patients, we will begin the efficacy phase of the study with treatment of up to 20 patients per stratum who are evaluable for efficacy.

Patients will enroll in one of three strata:

- Stratum A for patients with progressive, recurrent or refractory DIPGs (**Currently closed to enrollment**)
- Stratum B for patients with progressive, recurrent or refractory non-brainstem HGGs.
- Stratum C for patients with hypermutated brain tumors, including those with conditional mismatch-repair deficiency (CMMRD) syndrome

MK3475 (pembrolizumab) is supplied as an intravenous (IV) formulation. The starting dose level for this study is 2mg/kg given every 3 weeks (dose level 1). Patients will receive MK3475 (pembrolizumab). Intravenously at a dose based on their assigned dose level on day 1 of each course. Treatment can be administered on an outpatient basis. Each course will be 3 weeks (21 days) in length. Subsequent courses will immediately follow with no break in the absence of toxicity or disease progression. Therapy may continue for 34 courses (approximately 2 years) in the absence of significant toxicity or disease progression.

The primary endpoints for the first phase of the study will be toxicity and safety monitoring of MK3475 (pembrolizumab), and for the second phase of the study will be to estimate the sustained objective response for at least 9 weeks.

Potential biomarkers will be measured and correlated to outcome, including PD-L1 and PD-1 tumor expression, patient immunophenotype, cytokine expression profiles, RNA signature profile, and tumor gene expression profile (all tumor-derived studies will be completed in non-brainstem HGG patients only). These possible markers will define the immunologic phenotype of each patient, the response of the systemic immune system to PD-1 blockade, and potential tumor-derived predictors of response.

Quantitative MR spectroscopy and diffusion-weighted imaging will be obtained to attempt early prediction of tumor response and differentiation between pseudoprogression and true progressive disease. Serial MR permeability and MR perfusion will also be obtained to determine if elevated rCBV and ktrans can distinguish pseudoprogression/tumor inflammation from progression.

### **3. PATIENT SELECTION**

All subjects with the diagnosis of DIPG must meet the enrollment criteria in Sections 3.1 and 3.2. All subjects with the diagnosis of NB-HGG must meet the enrollment criteria in Sections 3.3 and 3.4.. Subjects with hypermutated brain tumors must meet the enrollment criteria noted in Sections 3.5 and 3.6. No exceptions will be given. Imaging studies to establish eligibility must be done within three (3) weeks prior to enrollment. All other clinical evaluations to establish eligibility must be done within 7 days prior to enrollment.

#### **3.1 Inclusion Criteria for DIPG (Stratum A– currently closed to accrual)**

##### **3.1.1 Tumor**

A diffuse intrinsic pontine glioma (DIPG) that is recurrent, progressive or refractory. Histologic diagnosis is not required for patients with typical imaging findings of DIPG (defined as patients with a diffuse expansile mass centered in and involving at least 2/3 of the pons.) Patients with brainstem tumors who have undergone biopsy with a diagnosis of high-grade glioma or diffuse infiltrating glioma are also eligible

##### **3.1.2 Available Pre-trial Tumor Tissue:**

Patients with DIPG who have tissue available are requested to submit similar tissue as patients with NB-HGG; however, this is not required for eligibility.

##### **3.1.3 Disease status:**

All subjects must have measurable disease in 2-dimensions on MRI scan of the brain. Disease should be consistently measured with the two largest perpendicular dimensions.

##### **3.1.4 Age**

Patient must be  $\geq 1$  but  $\leq 18$  years of age at the time of enrollment during the safety portion. Patients  $< 22$  may be enrolled during the efficacy portion of the study.

##### **3.1.5 Prior Therapy**

Patients must have received prior radiation therapy and/or chemotherapy and recovered from the acute treatment related toxicities (defined as  $< \text{grade } 1$  if not defined in eligibility criteria) of all prior chemotherapy, immunotherapy or radiotherapy prior to entering this study. There is no upper limit to the number of prior therapies that is allowed.

##### **3.1.5.1 Myelosuppressive chemotherapy:**

Patients must have received their last dose of known myelosuppressive anticancer therapy at least three (3) weeks prior to study enrollment or at least six (6) weeks if prior nitrosourea.

##### **3.1.5.2 Investigational/Biologic Agent:**

- **Biologic or investigational agent (anti-neoplastic):**

Patient must have received their last dose of the investigational or biologic agent  $\geq 7$  days prior to study enrollment.

- 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 must be discussed with and approved by the study chair.

- Monoclonal antibody treatment and/or agents with prolonged half-lives: At least three half-lives must have elapsed prior to enrollment.

Note: A list of commonly used agents with prolonged half-lives is available on the PBTC webpage under Generic Forms and Templates.

#### 3.1.5.3 Immunotherapy:

Patient must have completed immunotherapy (e.g. tumor vaccines, oncolytic viruses. etc.) at least 42 days prior to enrollment.

#### 3.1.5.4 Radiation

Patients must have had their last fraction of:

- Craniospinal irradiation  $\geq 3$  months prior to enrollment.
- Other substantial bone marrow irradiation  $\geq 6$  weeks prior to enrollment
- Local palliative XRT (small port)  $\geq 2$  weeks

#### 3.1.5.5 Bone Marrow Transplant

Patient must be:

$\geq 12$  weeks since autologous bone marrow/stem cell transplant prior to enrollment

#### 3.1.6 Surgery

Patients must be fully recovered from all acute effects of prior surgical intervention.

#### 3.1.7 Inclusion of Women and Minorities

Both males and females of all races and ethnic groups are eligible for this study.

#### 3.1.8 Neurologic Status

Patients with neurological deficits should have deficits that are completely stable for a minimum of 1 week (7 days) prior to enrollment.

#### 3.1.9 Performance Status

Karnofsky Performance Scale (KPS for  $> 16$  years of age) or Lansky Performance Score (LPS for  $\leq 16$  years of age) assessed within two weeks of enrollment must be  $\geq 70$ . Patients who are unable to walk because of neurologic deficits, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

#### 3.1.10 Organ Function

Patients must have adequate organ and marrow function as defined below:

- Absolute neutrophil count  $> 1.0 \times 10^9$  cells/L
- Platelets  $> 100,000/ \text{mm}^3$  (unsupported, defined as no platelet transfusion within 7 days)
- Hemoglobin  $\geq 8 \text{g/dl}$  (may receive transfusions)
- Total bilirubin  $\leq 1.5$  times institutional upper limit of normal (ULN)
- ALT(SGPT)  $< 3 \times$  institutional upper limit of normal
- Albumin  $\geq 2 \text{g/dl}$

- Serum creatinine based on age/gender as noted in Table 3. Patients that do not meet the criteria below but have a 24hour Creatinine Clearance or GFR (radioisotope or iothalamate)  $\geq$  70 ml/min/1.73 m<sup>2</sup> are eligible.

Table 3

Serum Creatinine for Age/Gender		
Age	Maximum Serum Creatinine (mg/dl)	
	Male	Female
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
$\geq$ 16 years	1.7	1.4
The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.		

- Pulmonary function - Pulse oximetry > 93% on room air and no evidence of dyspnea at rest.

#### 3.1.11 Growth Factors

Patients must be off all colony-forming growth factor(s) for at least 1 week prior to registration (i.e. filgrastim; sargramostim; erythropoietin). 2 weeks must have elapsed for long-acting formulations.

#### 3.1.12 Steroid Use

Patients must be willing to use brief courses (at least 72 hours) of steroids as directed for potential inflammatory side effects of the therapy if recommended by their treating physician.

#### 3.1.13 Pregnancy Status

Female subjects of childbearing potential must not be pregnant or breast-feeding. Female patients of childbearing potential must have a negative serum or urine pregnancy test within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

Pregnant women are excluded from this study because MK-3475 (pembrolizumab) is an agent with the potential for teratogenic effects. Because there is unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MK-3475 (pembrolizumab), breastfeeding should be discontinued if the mother is to be treated with MK-3475 (pembrolizumab).

#### 3.1.14 Pregnancy Prevention

Patients of childbearing or child fathering potential must be willing to use 2 methods of birth control or be surgically sterile or abstain from heterosexual activity while being treated on this study and for 4 months after the last dose of study medication.

### 3.1.15 Informed Consent

The patient or parent/guardian is able to understand the consent and is willing to sign a written informed consent document, inclusive of assent where appropriate, according to institutional guidelines.

## 3.2 Exclusion Criteria for DIPG (Stratum A)

### 3.2.1 Concurrent Illness

- Patients with active autoimmune disease or documented history of autoimmune disease/syndrome that requires ongoing systemic steroids or systemic immunosuppressive agents, except
  - Patients with vitiligo or resolved asthma/atopy
  - Patients with hypothyroidism stable on hormone replacement or Sjogren's syndrome
- History of or ongoing pneumonitis or significant interstitial lung disease
- Note: This would include non-infectious pneumonitis that required steroid use.
- Patients with any clinically significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction), that in the opinion of the investigator would compromise the patient's ability to tolerate protocol therapy, put them at additional risk for toxicity or would interfere with the study procedures or results.
- Patients with other malignancies.
- Patients with known hypermutated brain tumors including those with CMMRD and Lynch syndrome are ineligible for enrollment in Stratum A.
- Patients who have received a solid organ transplant.

### 3.2.2 Presence of Bulky Tumor

Patients with bulky tumor on imaging are ineligible. Bulk tumor is defined as:

- Tumor with any evidence of uncal herniation or midline shift
- Tumor with diameter of >4cm in one dimension on T2/FLAIR
- Tumor that in the opinion of the site investigator, shows significant mass effect
- Multi-focal tumor is defined as patients with metastatic disease or a clearly dis-contiguous second focus of disease. However, a second focus of enhancement in a single FLAIR abnormality is permissible and will not exclude the subject

Treating physicians are encouraged to contact the Study Chair to request a rapid central imaging review to confirm fulfilment of these eligibility criteria, if they have concerns.

### 3.2.3 Concurrent therapy

Patients who are receiving any other anti-cancer or investigational drug therapy are ineligible.

### 3.2.4 Infectious Diseases

Patients who have a known active Hepatitis B or Hepatitis C infection are ineligible. Patient must have documented evidence of negative tests for the presence of Hepatitis B surface antigen and Hepatitis C (anti-HCV antibody OR Hep C RNA-qualitative). HIV-positive patients are eligible if the following criteria are met:

- Stable on their antiretroviral agents
- Have CD4 counts above 400
- Undetectable viral loads, and

- No need for prophylactic medications for an opportunistic infections

### 3.2.5 Recent Live Vaccination

Patients who have received the last vaccination of a live vaccine  $\leq 30$  days prior to enrollment are ineligible. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and must meet timeline for live vaccine.

### 3.2.6 Allergy

Patients with a history severe ( $\geq$ Grade 3) hypersensitivity reaction to a monoclonal antibody are ineligible.

### 3.2.7 Prior Therapy

Patients who have received previous therapy with an anti-CTLA4, anti-CD137, anti-PD-L1 or anti-PD-1 antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).

### 3.2.8 Seizures

Patients with uncontrolled seizures defined as seizures that require regular use of rescue medications or in the opinion of the investigator require increasing doses of antiepileptic medications or would compromise the ability to tolerate study therapy or interfere with protocol therapy or procedures. Patients with seizures that are well controlled are eligible and may be on antiepileptic medications if on a stable dose.

### 3.2.9 Concomitant Medications

Patients may not be on immunosuppressive therapy, including corticosteroids (with the exception of physiologic replacement, defined as 0.75mg/m<sup>2</sup>/day) at time of enrollment. However, patients who require intermittent use of bronchodilators or local steroid injections will not be excluded from the study. Please see section 5.3 for a list of acceptable and unacceptable concomitant medications as well as reporting requirements.

## 3.3 Inclusion Criteria for NB-HGG (Stratum B)

### 3.3.1 Tumor

Patients must have a histologically confirmed diagnosis of a non-brainstem high-grade glioma that is recurrent, progressive or refractory. Spinal primary disease is eligible.

### 3.3.2 Available Pre-trial Tumor Tissue:

Patients with non-brainstem high grade glioma must have adequate pre-trial FFPE tumor material available for use in the Biology studies and genome wide sequencing (Section 9.2, 9.3 and 9.5). While tissue is required for PD-1, PD-L1 and immunophenotyping, patients will be deemed eligible for the study with a minimum of 10 unstained slides for the PD-1/PD-L1 immunohistochemical analysis.

### 3.3.3 Disease status:

All subjects must have measurable disease in 2-dimensions on MRI scan of the brain and/or spine. Disease should be consistently measured with the two largest perpendicular dimensions.

### 3.3.4 Age

Patient must be  $\geq 1$  but  $\leq 18$  years of age at the time of enrollment during the safety portion. Patients  $< 22$  may be enrolled during the efficacy portion of the study.

### 3.3.5 Prior Therapy

Patients must have received prior radiation therapy and/or chemotherapy and recovered from the acute treatment related toxicities (defined as  $<$  grade 1 if not defined in eligibility criteria) of all prior chemotherapy, immunotherapy or radiotherapy prior to entering this study. There is no upper limit to the number of prior therapies that is allowed.

#### 3.3.5.1 Myelosuppressive chemotherapy:

Patients must have received their last dose of known myelosuppressive anticancer therapy at least three (3) weeks prior to study enrollment or at least six (6) weeks if prior nitrosourea.

#### 3.3.5.2 Investigational/Biologic Agent:

- Biologic or investigational agent (anti-neoplastic):

Patient must have received their last dose of the investigational or biologic agent  $\geq 7$  days prior to study enrollment.

- 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 must be discussed with and approved by the study chair.
- Monoclonal antibody treatment and/or agents with prolonged half-lives: At least three half-lives must have elapsed prior to enrollment.

Note: A list of commonly used agents with prolonged half-lives is available on the PBTC webpage under Generic Forms and Templates.

#### 3.3.5.3 Immunotherapy:

Patient must have completed immunotherapy (e.g. tumor vaccines, oncolytic viruses. etc.) at least 42 days prior to enrollment.

#### 3.3.5.4 Radiation

Patients must have had their last fraction of:

- Craniospinal irradiation  $\geq 3$  months prior to enrollment.
- Other substantial bone marrow irradiation  $\geq 6$  weeks prior to enrollment
- Local palliative XRT (small port)  $\geq 2$  weeks

#### 3.3.5.5 Bone Marrow Transplant

Patient must be:

$\geq 12$  weeks since autologous bone marrow/stem cell transplant prior to enrollment

#### 3.3.5.6 Surgery

Patients must be fully recovered from all acute effects of prior surgical intervention.

### 3.3.6 Inclusion of Women and Minorities

Both males and females of all races and ethnic groups are eligible for this study

### 3.3.7 Neurologic Status

Patients with neurological deficits should have deficits that are completely stable for a minimum of 1 week (7 days) prior to enrollment.

### 3.3.8 Performance Status

Karnofsky Performance Scale (KPS for > 16 years of age) or Lansky Performance Score (LPS for ≤ 16 years of age) assessed within two weeks of enrollment must be ≥ 70. Patients who are unable to walk because of neurologic deficits, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

### 3.3.9 Organ Function

Patients must have adequate organ and marrow function as defined below:

- Absolute neutrophil count >1.0 x 10<sup>9</sup> cells/L
- Platelets >100,000/ mm<sup>3</sup> (unsupported, defined as no platelet transfusion within 7 days)
- Hemoglobin ≥8g/dl (may receive transfusions)
- Total bilirubin ≤1.5 times institutional upper limit of normal (ULN)
- ALT(SGPT) <3 x institutional upper limit of normal
- Albumin ≥2 g/dl
- Serum creatinine based on age/gender as noted in Table 4. Patients that do not meet the criteria below but have a 24hour Creatinine Clearance or GFR (radioisotope or iothalamate) ≥ 70 ml/min/1.73 m<sup>2</sup> are eligible.

Table 4

Serum Creatinine for Age/Gender		
	Maximum Serum Creatinine (mg/dl)	
Age	Male	Female
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

- Pulmonary function - Pulse oximetry > 93% on room air and no evidence of dyspnea at rest.

### 3.3.10 Growth Factors

Patients must be off all colony-forming growth factor(s) for at least 1 week prior to registration (i.e. filgrastim; sargramostim; erythropoietin). 2 weeks must have elapsed for long-acting formulations.

### 3.3.11 Steroid Use

Patients must be willing to use brief courses (at least 72 hours) of steroids as directed for potential inflammatory side effects of the therapy if recommended by their treating physician.

### 3.3.12 Pregnancy Status

Female subjects of childbearing potential must not be pregnant or breast-feeding. Female patients of childbearing potential must have a negative serum or urine pregnancy test within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

Pregnant women are excluded from this study because MK-3475 (pembrolizumab) is an agent with the potential for teratogenic effects. Because there is unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MK-3475 (pembrolizumab), breastfeeding should be discontinued if the mother is to be treated with MK-3475 (pembrolizumab).

### 3.3.13 Pregnancy Prevention

Patients of childbearing or child fathering potential must be willing to use 2 methods of birth control or be surgically sterile or abstain from heterosexual activity while being treated on this study and for 4 months after the last dose of study medication.

### 3.3.14 Informed Consent

The patient or parent/guardian is able to understand the consent and is willing to sign a written informed consent document, inclusive of assent where appropriate, according to institutional guidelines.

## 3.4 Exclusion Criteria for NB-HGG (Stratum B)

### 3.4.1 Concurrent Illness

- Patients with active autoimmune disease or documented history of autoimmune disease/syndrome that requires ongoing systemic steroids or systemic immunosuppressive agents, except
  - Patients with vitiligo or resolved asthma/atopy
  - Patients with hypothyroidism stable on hormone replacement or Sjogren's syndrome

- History of or ongoing pneumonitis or significant interstitial lung disease

Note: This would include non-infectious pneumonitis that required steroid use.

- Patients with any clinically significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction), that in the opinion of the investigator would compromise the patient's ability to tolerate protocol therapy, put them at additional risk for toxicity or would interfere with the study procedures or results.
- Patients with other malignancies.
- Patients with known hypermutated brain tumors including those with CMMRD and Lynch syndrome are ineligible for enrollment in Stratum B.
- Patients who have received a solid organ transplant.

### 3.4.2 Presence of Bulky Tumor

Patients with bulky tumor on imaging are ineligible. Bulk tumor is defined as:

- Tumor with any evidence of uncal herniation or midline shift
- Tumor with diameter of >4cm in one dimension on T2/FLAIR
- Tumor that in the opinion of the site investigator, shows significant mass effect
- Multi-focal tumor is defined as patients with metastatic disease or a clearly dis-contiguous second focus of disease. However, a second focus of enhancement in a single FLAIR abnormality is permissible and will not exclude the subject

Treating physicians are encouraged to contact the Study Chair to request a rapid central imaging review to confirm fulfilment of these eligibility criteria, if they have concerns.

#### 3.4.3 Concurrent therapy

Patients who are receiving any other anti-cancer or investigational drug therapy are ineligible.

#### 3.4.4 Infectious Diseases

Patients who have a known active Hepatitis B or Hepatitis C infection are ineligible. Patient must have documented evidence of negative tests for the presence of Hepatitis B surface antigen and Hepatitis C (anti-HCV antibody OR Hep C RNA-qualitative). HIV-positive patients are eligible if the following criteria are met:

- Stable on their antiretroviral agents
- Have CD4 counts above 400
- Undetectable viral loads, and
- No need for prophylactic medications for an opportunistic infections

#### 3.4.5 Recent Live Vaccination

Patients who have received the last vaccination of a live vaccine  $\leq 30$  days prior to enrollment are ineligible. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and must meet timeline for live vaccine.

#### 3.4.6 Allergy

Patients with a history severe ( $\geq$ Grade 3) hypersensitivity reaction to a monoclonal antibody are ineligible.

#### 3.4.7 Prior Therapy

Patients who have received previous therapy with an anti-CTLA4, anti-CD137, anti-PD-L1 or anti-PD-1 antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).

#### 3.4.8 Seizures

Patients with uncontrolled seizures defined as seizures that require regular use of rescue medications or in the opinion of the investigator require increasing doses of antiepileptic medications or would compromise the ability to tolerate study therapy or interfere with protocol therapy or procedures. Patients with seizures that are well controlled are eligible and may be on antiepileptic medications if on a stable dose.

### 3.4.9 Concomitant Medications

Patients may not be on immunosuppressive therapy, including corticosteroids (with the exception of physiologic replacement, defined as 0.75mg/m<sup>2</sup>/day) at time of enrollment. However, patients who require intermittent use of bronchodilators or local steroid injections will not be excluded from the study. Please see section 5.3 for a list of acceptable and unacceptable concomitant medications as well as reporting requirements.

## 3.5 Inclusion Criteria for Hypermutated brain tumors (Stratum C)

### 3.5.1 Diagnosis of Hypermutated Brain Tumors

Patients with brain tumors and increased tumor mutation burden as determined by

- Confirmed diagnosis of CMMRD syndrome by CLIA-certified germline gene sequencing OR
- Confirmation of high mutation burden by whole genome/exome sequencing performed in a CLIA-certified laboratory and/or the use of Foundation One next generation sequence panel or another CLIA approved targeted sequencing lab with publicly available correlations between number of mutations found in the panel and mutations per megabase and/or genome. For protocol purposes a high mutation burden will be defined as at least 100 non-synonymous coding-region mutations by whole exome/genome sequencing (well above two standard deviations of the number of median similar mutations described in pediatric CNS cancers) AND/OR a high tumor mutation burden (TMB) or intermediate TMB based on the reporting parameters of the panel. TMB parameters provided for the Foundation One reports are high tumor mutation burden is  $\geq 20$  mutations per megabase or intermediate TMB is between 6 to 19 mutations per megabase.
- Confirmed diagnosis of Lynch syndrome by CLIA-certified germline gene sequencing. Patients with Lynch syndrome will not be accounted for in primary objective 1.1.3 unless their tumors are determined to have the minimum number of mutations described above but they will still be eligible for this study.
  - Low-grade tumors in patients with CMMRD or Lynch syndrome do not have to reach the threshold of 100 mutations for study inclusion.

### 3.5.2 Tumor Type

Patients must have a histologically confirmed primary brain tumor that is recurrent, progressive or refractory. Inclusion criteria encompasses all types of brain tumors (e.g. gliomas, embryonal tumors or any other type of brain tumor as long as other eligibility criteria are met.

Of note:

- Patients with high-grade gliomas will be eligible for this clinical trial at least 6 weeks from completion of radiotherapy independent of tumor progression/recurrence as long as they are not enrolled on any other therapeutic clinical trial and there is macroscopic residual disease

Patients with other concomitant tumors associated with CMMRD syndrome including gastrointestinal polyps/adenomas and carcinomas, lymphomas and leukemias will be eligible as long as they are not requiring anticancer therapy directed against these other cancers and meet all other eligibility criteria.

### 3.5.3 Available Pre-trial Tumor Tissue:

Patients must have adequate pre-trial FFPE tumor material available and be willing to provide a blood sample for use in the genome wide sequencing studies (Section 9.5). While tissue is required for Genome-wide sequencing of tumor and germline samples, patients will be deemed eligible for the study with a minimum of approximately 10 unstained slides for the planned analysis.

### 3.5.4 Disease status:

All subjects must have measurable disease in 2-dimensions on MRI scan of the brain and/or spine. Disease should be consistently measured with the two largest perpendicular dimensions.

### 3.5.5 Age

Patient should be <30 years at the time of enrollment.

### 3.5.6 Prior Therapy

Patients must have received prior radiotherapy and/or chemotherapy with the following exceptions:

- Patients with secondary CNS cancers after a previous medical problem/malignancy if they cannot receive full dose of radiotherapy (>50Gy) as long as they meet all other eligibility criteria
- Patients with progressive low-grade gliomas and CMMRD or Lynch syndrome

Patients must have recovered from the acute treatment related toxicities (defined as < grade 1 if not defined in eligibility criteria) of all prior chemotherapy, immunotherapy or radiotherapy prior to entering this study. There is no upper limit to the number of prior therapies that is allowed.

The required intervals for prior therapy are identical to those noted below except for patients with HGG who have not experienced disease progression (Section 3.5.2).

#### 3.5.6.1 Myelosuppressive chemotherapy:

Patients must have received their last dose of known myelosuppressive anticancer therapy at least three (3) weeks prior to study enrollment or at least six (6) weeks if prior nitrosourea.

#### 3.5.6.2 Investigational/Biologic Agent:

- Biologic or investigational agent (anti-neoplastic):  
Patient must have received their last dose of the investigational or biologic agent  $\geq 7$  days prior to study enrollment.
  - 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 must be discussed with and approved by the study chair.
  - Monoclonal antibody treatment and/or agents with prolonged half-lives: At least three half-lives must have elapsed prior to enrollment.

Note: A list of commonly used agents with prolonged half-lives is available on the PBTC webpage under Generic Forms and Templates.

### 3.5.6.3 Immunotherapy:

Patient must have completed immunotherapy (e.g. tumor vaccines, oncolytic viruses. etc.) at least 42 days prior to enrollment.

### 3.5.6.4 Radiation

Patients must have had their last fraction of:

- Craniospinal irradiation  $\geq 3$  months prior to enrollment.
- Other substantial bone marrow irradiation  $\geq 6$  weeks prior to enrollment
- Local palliative XRT (small port)  $\geq 2$  weeks

### 3.5.6.5 Bone Marrow Transplant

Patient must be:

- $\geq 12$  weeks since autologous bone marrow/stem cell transplant prior to enrollment
- $\geq 5$  years since allogeneic bone marrow transplant prior to enrollment with no evidence of active graft vs. host disease.

### 3.5.7 Inclusion of Women and Minorities

Both males and females of all races and ethnic groups are eligible for this study.

### 3.5.8 Neurologic Status

Patients with neurological deficits should have deficits that are completely stable for a minimum of 1 week (7 days) prior to enrollment.

### 3.5.9 Performance Status

Karnofsky Performance Scale (KPS for  $> 16$  years of age) or Lansky Performance Score (LPS for  $\leq 16$  years of age) assessed within two weeks of enrollment must be  $\geq 60$ . Patients who are unable to walk because of neurologic deficits, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

### 3.5.10 Organ Function

Patients must have adequate organ and marrow function as defined below:

- Absolute neutrophil count  $> 1.0 \times 10^9$  cells/L
- Platelets  $> 100,000/ \text{mm}^3$  (unsupported, defined as no platelet transfusion within 7 days)
- Hemoglobin  $\geq 8 \text{g/dl}$  (may receive transfusions)
- Total bilirubin  $\leq 1.5$  times institutional upper limit of normal (ULN)
- ALT(SGPT)  $< 3 \times$  institutional upper limit of normal
- Albumin  $\geq 2 \text{g/dl}$
- Serum creatinine based on age/gender as noted in Table 5. Patients that do not meet the criteria below but have a 24hour Creatinine Clearance or GFR (radioisotope or iothalamate)  $\geq 70 \text{ ml/min/1.73 m}^2$  are eligible.

Table 5

Serum Creatinine for Age/Gender		
	Maximum Serum Creatinine (mg/dl)	
Age	Male	Female
1 to $< 2$ years	0.6	0.6

2 to < 6 years	0.8	0.8
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10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4
The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.		

- Pulmonary function - Pulse oximetry > 93% on room air and no evidence of dyspnea at rest.

### 3.5.11 Growth Factors

Patients must be off all colony-forming growth factor(s) for at least 1 week prior to registration (i.e. filgrastim; sargramostim; erythropoietin). 2 weeks must have elapsed for long-acting formulations.

### 3.5.12 Steroid Use

Patients must be willing to use brief courses (at least 72 hours) of steroids as directed for potential inflammatory side effects of the therapy if recommended by their treating physician.

### 3.5.13 Pregnancy Status

Female subjects of childbearing potential must not be pregnant or breast-feeding. Female patients of childbearing potential must have a negative serum or urine pregnancy test within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

Pregnant women are excluded from this study because MK-3475 (pembrolizumab) is an agent with the potential for teratogenic effects. Because there is unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MK-3475 (pembrolizumab), breastfeeding should be discontinued if the mother is to be treated with MK-3475 (pembrolizumab).

### 3.5.14 Pregnancy Prevention

Patients of childbearing or child fathering potential must be willing to use 2 methods of birth control or be surgically sterile or abstain from heterosexual activity while being treated on this study and for 4 months after the last dose of study medication.

### 3.5.15 Informed Consent

The patient or parent/guardian is able to understand the consent and is willing to sign a written informed consent document, inclusive of assent where appropriate, according to institutional guidelines.

## 3.6 Exclusion criteria for Hypermutated Brain Tumors (Stratum C)

### 3.6.1 Diagnosis

Patients with diffuse intrinsic pontine or other brainstem high-grade glioma and those with primary spinal cord tumors.

### 3.6.2 Presence of Bulky Tumor

Patients with bulky tumor on imaging are ineligible. Bulky tumor is defined as:

- Tumor with any evidence of uncal herniation or midline shift
- Tumor with diameter of >4cm in one dimension on T2/FLAIR
- Tumor that in the opinion of the site investigator, shows significant mass effect
- Multi-focal tumor is defined as patients with metastatic disease or a clearly dis-contiguous second focus of disease. However, a second focus of enhancement in a single FLAIR abnormality is permissible and will not exclude the subject

Treating physicians are encouraged to contact the Study Chair to request a rapid central imaging review to confirm fulfilment of these eligibility criteria, if they have concerns.

### 3.6.3 Concurrent Illness

Patients with active autoimmune disease or documented history of autoimmune disease/syndrome that requires ongoing systemic steroids or systemic immunosuppressive agents, except

- Patients with vitiligo or resolved asthma/atopy
- Patients with hypothyroidism stable on hormone replacement or Sjogren's syndrome
- History of or ongoing pneumonitis or significant interstitial lung disease

Note: This would include non-infectious pneumonitis that required steroid use.

- Patients with any clinically significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction), that in the opinion of the investigator would compromise the patient's ability to tolerate protocol therapy, put them at additional risk for toxicity or would interfere with the study procedures or results.
  - Exception to this is the presence of gastrointestinal polyps/adenomas and non-metastatic carcinomas, and history of any previous malignancies in patients with CMMRD which will be allowed in this study (as noted in section 3.5.2).
- Patients who have received a solid organ transplant.

### 3.6.4 Concurrent therapy

Patients who are receiving any other anti-cancer or investigational drug therapy are ineligible.

### 3.6.5 Infectious Diseases

Patients who have a known active Hepatitis B or Hepatitis C infection are ineligible. Patient must have documented evidence of negative tests for the presence of Hepatitis B surface antigen and Hepatitis C (anti-HCV antibody OR Hep C RNA-qualitative). HIV-positive patients are eligible if the following criteria are met:

- Stable on their antiretroviral agents
- Have CD4 counts above 400
- Undetectable viral loads, and
- No need for prophylactic medications for an opportunistic infections.

### 3.6.6 Recent Live Vaccination

Patients who have received the last vaccination of a live vaccine  $\leq 30$  days prior to enrollment are ineligible. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however,

intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and must meet timeline for live vaccine.

### 3.6.7 Allergy

Patients with a history severe ( $\geq$ Grade 3) hypersensitivity reaction to a monoclonal antibody are ineligible.

### 3.6.8 Prior Therapy

Patients who have received previous therapy with an anti-CTLA4, anti-CD137, anti-PD-L1 or anti-PD-1 antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).

### 3.6.9 Seizures

Patients with uncontrolled seizures defined as seizures that require regular use of rescue medications or in the opinion of the investigator require increasing doses of antiepileptic medications or would compromise the ability to tolerate study therapy or interfere with protocol therapy or procedures. Patients with seizures that are well controlled are eligible and may be on antiepileptic medications if on a stable dose.

### 3.6.10 Concomitant Medications

Patients may not be on immunosuppressive therapy, including corticosteroids (with the exception of physiologic replacement, defined as 0.75 mg/m<sup>2</sup>/day) at time of enrollment. However, patients who require intermittent use of bronchodilators or local steroid injections will not be excluded from the study. Please see section 5.3 for a list of acceptable and unacceptable concomitant medications as well as reporting requirements.

## 3.7 Treatment at Primary Institution

All experimental protocol therapy should be dispensed and all imaging studies should be obtained at a PBTC institution. Required exams during the safety phase must be completed at a PBTC institution. Laboratory studies, excluding pharmacokinetic and biologic assays, may be performed at a CLIA certified laboratory of the investigator's choice.

## 3.8 Criteria to Start Treatment

- Subjects must start therapy within seven (7) days of enrollment.
- Laboratory values must be no older than 7 days prior to the start of therapy except noted infectious disease evaluations. If a test that is repeated post enrollment and prior to the start of therapy is outside the limits for eligibility, it must be rechecked within 48 hours prior to the start of therapy. If rechecks are still outside the limits for eligibility, the patient may not receive protocol therapy and will be considered off study.
- Since patients with hypermutated brain tumors are expected to experience more severe inflammatory reactions after treatment with a PD-1 inhibitor, surgery to resect areas with mass effect will be strongly encouraged before enrollment on this trial.