NCI Protocol #: PBTC-051

Local Protocol #: PBTC-051

ClinicalTrials.gov Identifier: NCT03389802

Title: Phase I Study to Evaluate the Safety and Tolerability of the CD40 Agonistic Monoclonal Antibody APX005M in Pediatric Subjects with Recurrent/Refractory Brain Tumors and Newly Diagnosed Brain Stem Glioma

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Study Drug: APX005M (NSC#788188)
Company Name: Apexigen, Inc.
IND Number: 137045
IND Sponsor: St. Jude Children's Research Hospital
Protocol Type / Version # / Version Date: Amendment/ Version # 6.0/ September 23, 2022

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Abstract and Schema

This is a multicenter phase 1 trial of APX005M for children with recurrent or refractory primary malignant CNS tumor or newly diagnosed DIPG.

APX005M is a humanized immunoglobulin (Ig)G1κ-agonistic monoclonal antibody (mAb) with the S267E mutation at the Fc region. APX005M binds with high affinity to human CD40 (K_d = 1.2×10^{-10} M) and monkey CD40 ($K_d = 3.5 \times 10^{-10}$ M), but does not cross-react with mouse or rat CD40. APX005M blocks the binding of CD40 to CD40L. It has been shown that CD40L-blocking antibodies tend to have more potent CD40 agonistic activities than CD40L-non-blocking antibodies.

Pre-clinical studies of APX005M in monkeys revealed the most significant toxicity to be a prolonged reduction (no recovery over 28 days) in the number of B-cells at doses > 3 mg/kg/dose. Apexigen notes that there was no evidence of hepatotoxicity and "no evidence of any other clinical pathology effects".

Based on the pre-clinical observations, Apexigen initiated a first in human study for adults with solid tumors in 2015. APX005M induces a dose-dependent activation of antigen-presenting cells (as demonstrated by increases in expression of CD54, CD70, CD80, CD86, HLA-DR) and increases in circulating levels of IL12, interferon gamma, TNF-alpha and IL6. The most common symptoms observed during the first 48 hours following infusion of APX005M include: rigors/chills, fever, flushing, itching/pruritis, nausea/vomiting, headache, and rash, as well as hypotension/hypertension; the majority of these symptoms were mild (≤ Grade 2) and responded promptly to symptomatic treatment. Transient transaminase and total bilirubin elevations have been observed in several subjects with liver metastases or with pre-existing biliary tract stenosis due to the location of the tumor. A reversible decrease in peripheral blood lymphocyte counts in general, and B-cell count in particular, have been observed for APX005M and are believed to be a pharmacodynamic (PD) effect. Transient decreases in platelets with no clinical consequences were observed in some subjects.

Apexigen has declared the adult recommended phase 2 dose to be 0.3 mg/kg because no dose limiting toxicities were encountered at that dose and the pharmacodynamic profile was similar to the 1 mg/kg maximally tolerated dose.

The primary objectives of the study are to (1) evaluate the safety of APX005M administered intravenously every 3 weeks to children with central nervous system tumors; (2) determine the maximum tolerated dose and/or the recommended phase II dose of APX005M; (3) determine the pharmacokinetics of APX005M.

Only patients with recurrent or refractory primary malignant CNS tumor will be enrolled initially, newly diagnosed DIPG patients will be on-hold until pediatric RP2D has been established in Stratum 1. APX005M dosing will begin at 0.1 mg/kg, which is one dose level below the adult recommended phase 2 dose. The APX005M dose will be increased in subsequent cohorts until the MTD is reached or until dose level 3 is complete without MTD being defined. APX005M will

be administered at the assigned dose level by intravenous infusion in about 60 minutes every 21 days for 36 courses (2 year) or until disease progression, unacceptable toxicity or death, whichever occurs first.

The starting dose (dose level 1) is 0.1 mg/kg. The table below lists the proposed dose levels to be studied:

Dose Escalation Schedule	
Dose Level	**Dose of APX005M (mg/kg, Q3 weeks, IV)**
0	0.03 mg/kg
1* (starting dose level)	0.1 mg/kg
2	0.3 mg/kg
2.5	0.45 mg/kg
3	0.6 mg/kg

Note: Dose level 2.5 will only be studied if dose level 3 is deemed to be too toxic.

Accrual to Stratum 1 was completed as of May 14, 2020. Dose level 3 (0.6mg/kg) was determined to be the recommended phase 2 dose for Stratum 1. The starting dose for Stratum 2 (the DIPG cohort) was set to be one dose level below the RP2D determined in the non-DIPG patients (Stratum 1) (also see Section 13.1.3). Therefore, accrual to Stratum 2 was initiated at dose level 2 (0.3mg/kg). Accrual to Stratum 2 remains open at the time of this amendment.

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1 OBJECTIVES

1.1 Primary Objectives

1.1.1 To evaluate the safety of APX005M administered intravenously every 3 weeks to children with central nervous system tumors.

1.1.2 To determine the maximum tolerated dose and/or the recommended phase II dose of APX005M.

1.1.3 To determine the pharmacokinetics of APX005M.

1.2 Secondary Objective

1.2.1 To make a preliminary assessment of efficacy via overall response rate, duration of response, progression-free survival and overall survival for DIPG patients.

1.3 Exploratory Objectives

1.3.1 To assess the incidence of anti-drug antibodies.

1.3.2 To determine the immune pharmacodynamics of APX005M.

1.3.3 To identify tumor and blood efficacy and/or resistance biomarkers.

2 BACKGROUND

2.1 Pediatric Primary Malignant Central Nervous System (CNS) Tumors

2.1.1 Unmet medical need

Developing effective treatments for pediatric CNS tumors arguably represents the major remaining unmet need in pediatric oncology. CNS tumors as a group are the second most frequently encountered pediatric malignancy, the most common solid tumor in children, and are the leading cause of cancer-related death in children. In the US, approximately 4,300 children younger than 20 years of age were diagnosed with a CNS tumor in 2013.

The pediatric CNS tumors include a relatively wide variety of specific tumor types with the most common being high- and low-grade gliomas, medulloblastoma, and ependymoma. In general, pediatric patients with CNS tumors do not share the favorable prognosis of those with other common pediatric malignancies. Patients with newly diagnosed diffuse intrinsic pontine gliomas or high-grade astrocytomas essentially do not have any curative treatment options, and most patients with recurrent malignant brain tumors such as ependymoma, medulloblastoma, and others will die of their disease.

2.1.2 Immunotherapy

Immunotherapy is currently considered a very promising area of investigation in clinical oncology. Checkpoint inhibitors such as ipilimumab (anti-CTLA4) and pembrolizumab and nivolumab (anti-PD1) have revolutionized the treatment of adults with advanced melanoma and have also demonstrated promise for other high-risk malignancies in adults such as non-small cell lung cancer. There is only very limited experience with these agents in pediatrics: a pediatric phase I trial of ipilimumab monotherapy concluded that ipilimumab may be safely administered to pediatric patients[1] and a phase I study of nivolumab +/- ipilimumab is on-going through the Children's Oncology Group (COG ADVL 1412). Neither of these studies allow(ed) children with brain tumors to participate. PBTC-045 is a recently opened safety and preliminary efficacy study of the anti-PD1 agent pembrolizumab for children with recurrent/refractory high-grade astrocytoma or DIPG. It does not involve a CD40 agonist and so we believe that this current proposal will be complementary to that study.

Although antibodies are not thought to cross the blood-brain barrier, APX005M seem to be worthy of investigation for brain tumors since activated T-cells are capable of entering the central nervous system and several studies have shown evidence of activity of ipilimumab in adult melanoma patients with CNS lesions[2]. Additionally, preliminary evidence of safety and efficacy of nivolumab +/- ipilimumab in adults with recurrent glioblastoma multiforme treated on the CHECKMATE-143 study (NCT 02017717) was reported at the 2015 ASCO meeting[3]. Twenty patients with first recurrence of their GBM following initial treatment with surgery, RT and temozolomide were reported. Their median age was 57 years (range 37 to 73) and median time from original GBM diagnosis was 9 months. Overall survival at 6 months was 75% which was felt by the authors to be encouraging relative to historical controls. Updated results were presented at the 2016 ASCO meeting[4]. No new safety signals were identified and the 12 month overall survival rates were considered to be encouraging.

2.1.3 CD40 agonists

The PBTC immunotherapy committee has identified CD40 agonists as a class of agents that we are anxious to study in children with brain tumors. CD40 is expressed on antigen-presenting cells such as dendritic cells, as well as on B cells and macrophages. Agonist CD40 antibodies can activate antigen-presenting cells and stimulate anti-tumor CD8 T-cell responses. CD-40 activated macrophages can also become tumoricidal. It is important to note that these mechanisms do **NOT** require CD40 expression on the tumor cells.

While we are not aware of any experience investigating CD40 agonist antibodies as anti-tumor agents in children, several such antibodies (CP-870,893 [Pfizer], dacetuzmumab [Seattle Genetics], Chi Lob 7/4 [University of Southampton]) have been investigated in adults with cancer. In general the agents have been well-tolerated and have demonstrated some evidence of efficacy.

CP-870,893 seems to be the agent that has been most studied. A report of the first in human, phase I study included 29 adults with advanced solid tumors[5]. They received single doses of the agent intravenously, at doses ranging from 0.01 to 0.3 mg/kg. The maximum tolerated dose (MTD) was considered to be 0.2 mg/kg because 2/7 subjects treated at the 0.3 mg/kg dose level experienced DLT (venous thromboembolism, grade 3 headache). The most common adverse event was transient grade 1 or 2 cytokine release syndrome (chills, rigors, fever, rash, nausea, vomiting, muscle aches, back pain) associated with the infusion and elevated of serum TNF-alpha and IL-6. Other non-DLT toxicities included grade 3 or 4 lymphopenia, grade 2 thrombocytopenia (at the 0.3 mg/kg dose level), grade 3 AST/ALT elevations, grade 1 or 2 hyperbilirubinemia, and grade 1 or 2 proteinuria. Anti-tumor activity was demonstrated in 4 patients with melanoma (14% of all subjects; 27% of melanoma subjects) that achieved partial response at day 43. One of these patients received 9 subsequent doses (about every 8 weeks) and was in complete remission more than 5 years later[6]. CP-870,893 was subsequently used in combination with (1) carboplatin and paclitaxel for patients with advanced cancer, (2) gemcitabine for patients with metastatic pancreatic carcinoma with response rates of about 20% (thought to be promising in the context of these patients). Trials also have been initiated or done in combination with an anti-CTLA4 antibody (tremelimumab) for melanoma and cisplatin and pemetrexed for advanced mesothelioma.

Theoretical concerns associated with CD40 agonists beyond cytokine release include autoimmune reactions, thromboembolic phenomena (CD40 is expressed on platelets and endothelial cells), hyper-immune stimulation leading to cell death or tolerance, and tumor angiogenesis, but the Vonderheide and Glennie review article cited above notes that "overall, toxicity has not been a major issue with CD40 agonists in the clinic".

2.2 APX005M

APX005M is a humanized immunoglobulin (Ig)G1κ-agonistic monoclonal antibody (mAb) with the S267E mutation at the Fc region. APX005M binds with high affinity to human CD40 (K_d = 1.2×10^{-10} M) and monkey CD40 ($K_d = 3.5 \times 10^{-10}$ M), but does not cross-react with mouse or rat CD40. APX005M blocks the binding of CD40 to CD40L. The APX005M binding epitope has been mapped to 2 specific regions on CD40. These are 92TSEACESCVLHRSCSP107 and 125PCPVGFFSNVSSAFEKCHPW144. The region 92TSEACESCVLHRSCSP107 is known as a CD40L binding domain. It has been shown that CD40L-blocking antibodies tend to have more potent CD40 agonistic activities than CD40L-non-blocking antibodies[7].

Pre-clinical studies of APX005M in monkeys revealed the most significant toxicity to be a prolonged reduction (no recovery over 28 days) in the number of B-cells at doses > 3 mg/kg/dose. Apexigen notes that there was no evidence of hepatotoxicity and "no evidence of any other clinical pathology effects".

Based on these pre-clinical observations, in 2015 Apexigen initiated a first in human study for adults with solid tumors, APX005M-001. There were no adults with primary brain tumor or CNS metastases in the adult phase 1 trial. The dose escalation scheme called for enrollment of 1 subject each at dose levels 1-3 (0.0001, 0.001, 0.01 mg/kg) and then have a standard 3+3 design for dose levels 4 to 8 (0.03, 0.1, 0.3, 1, 3 mg/kg).

They enrolled the first subject on June 01, 2015. APX005M was administered to study subjects at doses up to 1 mg/kg. At the 1 mg/kg dose level, 1 out of 6 DLT-evaluable subjects experienced a DLT (grade 4 cytokine release syndrome). Two additional subjects at the 1mg/kg dose level experienced SAEs in later courses (Grade 3 cytokine release syndrome and Grade 4 thrombocytopenia). On May 2, 2016 Apexigen decided to discontinue dose escalation and enroll up to 6 subjects in dose level 0.6 mg/kg (an intermediate de-escalation dose level) and an additional 3 subjects at the previously completed dose level 0.3 mg/kg to better characterize the safety and pharmacodynamics of APX005M and to help establish the single agent recommended phase 2 dose.

As of the data cutoff date of 14 April 2017, 432 AEs have been reported in the 30 subjects enrolled in Study APX005M-001. :

- 371/432 (85.9%) AEs were ≤ Grade 2
- 44 (10.2%) AEs were Grade 3
- 8 (1.9%) AEs were Grade 4
- No Grade 5 events were reported
- 237/432 (55%) AEs were considered unrelated to APX005M by the Investigator

As of April 14 2017, infusion-related reactions including cytokine release syndrome have been reported in 10/30 (33%) of subjects. There has been 1 Grade 4 event of cytokine release syndrome that was considered related to APX005M. Three other subjects receiving APX005M experienced Grade 3 infusion-related reactions. All of these events occurred in subjects receiving APX005M at doses ≥ 0.6 mg/kg. The most common symptoms observed during the first 48 hours following infusion of APX005M include: rigors/chills, fever, flushing, itching/pruritis, nausea/vomiting, headache, and rash, as well as hypotension/hypertension; the majority of these symptoms were mild (≤ Grade 2) and responded promptly to symptomatic treatment. Asymptomatic transient transaminase and total bilirubin elevations have been observed in several subjects. Subjects with liver metastases or with pre-existing biliary tract stenosis due to the location of the tumor are more prone to develop this kind of liver toxicity. A reversible decrease in peripheral blood lymphocyte counts in general, and B-cell count in particular, have been observed for APX005M and are believed to be a pharmacodynamic (PD) effect. Transient decreases in platelets with no clinical consequences were observed in some subjects.

According to the communication with Apexigen, there were 9 patients that had grade 1 headache in adult phase 1 study, 5 of which were considered to be related to the study drug. No grade ≥ 2 headaches have been observed in adult phase 1 study.

APX005M induces a dose-dependent activation of antigen-presenting cells (as demonstrated by increases in expression of CD54, CD70, CD80, CD86, HLA-DR) and increases in circulating levels of IL12, interferon gamma, TNF-alpha and IL6.

Apexigen has declared the adult recommended phase 2 dose to be 0.3 mg/kg because no dose limiting toxicities were encountered at that dose and the pharmacodynamic profile was similar to the 1 mg/kg maximally tolerated dose.

As of April 3 2021, APX005M has been evaluated either as monotherapy or in combination with other agents in a total of 14 trials excluding PBTC-051. Most treatment-emergent adverse events occurred within 48 hours of APX005M dosing. The most common were asthenia, chills, cytokine release syndrome, decreased appetite, diarrhea, elevated ALT, elevated AST, elevated GGT, fatigue, headache, infusion-related reaction, nausea, pruritis, pyrexia, rash, and vomiting.

Additional information about APX005M is available in the Investigator Brochure (IB), which is posted on PBTC-051 webpage.

2.2.1 Preclinical data relevant to pediatric CNS tumors

We are not aware of any pre-clinical data regarding APX005M or any other CD40 agonist antibody for pediatric CNS tumors or any pediatric malignancy. However, pre-clinical efficacy data are often lacking for immunotherapeutic agents due to inter-species immune system differences and because of the need for the host to have an intact immune system. Discussions regarding this issue with a number of experts including Drs. Jedd Wolchok (MSKCC immunotherapeutics chief) and Duane Mitchell (PBTC immunotherapy chair), confirmed that progress to date in checkpoint inhibitor immunotherapy for non-melanoma tumors has usually been made via clinical investigations without pre-clinical efficacy data for a specific tumor type.

2.2.2 PBTC-051 Clinical Trial Experience

A total of 21 patients were enrolled on stratum 1. One patient was deemed ineligible after starting treatment on dose level 3 due to an incomplete eligibility assessment, leaving 20 eligible stratum 1 patients. Three were enrolled on dose level 1, 3 on dose level 2 and 14 on dose level 3. No dose-limiting toxicities (DLT) were noted in dose levels 1 or 2. Seven (6 evaluable) patients were subsequently enrolled on dose level 3 with no DLTs noted. Consequently, dose level 3 (0.6 mg/kg) was determined to be the RP2D. Seven additional (6 evaluable) patients were subsequently treated at dose level 3 in a dose expansion cohort to further define APX005M toxicities. Two DLTs were noted in this dose expansion cohort, for a total of 2 DLTs in 12 evaluable patients at the maximum dose level. Both DLTs were grade 3 infusion-related reactions. One occurred in cycle 1 and the other in cycle 2 of therapy. The most common adverse events recorded were lymphopenia (maximum grade 4), neutropenia (maximum grade 3), and leukopenia (maximum grade 4). The starting dose for the DIPG cohort (stratum 2) was chosen to be one dose level below the RP2D determined in stratum 1 as stated in Section 13.1.3 of the protocol document. This was driven by a concern that APX005M might be more toxic in DIPG patients, so we took a conservative yet also efficient approach for the stratum 2 safety study.

2.3 Correlative Studies Background

2.3.1 Potential impact on future trials and practice

We expect that this study will allow us to determine the safety and tolerability of APX005M in children with recurrent/refractory brain tumors and that we will obtain preliminary data regarding the efficacy of this treatment approach. These data should be valuable for the design of a subsequent phase II study in children with brain tumors and also for children with other poor prognosis tumors. This would also be an important first step towards the development of trials combining APX005M with another agent such as a checkpoint inhibitor or a tumor vaccine.

Patients with malignant glioma exhibit a variety of immune defects, many of which are related to impaired T-cell function[8]. Recent publications show that CD40/CD40L expression correlates with the survival of patients with glioblastomas[9] and activation of CD40 augments the efficacy of vaccinations against glioma models[9,10] or can exert immune mediated anti-glioma effects in combination with COX-2 inhibitors[11]. Moreover, ligation of CD40 can also inhibit human glioma cells proliferation via nuclear factor κB signaling pathway[12].

2.3.2 Rationale for Pharmacokinetic Studies

2.3.2.1 Hypothesis

The pharmacokinetics and tolerability of APX005M in pediatric patients with recurrent/progressive CNS tumor may differ from prior studies of APX005M in the adult population, and may be affected by prior treatment, age, gender, body weight, race, or concomitant medications.

2.3.2.2 Preclinical and Clinical Data

Because there is no previous pediatric pharmacokinetic (PK) studies of APX005M, PK information from this study will be essential for evaluating toxicity and disease response and for refining dosing in future clinical trials of APX005M. Mandatory pharmacokinetic studies are needed to characterize the PK profile of APX005M in this patient population, correlate PK with toxicities, and evaluate effects of concomitant medications. Insights into the biologically active dosage will be gained by relating APX005M systemic exposure (e.g., AUC) to results of pharmacodynamic studies.

The PK parameters of APX005M were evaluated in cynomolgus monkeys in both the single- and repeat-dose toxicology studies. The PK properties of APX005M appear typical of other mAbs, and comprise low CL, small V_z, and long $t_{1/2}$ at 3 and 30 mg/kg. There are limited human PK data with APX005M at this time. Exposures to APX005M at dose levels of 0.03 mg/kg or less were for the most part below the limit of quantitation (BLOQ). Increases in the dose of APX005M (0.1 mg/kg to 1 mg/kg) led to approximately dose-proportional increases in C_{max} and AUC_{0-t}. Accumulation of APX005M was not observed upon every 3-week dosing. It will be important to correlate pharmacodynamic effects of APX005M with PK.

2.3.3 Rationale for Anti-Drug-Antibody Studies

2.3.3.1 Hypothesis

APX005M is a humanized immunoglobulin (Ig)G1κ-agonistic monoclonal antibody CD40-agonistic antibody, and is not possible to lead to production of ADA. The anti-drug-antibody of APX005M in pediatric patients with recurrent/progressive CNS tumor will be studied before and after APX005M treatment.

2.3.3.2 Preclinical and Clinical Data

Anti-drug-antibody is an immune response by a human against a therapeutic antigen (e.g., monoclonal antibody). ADA can inactivate the therapeutic effects of the treatment and in rare cases, induce adverse effects.

Chi Lob 7/4 is a chimeric IgG1 CD40-agonistic antibody tested in a Phase 1 clinical trial in patients with solid tumors. Significant anti-drug-antibodies (ADA) were detected in patients treated with Chi Lob 7/4 due to its mouse-human chimeric structures. It is important to investigate whether the ADA is generated after continuous APX005M treatment.

2.3.4 Rationale for Measurement of Circulating Cytokines in Human Plasma

2.3.4.1 Hypothesis

Treatment of patients with agonistic anti-CD40 Ab may result in increased levels of inflammatory cytokines given the immune stimulatory effect of this therapy.

2.3.4.2 Preclinical and Clinical Data

Preliminary results from the adult Phase 1 study showed that APX005M induces a dose-dependent increase in circulating levels of IL12, interferon gamma, TNF-alpha and IL6.

2.3.5 Rationale for Characterization of T Cell Activation/Exhaustion Phenotype in Human PBMC

2.3.5.1 Hypothesis

Treatment of patients with agonistic anti-CD40 Ab may result in systemic activation of T cells and potential reversal of possible T cell exhaustion which may be evaluated by flow cytometric immune phenotyping using markers of T cell activation and exhaustion.

2.3.5.2 Preclinical and Clinical Data

In in vitro cultures with T cells and dendritic cells, APX005M was able to enhance antigen-specific T-cell proliferation and promote interferon (IFN)-gamma secretion. In combination with an antibody against programmed cell death ligand-1 (PD-L1), APX005M synergistically enhances antigen-specific T-cell responses. In addition, preliminary results from the adult Phase 1 study showed that APX005M induces a dose-dependent activation of T cells (as demonstrated by increases in expression of CD54 and HLA-DR).

2.3.6 Rationale for Tumor Mutational Burden and Immunogenomic Assessment

2.3.6.1 Hypothesis

It is now widely accepted that genomic features of the tumor or microenvironment are critical determinants of response to immunotherapy agents. For example, we and others have shown that

tumor mutation burden, tumor clonality, mutational signature profile, and other genetic features can determine clinical benefit from many tumor types. We hypothesize that critical immunogenomic features in the tumor cell and/or T cell repertoire will associate with the ability of anti-CD40 treatment to impart clinical benefit.

2.3.6.2 Preclinical and Clinical Data
Not available at this time.

3 PATIENT SELECTION

All subjects must meet the following inclusion and exclusion criteria. No exceptions will be given. Imaging studies to establish eligibility must be done within three weeks prior to enrollment. All other clinical evaluations to establish eligibility must be done within 7 days prior to enrollment.

3.1 Eligibility Criteria for Enrollment

3.1.1 Diagnosis

Stratum 1: Recurrent or refractory primary malignant CNS tumor patients
Patients with a histologically confirmed diagnosis of a primary malignant non-brainstem CNS tumor (excluding DIPG patients) that is recurrent, progressive, or refractory. All tumors must have histologic verification at either the time of diagnosis or recurrence except patients with marker (+) CNS germ cell tumors.

Stratum 2: Newly diagnosed DIPG patients
Patients with diffuse intrinsic pontine gliomas (DIPGs) will be eligible 6 to 14 weeks post-completion of radiation therapy if they do not have any evidence of progression. Patients with newly diagnosed DIPGs, defined as tumors with a pontine epicenter and diffuse involvement of 2/3 or more of the pons, are eligible without histologic confirmation. Patients with pontine tumors that do not meet these criteria or not considered to be typical intrinsic pontine gliomas will only be eligible if the tumors have been biopsied and (1) are proven to be an anaplastic astrocytoma, glioblastoma multiforme, gliosarcoma, anaplastic mixed glioma or fibrillary astrocytoma or (2) have a histone mutation typically seen in DIPG. Patients with disseminated disease are not eligible, and MRI of spine must be performed if disseminated disease is suspected by the treating physician.

3.1.2 Available Pre-trial Tumor Tissue

Stratum 1: Recurrent or refractory primary malignant CNS tumor patients must have adequate pre-trial frozen or FFPE tumor material (minimum of 10 unstained slides) available for use in the tumor mutation burden studies (section 9.1.5).

Stratum 2: Patients with DIPG who have pre-trial tumor tissue available are requested to submit tissue; however, this is not required for eligibility.

3.1.3 Age

Patient must be ≥ 1 and ≤ 21 years of age at the time of enrollment.

3.1.4 Prior Therapy

- Newly Diagnosed DIPG patients

Patients must have not received any prior therapy for treatment of their current CNS malignancy other than radiation therapy.

- Refractory/Recurrent patients

Patients must have recovered from the acute treatment related toxicities (defined as < grade 1) of all prior chemotherapy, immunotherapy, radiotherapy or any other treatment modality prior to entering this study.

3.1.4.1 Myelosuppressive chemotherapy

Patients must have received their last dose of known myelosuppressive anticancer therapy at least 21 days prior to enrollment or at least 42 days if nitrosourea.

3.1.4.2 Biological Agent/Monoclonal Antibody

- Biological agent:

Patient must have recovered from any acute toxicity potentially related to the agent and received their last dose of the biologic agent ≥ 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.

- Monoclonal antibody treatment and agents with known prolonged half-lives: Patient must have recovered from any acute toxicity potentially related to the agent and received their last dose of the agent ≥ 28 days prior to study enrollment.

3.1.4.3 Radiation

Patients must have had their last fraction of:

- Craniospinal irradiation (>24Gy) or total body irradiation or radiation to ≥ 50% of pelvis ≥ 42 days prior to enrollment.
- Focal irradiation ≥ 14 days prior to enrollment
- Local palliative irradiation (small port) ≥ 14 days prior to enrollment

3.1.4.4 Autologous Stem Cell Transplant

If the patient has previously received an autologous stem cell transplant, patient must be ≥ 6 months since autologous bone marrow/stem cell transplant prior to enrollment and have CD4 counts above 200/mm^3.

3.1.5 Surgery

Patients must be at least 4 weeks (28 days) from major surgery and fully recovered from all acute effects of prior surgical intervention.

3.1.6 Inclusion of Women and Minorities

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

3.1.7 Neurologic Status

- Patients with neurological deficits should have deficits that are stable for a minimum of 1 week prior to enrollment.

- Patients with seizure disorders may be enrolled if seizures are well controlled.

3.1.8 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 ≥ 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.

3.1.9 Organ Function

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

- Absolute Neutrophil Count (ANC) ≥ 1.0 x 10^9 cells/ L
- Platelets ≥ 100 x 10^9 cells/L (unsupported, defined as no platelet transfusion within 7 days)
- Hemoglobin ≥ 8 g/dL (may receive transfusions)
- Total bilirubin ≤1.5 times institutional upper limit of normal (ULN)
- AST(SGOT)/ALT(SGPT) ≤ 3 x institutional upper limit of normal (ULN)
- Albumin ≥ 3 g/dl
- Serum creatinine based on age/gender as noted in Table1. Patients that do not meet the criteria in Table 1 but have a 24 hour Creatinine Clearance or GFR (radioisotope or iothalamate) ≥ 70 mL/min/1.73 m^2 are eligible.

Table 1

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
≥ 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.		

- Cardiac Function:
  - Left Ventricular Ejection Fraction (LVEF) > 50%
  - ECG QTc ≤ 450 msec
- Pulmonary Function:
  - Oxygen saturation as measured by pulse oximetry is > 93% on room air and no evidence of dyspnea at rest

3.1.10 Growth Factors

Patients must be off all colony- forming growth factor(s) for at least 1 week prior to enrollment (i.e., filgrastim, sargramostim or erythropoietin). 2 weeks must have elapsed if patients received PEG formulations.

3.1.11 Pregnancy Status
Female patients of childbearing potential must have a negative serum or urine pregnancy test.

3.1.12 Pregnancy Prevention
Female subjects with childbearing potential and male subjects should use effective contraception methods (or abstain from sexual activity) while being treated with APX005M and for 30 days following treatment.

3.1.13 Informed Consent
The patient or parent/guardian is able to understand the consent and is willing to sign a written informed consent document according to institutional guidelines.

3.2 Exclusion Criteria

3.2.1 Concurrent Illness

- Patients with any clinically significant unrelated systemic illness (serious infections Grade ≥ 2 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 a history of any other malignancy, except patients with a secondary brain tumor if the patient's first malignancy has been in remission for at least 5 years from the end of treatment.

3.2.2 Concurrent Therapy

- Patients who are receiving any other anticancer or investigational drug therapy.
- Patients requiring systemic treatment with either corticosteroids (greater than dexamethasone 0.75 mg/m^2/day or the equivalent dose of other steroids) or other immunosuppressive medications within 14 days of study drug administration will be excluded. 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.

3.2.3 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 that in the opinion of the site investigator, shows significant mass effect

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.

3.2.4 Allergy
Patients with a history of severe (Grade ≥ 3) hypersensitivity reaction to a monoclonal antibody are ineligible.

3.2.5 Allogeneic Hematopoietic Stem Cell Transplantation
Patients who have received allogeneic hematopoietic stem cell transplantation are ineligible.

3.2.6 Autoimmune Diseases
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 well controlled asthma/atopy
- Patients with hypothyroidism stable on hormone replacement or Sjogren's syndrome

3.2.7 Inability to Participate
Patients who in the opinion of the investigator are unwilling or unable to return for required follow-up visits or obtain follow-up studies required to assess toxicity to therapy or to adhere to drug administration plan, other study procedures, and study restrictions.

3.2.8 Bleeding Disorder
Patients with a known coagulopathy or bleeding diathesis or require the use of systemic anticoagulant medication are not eligible.

3.2.9 Lactation Status
Female patients must not be breast-feeding.

3.3 Treatment at the Primary Institution
All experimental protocol therapy should be dispensed and all on treatment imaging studies should be obtained at a PBTC institution. Laboratory studies, excluding pharmacokinetic and biologic assays, may be performed at a CLIA certified laboratory of the investigator's choice. Imaging utilized to determine eligibility may be performed at an outside institution if all required imaging sequences are included and the study is deemed of adequate quality by the treating team. All required physical examinations, laboratory parameters need to be performed at the primary PBTC institution during the dose finding period of the protocol.

3.4 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. 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.

4 REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP
Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to any NCI-sponsored clinical trial to register and renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual

registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr).
RCR utilizes five-person registration types.

- IVR — MD, DO, or international equivalent;
- NPIVR — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD);
- AP — clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications such as Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges;
- Associate (A) — other clinical site staff involved in the conduct of NCI-sponsored trials; and
- Associate Basic (AB) — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	**IVR**	**NPIVR**	**AP**	**A**
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL), if required.

In addition, all investigators acting as the Site-Protocol PI (Investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the Clinical Investigator (CI) on the DTL must be rostered at the enrolling site with a participating organization.

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov.

4.2 Cancer Trials Support Unit Registration Procedures

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

4.2.1 IRB Approval

For CTEP and Division of Cancer Prevention (DCP) studies open to the National Clinical Trials Network (NCTN) and NCI Community Oncology Research Program (NCORP) Research Bases after March 1, 2019, all U.S.-based sites must be members of the NCI Central Institutional Review Board (NCI CIRB). In addition, U.S.-based sites must accept the NCI CIRB review to activate new studies at the site after March 1, 2019. Local IRB review will continue to be accepted for studies that are not reviewed by the CIRB, or if the study was previously open at the site under the local IRB prior to March 1, 2019. International sites should continue to submit Research Ethics Board (REB) approval to the CTSU Regulatory Office following country-specific regulations.

Sites participating with the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet for Local Context (SSW) to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by email or calling [redacted]).

In addition, the site-protocol Principal Investigator (PI) (i.e., the investigator on the IRB/REB approval) must meet the following criteria in order for the processing of the IRB/REB approval record to be completed:

- Holds an active CTEP status;
- Rostered at the site on the IRB/REB approval (applies to US and Canadian sites only) and on at least one participating roster;
- If using NCI CIRB, rostered on the NCI CIRB Signatory record;
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile; and
- Holds the appropriate CTEP registration type for the protocol.

4.2.2 Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federalwide Assurance (FWA) number;
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO); and
- Compliance with all protocol-specific requirements (PSRs).

4.2.3 Protocol-Specific Requirements for PBTC-051 Site Registration

Upon site registration approval in RSS, the enrolling site may access OPEN to complete enrollments. The enrolling site will select their credentialed provider treating the subject in the OPEN credentialing screen and may need to answer additional questions related to treatment in the eligibility checklist.

4.2.4 Downloading Site Regulatory Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted based on person and site roster assignment. To participate, the institution and its associated investigators and staff must be associated with the LPO or a Protocol Organization (PO) on the protocol. One way to search for a protocol is listed below.

- Log in to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password;
- Click on *Protocols* in the upper left of the screen
    - Enter the protocol number in the search field at the top of the protocol tree; or
    - Click on the *By Lead Organization* folder to expand, then select *Pediatric Brain Tumor Consortium (PBTC)* and protocol number *PBTC-051*.
- Click on *Documents*, select *Site Registration*, and download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

4.2.4.1 Submitting Regulatory Documents:

Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal on the CTSU website.

To access the Regulatory Submission Portal, log in to the CTSU members' website, go to the Regulatory section, and select Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately at [redacted] in order to receive further instruction and support.

4.2.4.2 Checking Site Registration Status

Site registration status may be verified on the CTSU members' website.

- Click on *Regulatory* at the top of the screen;
- Click on *Site Registration*; and
- Enter the site's 5-character CTEP Institution Code and click on Go.
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for

individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.2.5 Delegation of Task Log (DTL)

Each site must complete a protocol-specific Delegation of Tasks Log (DTL) using the DTL application in the Delegation Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and include a Master Task List, which describes DTL task assignments, CI signature, and CTEP registration requirements.

4.2.6 Checking Site Registration Status

You can verify your site registration status on the members' section of the CTSU website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient Enrollment

4.3.1 OPEN/IWRS

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the Lead Protocol Organization (LPOs) registration/randomization systems. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account;
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or participating organization roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type;
- If a Delegation of Tasks Log (DTL) is required for the study, the registrars must hold the OPEN Registrar task on the DTL for the site; and
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes; and
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. You may print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at [redacted] or ctsucontact@westat.com.

Patient enrollment for this study will be facilitated using the Slot Reservation System in conjunction with the registration system in OPEN. Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

4.3.2 OPEN Questions?

Further instructional information on OPEN is provided on the OPEN link of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at [redacted] or ctsucontact@westat.com.

5 TREATMENT PLAN

Treatment will generally be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's tumor.

Timing of protocol therapy administration and response assessment studies are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable.

5.1 Agent Administration

5.1.1 APX005M (CD40 agonistic mAb)

APX005M dosing will begin at 0.1 mg/kg, which is one dose level below the adult recommended phase 2 dose. For APX005M, both 1 mg/kg and 0.6 mg/kg were explored in adult participants, and the adverse events at 0.6 mg/kg were manageable and reversible. The APX005M dose will be increased in subsequent cohorts until the MTD is reached or until dose level 3 is complete without MTD being defined (
Table 2). No intrapatient dose escalation will be permitted on the protocol. Only DLTs observed during the dose-finding period (the first 2 courses, i.e., the first 6 weeks of treatment) will be used to guide dose escalation. Dose escalation will be governed by the statistical design as described in section 13 of the protocol.

Table 2

Dose Escalation Schedule		
Dose Level	**Dose of APX005M (mg/kg, IV, Q3 weeks)**	**round final dose to**
0	0.03 mg/kg	2 decimals
1* (starting dose level)	0.1 mg/kg	1 decimal
2	0.3 mg/kg	1 decimal
2.5	0.45 mg/kg	1 decimal
3	0.6 mg/kg	1 decimal
Note: Dose level 2.5 will only be studied if dose level 3 is deemed to be too toxic.		

Subjects will be assigned a dose level at the time of enrollment. APX005M will be administered at the assigned dose level on Day 1 of each course 21 day. Patients may continue to receive APX005M for 36 courses (approximately 2 years) or until disease progression, unacceptable toxicity or death, whichever occurs first. Delays between courses for reasons other than toxicity may occasionally occur however dosing should follow the treatment plan as closely as possible.

Dosing should be adjusted based on weight (kg) calculated at the beginning of each course of therapy. The dose may be prescribed using the weight that was determined at the prior visit. However, if the current weight is ≥ 5% different from the prior weight, the prescription should be revised.

Accrual to Stratum 1 was completed as of May 14, 2020. Dose level 3 (0.6mg/kg) was determined to be the recommended phase 2 dose for Stratum 1. The starting dose for Stratum 2 (the DIPG cohort) was set to be one dose level below the RP2D determined in the non-DIPG patients (Stratum 1) (also see Section 13.1.3). Therefore, accrual to Stratum 2 was initiated at dose level 2 (0.3mg/kg). Accrual to Stratum 2 remains open at the time of this amendment.

5.1.2 APX005M Infusion

APX005M is supplied in 20 mL (10 mg/mL) type 1 clear glass vials for intravenous injection. The target fill of each vial is 16.9 mL; the amount of APX005M that can be reliably extracted is 16.3 mL (163 mg)/vial. The calculated APX005M is prepared in 0.9% Sodium Chloride to a final volume of 50 mL, and administered as a 60 minute IV infusion. Sites should make every effort to

target infusion timing to be as close to 60 minutes as possible. A window between -5 minutes and +10 minutes is permitted (i.e., infusion time between 55 minutes and 70 minutes).

The APX005M infusion can be interrupted in the case of infusion related reaction/cytokine release syndrome (see Section 6.2.3). Once symptoms resolve, infusion should be restarted at 50% of the initial infusion rate (e.g., from 50 mL/hr to 25 mL/hr). If tolerated 10 minutes later it should be increased to the initial infusion rate. If symptoms recur with the increase in rate but the infusion is tolerated at the 50% rate, complete the infusion at the 50% rate without further attempts to increase the rate. It is recommended that the actual infusion time does not exceed 90 minutes. However, the infusion may last for a total of 2 hours in cases where the infusion is run at the 50% rate for the entire infusion. If infusion related reaction symptoms recur at the 50% rate despite decreased infusion rate and symptomatic treatment (Section 6.2.4) then permanently discontinue dosing.

For the infusion following one in which a patient developed infusion related reaction/cytokine release syndrome, initiate the infusion at the 50% rate. If the infusion is tolerated, increase the rate 10 minutes later to the 100% rate. If an infusion related reaction/cytokine release syndrome develops, interrupt the infusion and provide symptomatic care. Once symptoms resolve, restart the infusion at the 50% rate and do not increase the rate during the current infusion or for any future infusions. If infusion related reaction symptoms recur at the 50% rate despite decreased infusion rate and symptomatic treatment (Section 6.2.4), permanently discontinue dosing.

Subjects will be assigned a dose level at the time of enrollment. See section 5.1.1 for details of dose escalation schedule. See section 6.2 for dose modifications.

5.1.2.1 Pre-medication

Subjects are encouraged to have good pre-treatment hydration (good oral intake and/or IV fluids) prior to infusion. Subjects are encouraged to drink volume-increasing fluids (e.g., Gatorade, broth) for the reminder of the infusion day and maintain an adequate oral fluid intake for the first 24-48 hours after APX005M administration. Subjects must be premedicated prior to all APX005M infusions.

Approximately 30-60 minutes before the infusion of APX005M premedicate subjects with a regimen containing the following medications at the appropriate pediatric doses:

- Oral nonsteroidal anti-inflammatory drug (e.g., ibuprofen)
- Oral acetaminophen

Intravenous formulations of these medications (rather than oral) may be administered approximately 10 minutes prior to infusion. When the time between premedication and scheduled APX005M administration exceeds 4 hours, subjects may receive an additional course of premedication prior to APX005M administration.

The investigator may consider withholding anti-hypertensive medications on the day of APX005M administration if in the opinion of the investigator such action poses no risk to the subject.

It is strongly suggested subjects continue anti-pyretics (e.g., ibuprofen every 8 hours, alternating with acetaminophen every 8 hours x 3 doses at the appropriate pediatric dose) for 24 hours following each dose. If there is any concern regarding renal function or anticoagulation, consider

using a lower dose of ibuprofen or eliminating it from the regimen. If the patient continues to be symptomatic, consider continuing the regimen after the 3 doses as needed.

Subjects must be observed for at least 48 hours following the completion of the first 2 infusions of APX005M. Patients should be admitted to the hospital for 48 hours or must agree to stay within 1 hour driving distance from PBTC center for 48 hours following the first 2 infusions. If not admitted, subjects must be discharged into the company of a caregiver or healthcare provider who will continue to observe the subject for at least a 48-hour period following the first 2 infusions. Subjects and caregivers will both be provided with discharge instructions to contact the investigational site and/or seek medical care if appropriate. Subjects should be told to bring investigational site contact information and/or a copy of the informed consent document when seeking medical care from anyone other than the investigational site staff.

5.1.3 Criteria to Start Subsequent Courses

A course may be repeated every 3 weeks if the patient has at least stable disease, all drug-related toxicities return to baseline or ≤ Grade 1 (excluding alopecia and Grade 2 fatigue) and has again met laboratory parameters as defined in Section 3.1.9. If a patient does not meet these parameters at the end of the treatment course then APX005M should be held until parameters meet the eligibility criteria.

5.2 Dose Limiting Toxicity

The Dose Limiting Toxicity (DLT) will be defined as any of the following events which are at least possibly attributed to APX005M and occur during the first 2 courses (6 weeks, 42 days) following the APX005M administration. Management and dose modifications associated with adverse events are outlined in Section 6.

Management and dose modifications for toxicities which occur outside of the dose-finding period should also follow section 6; however these will not be considered dose limiting for the purpose of dose escalation.

5.2.1 Dose-Limiting Toxicities (DLT's)

- Any APX005M-related adverse event during the first 2 courses of therapy (6 weeks, 42 days) that leads to a dose reduction or results in the permanent cessation of therapy will be considered dose limiting. All dose modifications in all courses are to follow the guidelines provided in Section 6.2.
- Any APX005M-related adverse event during the first 2 courses of therapy that results in a delay of treatment > 2 weeks.

5.2.1.1 Non-hematologic dose limiting toxicity is defined as:

- Any grade 4 non-hematologic toxicity
- Any grade 3 non-hematologic toxicity with the exception of:
  - Grade 3 nausea and vomiting < 5 days
  - Grade 3 diarrhea responding to optimal medical treatment within 5 days
  - Grade 3 elevation of transaminases that returns to levels meeting eligibility criteria within 7 days of study drug interruption and does not recur upon restarting drug.
  - Grade 3 fever or infection of fewer than 5 days in duration

- Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia that are asymptomatic and responsive to oral supplementation
- Grade ≥ 3 cytokine release syndrome
- Toxicity related to treatment with APX005M which delays scheduled re-treatment for > 2 weeks, or >1 week for cytokine release related complications.

5.2.1.2 Hematologic dose limiting toxicity is defined as:

- Any grade 4 hematologic toxicity with the exception of lymphopenia
- Grade 3 neutropenia with fever
- Grade 3 thrombocytopenia on 2 separate days or requiring a platelet transfusion on 2 separate days within a 7 day period.

5.2.2 Dose-finding period

The dose finding period begins with the initial dose of APX005M and ends on the last day of course 2, which is 6 weeks (42 days) in duration. Should there be a delay starting the subsequent course, dose finding will complete on the start date of the subsequent course. The recommended phase 2 dose (RP2D) will be based on the MTD and the totality of the safety/efficacy data.

5.2.3 Dose Escalation/ De-escalation

Dose escalations/de-escalation decisions will be based on the 3+3 design as described in the statistical section of the protocol. The initial cohort of three (3) patients will be treated at 0.1 mg/kg (the dose levels to be tested are listed in

Table 2). Only DLTs observed during the dose-finding period of therapy will be used to guide dose escalation/de-escalation.

5.3 Concomitant Medications and Supportive Care Guidelines

5.3.1 Steroids

Corticosteroids should be used at the lowest dose to control symptoms of edema and mass effect, and discontinued, if possible. Use of corticosteroids should be recorded in the PBTC-051 database.

5.3.2 Anticonvulsant drugs

Anticonvulsant drugs should be used, if indicated. Use of anticonvulsants should be recorded in the PBTC-051 database.

5.3.3 Growth Factors

Routine use of growth factors (e.g., G-CSF, GM-CSF and erythropoietin) is not permitted. However, therapeutic use of G-CSF or GM-CSF in patients with serious neutropenic conditions, such as sepsis, may be considered at the investigator's discretion. Use of growth factors should be recorded in the PBTC-051 database.

5.3.4 Anti-emetics

The use of anti-emetics will be at the investigator's discretion. Use of anti-emetics should be recorded in the PBTC-051 database. Steroid should not be used as anti-emetics.

5.3.5 Febrile neutropenia

Febrile neutropenia should be managed according to the local institutional guidelines.

5.3.6 Neurosurgical or other surgical procedures

If a neurosurgical procedure or other surgical procedure is required for a reason other than tumor progression (i.e. the onset of hydrocephalus), these procedures should be documented, but will not constitute criteria for declaring the patient "off therapy". APX005M should be held until the patient is clinically stable and has recovered from the acute effects of surgery.

5.3.7 Live vaccines NOT allowed

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 are not allowed.

5.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s) or disease progression, treatment may continue for 36 courses (approximately 2 years) or until one of the Off Treatment Criteria applies noted in section 5.4.2.

Patients will be followed weekly during the first 2 courses and prior to course 3 – 36 till the end of therapy, and at the time of disease progression or completion of treatment. The medical history, physical exam (including height, weight, vital signs, and performance status), dates of drug administration, adverse events, neurologic exam, relevant laboratory evaluations, and routine neuro-imaging scans will be assessed. Disease assessment studies should be uploaded if required per protocol (Section 9.2) Please review Section 10 of the protocol for the required assessments and intervals.

If the patients are tolerating APX005M and non-progressive at 2 years, the patients may request to continue non-protocol APX005M treatment from Apexigen.

5.4.1 On Study Data Submission Schedule

Pre-treatment, on-study and off-treatment data, as well as patient response data are to be recorded in the electronic data collection screens using the PBTC-051 database. See the Required Data and Timetable for Submission form located on the PBTC-051 Protocol webpage for the schedule. For assistance, contact the PBTC Protocol Coordinator listed on the cover page. An optional roadmap is located on the PBTC-051 Protocol Webpage.

5.4.2 Off Treatment Criteria

At the discontinuation of treatment, the "Off Treatment Date" is to be recorded in the eCRF and is to be consistent with the reason given for going off treatment. The "Last Treatment Date" is defined as the last date that the patient received protocol based therapy. Date of "off treatment" must be the greatest of the date of last treatment, date of procedure, date of patient assessment, notification of patient/family decision, or decision made by the physician that resulted in the patient being taken off protocol treatment. The reason for discontinuation of treatment must be documented by the attending investigator in the medical record and recorded in the eCRF.

Patients will be considered Off Treatment for the following reasons:

- Development of unacceptable toxicity as outlined in section 5.2.1. See section 7 for specific reporting requirements.
- Progressive disease (PD) as described in section 11.3.
- Development of a medical or psychiatric illness that in the investigator's judgment renders the patient incapable of further therapy on this protocol or the treating physician determines continuation on this study is not in the patient's best interest.
- The patient, parent or legal guardian refuses further treatment on this protocol. In this case the investigator should clarify if the family also wishes to withdraw consent for continued participation for data collection purposes.
- Completion of all protocol defined treatment
- Pregnancy
- Non Compliance that in the opinion of the investigator does not allow for ongoing participation.

Patients who are off protocol therapy must be followed until an "Off Study Criterion" is met.

5.4.3 Data Submission Schedule for Patients Off Treatment

All patients must be followed for a minimum of 30 daysfrom last treatment date. Toxicities that are considered at least possibly related to APX005M and are ongoing at the end of day 30 of last treatment date will be followed until resolution or return to baseline unless consent is withdrawn for further data submission or follow-up.

Patients in long term follow-up (Stratum 2 DIPG patients) will continue to be followed for 3 years from the initiation of protocol treatment to document any unexpected later developing toxicities or other morbidity and to document disease progression and survival. The requested follow-up data, including imaging uploads requested in Section 9.2, are to be submitted quarterly in the RAVE database from the date the patient went off treatment. Data to be recorded during this interval are the following:

- Any adverse events that are possibly, probably or definitely related to APX005M (see Section 7.3 for specific reporting timelines)
- Dates of disease progression
- Date of progression assessments and the associated results
- Date of commencement of new anticancer therapy
- Date of most recent contact
- Date of death

5.4.4 Criteria for Removal from Study

Patients who are off protocol therapy will be followed until they meet criteria for removal from study. The date and reason for the patient coming off study must be documented in the eCRF and the Operations, Biostatistics and Data Management Core (OBDMC) must be notified according to standard reporting guidelines (see section 5.4.3, sections 7, section 12, and the Required Data and Timetable Submission form located on the PBTC-051 protocol webpage).

Ongoing AEs, or AEs that emerge after the patient is removed from protocol therapy but within 30 days of the last dose of study treatment, must be followed and reported via Rave and CTEP-AERS (if applicable). Toxicities that are at least possibly related to the study treatment and are

ongoing at the end of day 30 of last treatment date will be followed until resolution or return to baseline, whichever is longer.

- Patient determined to be ineligible.
    - If the patient was found to be ineligible after starting treatment, follow-up should continue for 30 days from the last administration of study drug.
- Patient did not initiate treatment on study.
- Parent, patient, or guardian withdraws consent for further required observations or data submission.
- Patient death while on study. The IRB, Investigational Drug Branch (IDB), Study Chair and OBDMC must be notified as per section 7.
- Patient has completed study follow-up as defined per 5.4.3.
- Lost to follow-up
    - Sites must make every attempt to continue follow-up of patients during the study period as specified in Section 5.4.1. Multiple attempts must be made and documented to obtain required study data before patient can be declared lost to follow-up. The study Protocol Coordinator and Study Chair must be notified before a subject is declared lost to follow-up.
- DIPG patients treated in Stratum 2 who are removed from treatment for reasons other than withdrawal of consent or death will be followed until death or 3 years from diagnosis, whichever is earlier.

5.4.5 Data Submission for Patients Off study

No data will be collected documenting treatment or reporting events or disease status that occurs subsequent to the official "off study" date with the exception of adverse events with an attribution of possible, probable, or definite that occur after the "off study" date for agents being studied (see section 7).

6 DOSING DELAYS/DOSE MODIFICATION

If a subject fails to meet criteria to start subsequent course (section 5.1.3) then treatment with investigational product should be delayed and the subject should be re-evaluated weekly. Any subject who fails to recover from a disease or treatment-related toxicity to baseline or ≤ Grade 1 (except alopecia and Grade 2 fatigue) within 2 weeks of scheduled retreatment, or within 1 week of scheduled retreatment if toxicity is related to cytokine release, should discontinue the investigational product.

Subjects requiring more than 2 dose reductions, or dose reduction below dose level 0, must discontinue the investigational product.

Management of suspected adverse drug reactions may require temporary interruption and/or dose reduction of APX005M. If a subject experiences several toxicities, the recommended dose adjustment should be based on the highest grade toxicity. All dose reductions for APX005M, if required, will follow the dose levels defined in

Table **2**.

6.1 Notification of the Study Chair

The study chair or co-chair must be notified of any dosage modifications.

6.2 Hematologic and Non-hematologic Adverse Events and Management

6.2.1 Dose Modifications for Hematologic Toxicity

Dose adjustments are based on nadir blood counts since the preceding administration of investigational product.

If a patient experiences dose-limiting hematological toxicity as outlined in Section 5.2.1.2, APX005M treatment will be reduced to the next lower dose level. If the toxicity resolves to meet on study parameters within 14 days of drug administration, provided there is no current active bleeding in the case of dose-limiting thrombocytopenia, the patient may resume therapy at one dose level lower. Patients who are dose-reduced for toxicity will not have their dose re-escalated. If toxicity does not resolve to meet on study parameters within 14 days of drug administration, the patient must be removed from protocol therapy. Two dose-reductions per patient are allowed. If any dose-limiting toxicity occurs in a patient already dose-reduced twice for toxicity, the patient must be removed from protocol therapy. APX005M dose adjustments for hematologic toxicity during treatment are described in Table 3.

Table 3: APX005M Dose Modifications for Hematologic Toxicity

APX005M related toxicity	APX005M dose modification
Grade 4 Neutrophil count decrease	Dose reduction
Grade 3 Neutrophil count decrease with fever	Dose reduction
Grade 4 Thrombocytopenia	Dose reduction
Grade 3 Thrombocytopenia on 2 separate days or requiring a platelet transfusion on 2 separate days within a 7 day period	Dose reduction

6.2.2 Dose Modifications for Non-hematologic Toxicities

APX005M dose adjustments for non-hematologic toxicity during treatment are described in Table 4. All dose modifications should be made based on the worst preceding toxicity. For all toxicity ≤ Grade 2, the current dose level should be maintained.

Table 4: APX005M Dose Modifications for Non-hematologic Toxicity

APX005M-related toxicity	Action taken with APX005M
Nausea/Vomiting Grade 4, or Grade 3 ≥ 5 days	Dose reduction
Diarrhea Grade 4, or Grade 3 not responsive to optimal medical treatment for > 5 days	Dose reduction
Cytokine release syndrome Grade 3 ≤ 7 days	Dose reduction
Cytokine release syndrome Grade 3 > 7 days	Discontinue APX005M
Cytokine release syndrome Grade 4	Discontinue APX005M

Other Grade ≥ 3 toxicities (except alopecia) [§]	Adjust as medically indicated after discussion with study chair

[§] In the event of the "other" Grade 3 or 4 non-hematologic toxicities that, in the opinion of the Investigator, are unrelated to the investigational product, subject may continue on therapy with or without dose reduction only after documented discussion with the study chair.

6.2.3 Infusion Reaction/Cytokine Release Syndrome Precautions

All subjects receiving APX005M should be carefully monitored during and after the infusion. Infusion-related reaction/cytokine release syndrome precautions should be observed during the administration of APX005M. Emergency care agents including oxygen, oral and endotracheal airways, intubation equipment epinephrine, antihistamines, and corticosteroids should be available and used if required at the Investigator's discretion.

Subjects should be instructed that symptoms associated with cytokine release syndrome can occur at any time following the administration of the APX005M, and if such symptoms develop while they are at home, they should contact the Investigator and/or seek emergency medical care if appropriate.

The infusion can be interrupted in the case of an infusion related reaction/cytokine release syndrome (see Section 5.1.2).

6.2.4 Management of Infusion-Related Reaction/Cytokine Release Syndrome

Steroids should not be used routinely to prevent or treat infusion-related reaction/cytokine release syndrome, as steroidal therapy may significantly impair the therapeutic benefit, however, these suggestions do not contraindicate the use of any medicine clinically needed under emergency circumstances including epinephrine, diphenhydramine, methylprednisolone or other steroids, nebulized albuterol, or any other medicine needed, including additional narcotics to manage treatment-related symptoms, as clinically indicated.

In the event of toxicities consistent with infusion-related reaction/cytokine release syndrome, consider the following recommendations (Table 5Table 5)

Table 5:

Cytokine Release Syndrome/Infusion Related Reaction Management Recommendations	
Suspected Cytokine Release Syndrome/Infusion Related Reaction*	Recommended Treatment
• Grade 1 cytokine release syndrome/infusion related reaction – Mild toxicity requiring symptomatic treatment only (e.g., fever with or without constitutional symptoms, nausea, fatigue, headache, myalgia, malaise)	• Vigilant supportive care • Interrupt infusion, once symptoms resolve restart at 50% of the initial infusion rate if tolerable • Maintain adequate hydration • Antipyretics, nonsteroidal anti-inflammatory drugs, antihistamines, anti-emetics, analgesics as needed • In case of mild symptoms persisting for > 48 hours

<table>
<tr><th colspan="3">Cytokine Release Syndrome/Infusion Related Reaction Management Recommendations</th></tr>
<tr><td colspan="2">Suspected Cytokine Release Syndrome/Infusion Related Reaction*</td><td>Recommended Treatment</td></tr>
<tr><td colspan="2"></td><td>assess for infection, empiric treatment of concurrent bacterial infections</td></tr>
<tr><td rowspan="2">• Grade 2 cytokine release syndrome/infusion related reaction – Symptoms or clinical findings requiring and responding to moderate intervention, such as:
o O2 requirement < 40%
o Hypotension responsive to fluids</td><td>No extensive comorbidities</td><td>• All of the above
• Monitor cardiac and other organ functions closely</td></tr>
<tr><td>Extensive comorbidities</td><td rowspan="2">• All of the above
• Administer tocilizumab first, followed by corticosteroids if symptoms worsen or do not improve in < 4 hours.</td></tr>
<tr><td colspan="2">• Grade 3 cytokine release syndrome/infusion related reaction – Symptoms or clinical findings requiring aggressive intervention, such as:
o O2 requirement ≥ 40%
o Hypotension requiring low dose of 1 vasopressor (e.g., < 50 mg/min of phenylephrine)
o Ventilator support required
o CTCAE Grade ≥3 organ toxicity</td></tr>
<tr><td colspan="2">• Grade 4 cytokine release syndrome/infusion related reaction – Life-threatening consequences; urgent intervention indicated</td><td>• All of the above
• Permanently discontinue APX005M</td></tr>
</table>

* It can be very difficult to distinguish an infusion related reaction from cytokine release syndrome or allergy. In this study all reactions that occur during the infusion or shortly following completion of the infusion should be coded as infusion related reactions and the specific signs and/or symptoms should be noted.

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2 and 7.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) in addition to routine reporting. The coding of attribution in the PBTC-051 database pertains to adverse events related to APX005M.

- *Baseline Abnormalities*

Any baseline (pretreatment) abnormalities observed during the initial physical examination should be recorded in the PBTC-051 database.

- *Treatment or within 30 days of treatment*

Only record adverse events grades 1 and 2 if the attribution is at least possibly related to APX005M. Record all adverse events grades 3 through 4 and deaths, regardless of attribution on the electronic case report forms.

7.1 Potential Risks and Side Effects

7.1.1 Adverse Event Lists for APX005M

COMMON, SOME MAY BE SERIOUS In 100 people receiving APX005M, more than 10 and up to 100 may have:
• Fever, chills • Fatigue, asthenia • Nausea • Vomiting • Diarrhea • Headache • Pruritus • Rash • Abnormal liver function as seen on blood tests (transaminases e.g. ALT and/or AST, increased in your blood • Infusion related reaction during or following the infusion of the drug which may cause fever, chills, rash, hypotension (low blood pressure) • Blood alkaline phosphatase increased • Decreased appetite • Blood gamma-GT increase • Cytokine release syndrome which may cause fatigue, chills, fever, pruritus, vomiting, nausea, flushing, headache, diarrhea, rash, hypotension.

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving APX005M, from 5 to 10 may have:
• Hypotension (low blood pressure) • Hypertension (high blood pressure) • Anemia • Arthralgia • Tachycardia • Dyspnea • Blood bilirubin increased • Myalgia • Flushing

RARE, AND SERIOUS In 100 people receiving APX005M, 5 or fewer may have:
• Thrombocytopenia

RARE, AND SERIOUS In 100 people receiving APX005M, 5 or fewer may have:
• Abdominal pain • Amylase increased • Blood creatinine increased • Lipase increased • Dizziness • Hyperthyroidism • Urticaria • Malaise • Chest discomfort • Guillain-Barre Syndrome

Other symptoms: Other symptoms might also occur, including rarely, myocardial infarction and/or death.

Pregnancy: Females of reproductive potential should be advised to avoid becoming pregnant while being treated with APX005M. Female subjects with childbearing potential and male subjects should use effective contraception methods (or abstain from sexual activity) while being treated with APX005M and for 30 days following treatment. There are no reliable data on the effects of any of the CD40-agonistic mAbs in pregnant women. If APX005M is used during pregnancy, or if the subject (or male subject's partner) becomes pregnant while taking APX005M, the subject (and/or partner) should be apprised of the potential hazard to the fetus.

Overdose: There is no known specific antidote for an APX005M overdose. In the event of an overdose, monitor the subject and provide appropriate supportive care. Administration of APX005M after an overdose should only be resumed following consultation with study chair and in concordance with the retreatment criteria stated in the protocol.

7.2 Adverse Event Characteristics

7.2.1 CTCAE term (AE description) and grade:

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for recording AEs in the RAVE database. CTCAE version 5.0 will be utilized for expedited AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

7.2.2 Attribution of the AE:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.

- Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (Section 7.3.2).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to PBTC OBDMC Operations Office by telephone at [redacted]. Once Internet connectivity is restored, the 24-hour notification phoned-in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.1 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and the responsible Protocol Coordinator at the PBTC, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

The following must be copied on expedited reports (24-hour notification and the complete report) submitted via CTEP-AERS:

Study Chair:
Ira Dunkel, MD
Memorial Sloan Kettering Cancer Center
Department of Pediatrics
[redacted]
E-mail: dunkeli@mskcc.org

PBTC OBDMC:
Suresh "Rama" Ramanathan St. Jude
Protocol Coordinator-Pediatric Brain Tumor Consortium
Memorial Sloan Kettering Cancer Center
[redacted]
Email: suresh.ramanathan@stjude.org

Co-PI
Holly Lindsay, MS, MD
Texas Children's Hospital Feigin Center
Baylor College of Medicine
[redacted]
Email: hblindsa@txch.org

St. Jude Regulatory Affairs
St. Jude Children's Research Hospital
[redacted]
Email: regulatoryaffairsteam@stjude.org

The OBDMC will email or fax the SAE /CTEP-AERS report, within 48 hours of their awareness of the event to **Apexigen** by Email: drugsafety005@apexigen.com, drugsafety@apexigen.com,

dlsspvdrugsafety@eversana.com, or eFax: [redacted]. SAE reports will be automatically forwarded to EVERSANA, which is a Global Drug Safety Provider authorized by Apexigen to manage Serious Adverse Events on clinical trials using APX005M.

The IND holder, St. Jude Children's Research Hospital, shall notify the FDA of any event that is both a serious suspected adverse reaction and unexpected in accordance with FDA rules and regulations (21 CFR 312.32). Follow-up information to a safety report will be submitted, as requested.

The IND Sponsor or designee will submit a report of the unexpected, suspected adverse event to the FDA. The FDA prefers these reports to be made on a MedWatch 3500A form, but alternative formats are acceptable (e.g. a summary letter). The report should describe the event as fully as possible. Supporting documentation (lab reports, summary notes, and autopsy report) should accompany the report. A fatal or immediately life-threatening suspected adverse event will be reported to the FDA within 7 calendar days of the receipt of the initial report by the IND sponsor. A non-fatal, non-life threatening unexpected, suspected, serious adverse event will be reported to the FDA within 15 calendar days of receipt of the initial report by the IND Sponsor.

The PBTC OBDMC will post all IND Safety Letters on the PBTC-0**51** webpage. Sites will be notified via email of the receipt of the IND Safety Letter(s) and instructed to submit these to their local IRB in accordance with the institution's requirements.

A copy of the Annual Progress Reports is submitted by the IND holder as required by FDA. This submission will be cross referenced according to local regulations to the **Apexigen/APX005M** IND at the time of submission.

7.3.2 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:
1) Death
2) A life-threatening adverse event

3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.3.3 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must also be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

7.3.4 Second Malignancy and Secondary Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

A secondary malignancy is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

8 AGENT INFORMATION

A list of the adverse events and potential risks associated with the investigational agent APX005M administered in this study can be found in Section 7.1.

8.1 APX005M (CD40 agonistic monoclonal antibody)

Classification: Monoclonal Antibody

Dosage Form: APX005M is supplied in 20 mL Type 1 clear glass vials for IV injection. Each vial contains 10 mg/mL APX005M in a sterile, clear to slightly opalescent, colorless to slightly yellow, preservative-free solution (pH 5.5) containing 25 mM sodium acetate, 248 mM trehalose, and 0.02% polysorbate 20 in water for injection (WFI). The 20 mL vials are intended for single use. The target fill of each vial is 16.9 mL; the amount of APX005M that can be reliably extracted is 16.3 mL (163 mg)/vial.

Route of Administration: APX005M is intended for IV infusion. For use, APX005M is diluted in normal saline to a final volume of 50 mL and infused via IV infusion.

Structural Formula: APX005M is a full-length humanized mAb comprising 2 human gamma 1 heavy (H) and kappa light (L) chains. The molecular weight of APX005M is approximately 150 kDa. APX005M carries a mutation at position 267, from serine to glutamic acid (S267E), in the Fc region. M.W.: 150 kDa

Mechanism of Action: APX005M is a humanized IgG1κ-agonistic mAb that binds to CD40. APX005M binds to both human and cynomolgus monkey CD40 with high affinity, triggering activation of B cells, monocytes, and dendritic cells and stimulating cytokine release from both human and monkey lymphocytes and monocytes. APX005M does not bind to mouse or rat CD40. CD40 is also expressed on many human tumor cells, and APX005M can mediate a direct cytotoxic effect on CD40+ tumor cells.

Activation of CD40 on tumor cells results in tumor cell apoptosis and inhibition of tumor growth[13]. CD40 agonistic antibodies have demonstrated potent antitumor immune response stimulation in both animal models and cancer patients. Due to its action on both immune and tumor cells, CD40 has been studied as a target for novel cancer immunotherapy.

General Description of Manufacturing: APX005M is produced by mammalian cells (Chinese hamster ovary [CHO]) in serum-free cell culture production medium (Boehringer Ingelheim, Fremont, CA). APX005M is secreted into cell culture medium during cell culture production, recovered from the medium, and purified using a series of chromatographic and filtration steps.

Availability:
APX005M is provided by the Apexigen, Inc.

8.2 APX005M Storage

APX005M is supplied in 20 mL Type 1 clear glass vials for IV injection. The 20 mL vials are for single use. Vials of APX005M must be stored at 2°C to 8°C (36°F to 46°F) and stored in their original folding carton to protect from light. During preparation and administration of diluted APX005M product, protection from light is not required.

Preparing APX005M infusions requires the removal of vials of APX005M from refrigeration. Storage temperature excursions of:

- > 8ºC to 25ºC for up to 14 days (336 hours)
- > 25ºC to 40ºC for up to 2 days (48 hours)

Investigational product that has been exposed to temperature ≥ 40ºC must not be used.

The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations. It is the responsibility of the Investigator to ensure that the investigational product is only dispensed to study subjects. APX005M must be administered to study subjects by qualified personnel.

8.2.1 Safety Precautions

When handling investigational product, wear laboratory coats and disposable protective gloves. Avoid contact with eyes, skin, and clothing. Protect it from light and contamination.

8.3 Infusion Solution Preparation

8.3.1 Timing of Preparation and Administration

It is best to prepare IV infusion solutions as close to the anticipated time of infusion as possible.
IV infusion solutions are to be stored at room temperature prior to dosing. Prepared infusion solutions must be administered within 4 hours following preparation. Prepared solutions > 4 hours old should be discarded.

8.3.2 Preparation of APX005M Solutions

Due to the relative low amount of investigational product to be administered in the Dose Level 0 (0.03 mg/kg) or patients (weight < 40 Kg), infusion preparation in these cases will include an

intermediary dilution step where investigational product (APX005M 10 mg/mL) will be diluted with normal saline to provide an APX005M solution of a lower concentration to allow more accurate dose administration.

APX005M infusions for doses ≥ 5 mg could use original concentration APX005M as provided in the vial(s).

Lower concentrations of APX005M should be prepared as close to the intended solution preparation start time as possible (use 0.05 mg/mL concentration if amount of APX005M to be delivered is less than 1 mg). The 4 hours of solution stability begins with the preparation of a lower concentration.

Intravenous solutions of investigational product will be prepared using full sterile compounding procedure where both the exact amount of APX005M and the exact amount of base solution will be added to an empty IV or admixture bag or container. All containers (intermediary dilution and final solution) should be labeled with the exact amount of APX005M and volume/concentration.

8.3.2.1 Preparation of (Intermediary) Lower Concentration of APX005M

Table 6: Investigational Product Dilution Concentrations (Intermediary Dilutions)

	Amount	**Concentration (mg/mL)**
Solution #1 (0.05 mg/mL concentration) (APX005M doses < 1 mg should use 0.05 mg/mL concentration)		
Withdraw undiluted APX005M	0.5 mL	5 mg/0.5 mL
Dilute with Normal saline for injection	99.5 mL	-
	100 mL	0.05 mg/ mL
Solution #2 (0.1 mg/mL concentration) (APX005M doses 1 mg to < 2 mg should use 0.1 mg/mL concentration)		
Withdraw undiluted APX005M	1 mL	10 mg/mL
Dilute with Normal saline for injection	99 mL	-
Final solution volume	100 mL	0.1 mg/mL
Solution #3 (1 mg/mL concentration) (APX005M doses 2 to < 5 mg should use 1 mg/mL concentration)		
Withdraw undiluted APX005M	1 mL	10 mg/mL
Dilute with Normal saline for injection	9 mL	-
Final solution volume	10 mL	1 mg/mL
Original concentration 10 mg/mL (APX005M doses ≥ 5 mg should use 10 mg/mL undiluted solution)		

8.3.3 Preparation of Final APX005M Infusion Solution

Table 7: APX005M Infusion Solution Preparation

Step 0 is not required if the 10 mg/mL undiluted APX005M is used to prepare the final infusion.

Step 0:	Prepare Intermediary APX005M solution of appropriate concentration to allow the most accurate preparation of investigational product for administration.
Step 1:	Determine amount in mL of APX005M solution required to deliver desired amount of APX005M
Step 2:	Withdraw entire contents of a compatible Normal Saline for Injection 50 mL IV bag into a 60 mL syringe.
Step 3:	Inject the amount of APX005M solution into the IV bag
Step 4:	Inject a sufficient amount of Normal Saline in to the IV bag containing APX005M to provide a final volume of 50 mL for infusion
Step 5:	Label admixture per institutional policy including the Subject ID No., the name of study drug, the amount of APX005M (mg), and the final volume of the infusion (mL)

The goal of APX005M Infusion Solution preparation is to ensure that an appropriate dose (amount) of APX005M is admixed with the base solution to provide the accurate exposure of APX005M. Do not co-administer other drugs through the same infusion line

8.4 APX005M Infusion Procedure

APX005M solution for IV infusion may be administered by using an intravenous infusion pump using compatible IV bags, infusion sets, and materials.

The following IV solution containers and equipment have been evaluated for compatibility with APX005M by Apexigen. If a component of the infusion set-up planned for use is not listed in the table below, please contact Apexigen at APX005M-PM@apexigen.com to confirm acceptability of the component.

Prequalified Intravenous Equipment	
IV bag *, Normal Saline for Infusion	0.9% NaCl Viaflex 250 mL (PVC/DEHP) (Baxter P/N 2B1322Q)
IV tubing	Continuo-Flo® (Baxter P/N ACT8570)
IV Tubing	Paclitaxel® administration set (Baxter P/N 2CC8857)
IV Tubing/filter	Alaris Pump Infusion Set 0.2 Micron Filter (BD P/N 11532269)
* 50 mL or 100 mL IV bags are acceptable	

8.4.1 Inline filter (at least 0.2 micron)

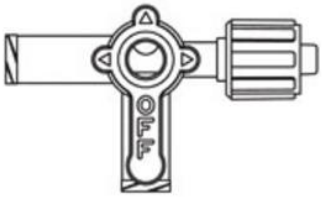
All infusions must administered using an in-line low protein-binding filter (0.2 or 0.22 micron).

Figure 1: Example of Inline Filter (not to scale)

8.4.2 Inline 3-way stop-cock

A 3-way stock-cock immediately before the IV access device and distal (downstream) of the APX005M infusion solution insertion site is recommended. In the event of an infusion-related reaction, the infusion can be immediately interrupted and supportive medications and fluids maybe administered through the "flush" port.

Figure 2: Example of 3-way Stopcock



8.4.3 APX005M Infusion Using an Intravenous Infusion Pump

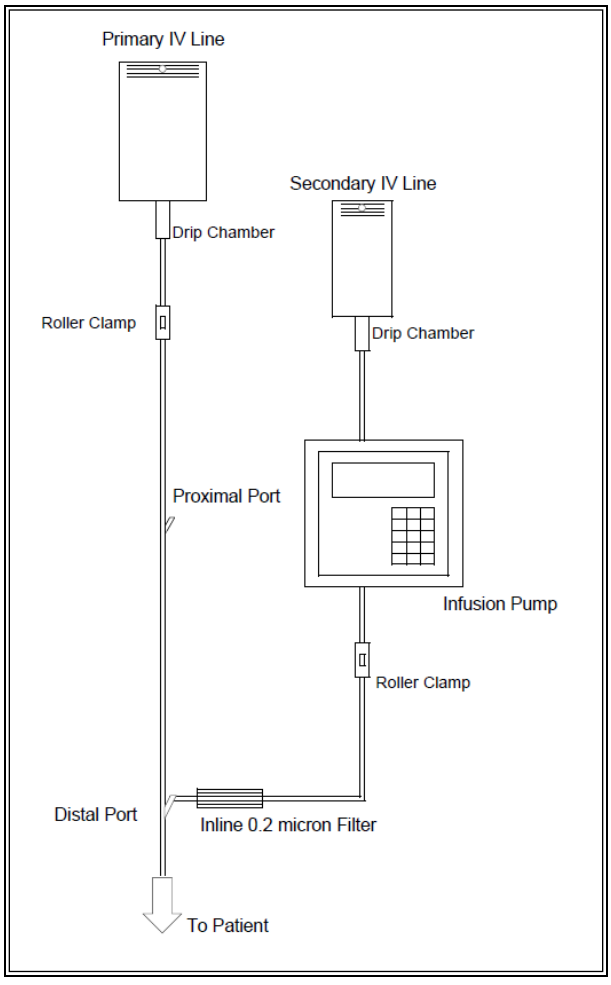
All IV preparation and infusion should be conducted under proper aseptic technique. Process steps with anticipated possible direct contact with APXM005 (e.g.: preparation and priming of APX005M) should include protective gloves. See Figure 3 for schematic of example IV infusion pump set-up.

Table 8: APX005M Administration Set-up (IV infusion Pump)

Step 1:	Assemble all equipment required for IV administration of APXM005
Step 2:	(Primary Line) Establish IV access with a primary IV line using Normal Saline for Injection; the primary IV line should be maintained at a KVO (keep vein open) rate. The purpose is to maintain constant low rate IV access
Step 3:	(Secondary Line) Using aseptic technique, puncture the IV infusion solution bag with the IV tubing (with built-in or added in-line low protein-binding 0.2 micron filter) and carefully prime the tubing and in-line filter to displace all air being careful not to waste any IV infusion solution
Step 4:	The secondary IV line (APX005M) should be connected as close the subject as possible. If using primary infusion tubing set with more than one injection port, use the most distal injection port. If using a 3-way stop-cock ensure that the stop-cock is as proximal to the subject as possible
Step 5:	Connect the primed IV tubing
Step 6:	Initiate the infusion at a rate intended to deliver the entire dose in approximately one hour.

	• Infusion is recommended to be 60 minutes (-5 to +10 minutes; i.e. 55 to 70 minutes) • Infusion may be interrupted for infusion-related reactions • Infusion is recommended to last no longer than 90 minutes • Infusion duration of > 90 minutes is acceptable based on subject tolerance but one should attempt to infuse within 60 to 90 minutes
Step 7:	Monitor for safety and tolerability
Step 8:	When IV infusion solution bag is empty, replace with second IV bag of plain NS
Step 9:	Continue infusion until sufficient amount of plain NS has been infused to ensure complete study drug administration (included in the 60-minute infusion period)
Step 10:	At completion of infusion and flushing procedure, remove the secondary IV tubing equipment and dispose of appropriately

Figure 3: Schematic IV infusion Setup with IV infusion Pump



8.5 Agent Ordering and Agent Accountability

8.5.1 Agent Ordering

The PBTC sites need to place an order for APX005M starter supplies upon site protocol activation. The shipment is anticipated to contain one-year supply for 2 subjects to minimize the amount of APX005M at member sites. For subsequent orders, sites are expected to request enough study drug to supply all potential patients for 6 months. Use the Apexigen Clinical Supply Shipment Request Form, which is posted on PBTC-051 webpage when ordering. Allow 7 business days from the request to receipt of APX005M.

Note: APX005M will not be shipped on Fridays.

Upon receipt, verify contents, complete drug acknowledgement form, and email back to Apexigen. Discrepancy or damage noted in the contents or if the shipment does not arrive within 7 business days, immediately notify Apexigen by the following addresses:
IPRequest@apexigen.com
Philadelphia.AOR@catalent.com

8.5.2 Agent Inventory Records and Accountability

The investigator, or a responsible party designated by the investigator, must maintain accurate records regarding study agent receipt, dispensing, use and disposal using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF). Store and maintain separate NCI Investigational Agent Accountability Records for each ordering investigator on this protocol. Records should include:

- Quantity of APX005M received and placed in storage area
- Quantity of APX005M currently in storage area
- APX005M label information
- Dates and initials of person responsible for each APX005M inventory entry/movement
- Quantity of APX005M administered to each subject. Documentation should include unique subject identifier
- Quantity of APX005M transferred to another area/site for dispensing or storage
- Non-study disposition of APX005M (e.g., lost, wasted, and/or broken)
- Quantity of APX005M destroyed at study site, if applicable
- Retention of used or partially used vials is not required.

8.5.3 Return and Destruction of APX005M

Upon completion or termination of the study, all unused APX005M can be destroyed at the site. It is the Investigator or designee's responsibility to dispose of all partial or empty containers, following procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures. Appropriate records of disposal are to be maintained in the site pharmacy.

9 BIOMARKER, CORRELATIVE AND SPECIAL STUDIES

This section contains the collection, shipping and handling information for all planned biomarker and exploratory correlative studies. The table below identifies the tests, sample type and amount, analyzing laboratory and whether it is required or optional. For additional details, please review the associated section below.

Test Name	Sample Type and Amount	Analyzing Laboratory	Required or Optional	Section ID
Pharmacokinetics (PK)	Serum from 2 mL whole blood (3.5 mL serum tubes)	PPD	Required	9.1.2
Anti-Drug Antibody (ADA) Assay	Serum from 2 mL whole blood (3.5 mL serum tubes)	PPD	Required	9.1.2
T cell phenotyping	PBMC (minimal 16 mL blood, ideal 32 mL blood) (8 mL CPT tubes) Plasma	P. Wong MSKCC IMF	Required	9.1.4
Circulating Cytokines		P. Wong MSKCC IMF	Required	9.1.3
Tumor Mutational Burden Assessment (Whole Exome Sequencing)	FFPE tumor tissue; PBMC (from 5 mL blood) (8 mL CPT tubes)	T. Chan Cleveland Clinic	Required	9.1.5
Repository Samples	PBMC and FFPE tumor tissue	PBTC	Optional	9.1.1

Blood sample prioritization: If there is limited blood samples to complete all mandatory assays, PK and ADA assays should be given priority.

9.1 Pathology and Exploratory Correlative Studies

The Pathology Central Review and Biorepository (CRB)'s function is to collect, distribute and store specimens for the planned correlative studies which support the laboratory objectives of this protocol.

The CRB will also serve as a central repository for specimens collected for future research and left over specimens (tumor tissue, blood) returned to the repository following the planned analysis from patients who consent to long term storage of unused specimen. These samples will be stored in the repository for undefined future studies which support the mission of the PBTC. **If the patient does not consent to participation in the repository, correlative study samples should be submitted following the guidelines in the appropriate correlative study section below. If the patient does not consent to long term storage, remaining correlative study samples will be destroyed once the PBTC-051 analysis is complete.**

9.1.1 PBTC CRB Submission Guidelines

If the patient consents to provide slides for submission to the repository at the time of participation in a PBTC trial the following should be submitted:

- Tumor material

Slides from the original and/or recurrent surgery should be prepared for storage. The site should provide up to twenty (20) unstained sections cut at 4µm in thickness on (+) slides from the most representative section. These submitted slides will be used for the conduct of studies outlined in section 9.1.5. Fewer unstained sections may be submitted based on size and availability of tissue. Preference is for tissue that has not previously been frozen. The corresponding pathology report (s) including immunohistochemical, special stains, and molecular/genetic results is to be

uploaded to the PBTC using the secure File Upload system. These reports will be made available to the pathologist via a link in the ProtoLab.

Suitability of sections would be established by preparing one (1) H&E to ensure that the sections meet the following criteria:

- histologically representative of the reported lesion
- contain at least 60% viable tumor
- no more than 40% necrosis

The following specimens may also be submitted to the CRB for distribution and storage:

- Scrolls: if sufficient tissue is available, 2 scrolls at 10 micron thickness as outlined in section 9.1.5
- Peripheral blood mononuclear cells: quantity and schedule of collection as outlined in section 9.1.4 and 9.1.5

- Peripheral Blood Mononuclear Cells

PBMC may be collected by processing a 2-5mL whole blood specimen with Ficoll or collecting the specimen in a BD Vacutainer™ CPT™ Cell Preparation Tube with Sodium Citrate or a similar tube which is available locally. Once separated, all pellets must be snap frozen and stored at least at -20ºC prior to dry ice shipment

9.1.1.1 Specimen Collection and Processing by Ficoll tube

- Collect 2 - 5mL of fresh blood into an EDTA tube.
- Transfer blood into a sterile 50-mL tube and add double the amount of PBS. MIX GENTLY.
- Set up another tube containing half of its total volume of Ficoll. For example, if there is 15 mL of blood + PBS, then use 7.5 mL of Ficoll (2:1 ratio).

At very slow pace (approx. 2 mL/minute), layer the blood + PBS mixture onto the Ficoll so that the solutions DO NOT MIX. Spin the blood/Ficoll at 750 g in slow mode for 30 minutes @ 25ºC. After spin you will see four distinct layers: plasma (top layer), white fluffy ring (2nd layer), Ficoll (3rd layer), and blood (bottom layer).

- Remove plasma layer down to about 1 mL above the white fluffy ring and discard.
- Collect the entire white fluffy ring. If ring is hard to see, also take extra liquid above. Then discard everything else.
- Place this fraction of white blood cells into a fresh 50 mL sterile tube with 20 mL of PBS. Spin down for 10 minutes @ 25ºC, 750 g in fast mode. Remove the supernatant. Add back to pellet 1 mL of PBS and spin for 5 min. at 4°C at 10000rpm. Remove supernatant.
- Freeze the pellet of WBCs in cryovials that are 2 mL or smaller and store at -80ºC until shipment.
- Ensure that all tubes are clearly labeled with the PBTC patient accession number, sample type, collection date and Study ID. Please ensure that the labeling system used is designed to withstand temperatures down to -80ºC. Samples should be stored at -80ºC until shipment. For short term storage (2-3 weeks) -20ºC is acceptable. NOTE 4ºC IS NOT ACCEPTABLE STORAGE.

If it is not possible to collect the PBMC by Ficoll gradient then separation of PBMC can be conducted using CPT tube separation as an alternative. However the PBMC pellet MUST BE frozen immediately and stored at -80ºC.

9.1.1.2 Collection and Processing by CPT tube

- Peripheral blood should be collected in a BD Vacutainer CPT™ Cell Preparation Tube with Sodium Citrate or similar tube which is available locally. 8 mL and 4 mL CPT tubes can be obtained from Fisher Scientific (Cat# 02-685-125, 02-688-81) or Becton-Dickinson (BD No.362761, 362760). The 8 mL tubes have a 6 mL minimum draw and the 4 mL tubes have a 3 mL minimum draw.
- Centrifuge the CPT™ tube at 1500 x g for 30 minutes at room temperature (20º C to 25º C). DO NOT APPLY THE BREAK ON THE CENTRIFUGE. Use acceleration 5, brake 0 ("slow mode").
- It may be necessary to spin the tube longer to ensure that all of the red blood cell components have been separated from the plasma layer through the polyester gel barrier.
- The tube should be removed immediately from the centrifuge. The mononuclear layer and plasma lie above the polyester gel plug.
- Using a sterile pipette, remove as much of the plasma component (upper half of the CPT tube) without disturbing the mononuclear layer if possible and discard.
- Transfer mononuclear cell layer (and some residual plasma layer) to a labeled 15- mL conical centrifuge tube and add 5 mL sterile room temperature magnesium or calcium-free phosphate buffered saline (PBS) to fill the conical tube and recap.
- Centrifuge at 450 x g for 10 minutes at room temperature (20º C to 25º C). Use acceleration 9, brake ("fast mode").
- Remove supernatant, being careful not to aspirate the cellular pellet at the bottom of the tube.
- Add 1 mL of sterile PBS to the pellet and gently re-suspend by pipetting up and down. Transfer the entire suspended pellet to the labeled cryovial that is 2 mL or smaller.
- Centrifuge the cryovial at 450 x g for 5 minutes (or spin down the microcentrifuge tube at 1300 x g for 5 minutes) at room temperature. Discard the supernatant. Store the cell pellet cryovial frozen at -80ºC. For short term storage (2-3 weeks) -20º C is acceptable.

9.1.1.3 Handling of Specimens

- Slides are to be labeled with the study ID and the patient PBTC Accession # and these slides should be designated as PBTCR # (where the # assigned from 1 to 20, or the highest number of unstained sections prepared, sequentially) or PBTCR H&E for the H&E stained section.

- Scrolls or Formalin Fixed, Paraffin Embedded (FFPE) tumor materials are to be labeled with the PBTC Accession # and the PBTC study for which the sample is provided. Scrolls or FFPE tumor material should be shipped at room temperature.

- PBMCs are to be labeled with the PBTC Accession #, sample type, date of collection and the PBTC study ID for which the sample is provided. The PBMCs should be put in

cryovials that are 2 mL or smaller. Samples should be shipped overnight in a separate box with a 2-day supply of dry ice.

9.1.1.4 Shipment of Specimens

Samples collected for the repository should be sent to the PBTC CRB overnight via FedEx by completing the Internet form at http://www.fedex.com/us/ and requesting FedEx to e-mail listed below. FedEx user ID and password for pathology shipping can be found at PBTC-051 protocol webpage. Samples should be shipped in the appropriate environment as described in Section 9.1.1. Samples are to be shipped to:

PBTC CRB
Research Support and Biorepository Services
Children's Hospital Los Angeles
[redacted]
Email: ClinicalLabResearchServices@chla.usc.edu

9.1.2 Pharmacokinetics (PK) and Anti-Drug Antibody (ADA) assays

Pharmacokinetics (PK) and Anti-Drug Antibody (ADA) studies will be obtained from all patients enrolled on this study.

9.1.2.1 Collection of Specimens

Sampling Strategy for Pharmacokinetic Study

Course 1 and 2: serial blood samples for APX005M pharmacokinetic studies will be collected at the following times: pre-dose, at the end of infusion, and at 4, 24 ± 1 (Day 2), and 168 ± 4 hours (Day 8) from the start of the infusion in that course.
Course 3 and 4: serial blood samples for APX005M pharmacokinetic studies will be collected at pre-dose and at the end of infusion.

Sampling Strategy for ADA Assays

Serial blood samples for Anti-APX005M antibodies (ADA) will be collected prior to dosing on Course 1, 2, 3, 4, then every 3rd Course (Course 7, 10), and then every 4 courses (Course 14, 18, 22, 26, 30, 34) till the end of therapy (EOT) visit.

9.1.2.2 Handling of Specimens

At each time point, two tubes of 2 mL of blood (one for PK, another for ADA assay) will be collected into serum tubes such as BD366431 or 366430 which are appropriately labeled. The PK and ADA sample collection form should be completed with the exact time that the sample is drawn as well as the exact time that the drug is administered. The PK and ADA sample collection form is located on the PBTC-051 webpage. The samples should be labeled with the PBTC Accession number and the Course, Day and Time information for each sample.

Here is the instruction for serum processing:

Blood must not be drawn from the intravenous line used for infusion and should not be drawn from any indwelling venous access device proximal to the infusion site. For PK and ADA assays, please collect one serum sample for PK and one serum sample for ADA assay at each designated time point.

1) Allow vacutainer to fill completely
2) Gently invert the vacutainer approximately 5 times to allow complete missing of additive
3) Allow sample to clot at room temperature
4) Centrifuge to separate the clot from the serum (refrigerated centrifuge is recommended but not required)
   a. Spin at 1200g for 10 minutes
5) Remove the stopper of the vacutainer
6) Using a pipette, aliquot into 2 polyethylene freezer storage tubes (approx. 0.25 mL/tube)
7) Ensure that the samples are properly labeled with Subject ID Number, protocol-specified time point, date of collection, and time of collection.
8) Place properly labeled aliquots in – 70/-80º C freezer until shipment. (Ensure sample is fully frozen prior to packaging and shipping)

9.1.2.3 Shipping of Specimens

Samples must be shipped from the site to the respective laboratory within 30 days of last sample collection in order to receive cost reimbursement. The sample collection and shipping dates must be documented in the eCRF.

- Please store serum samples for PK/ADA at -80°C until shipment and ship to PPD on dry ice using Federal Express as the courier.

PPD

[redacted]

- Include an electronic shipping manifest with each shipment.

- Notify PPD of all shipments via email and cc, Meghana Rao, , and Jamie Grayson at Apexigen on the day of shipment.

RichmondSMOpeners@ppdi.com
- MRao@apexigen.com

JGrayson@apexigen.com
richmond_data@ppd.com

9.1.2.4 Site Performing Correlative Study

Apexigen will have the PK and ADA analyses performed by the Pharmaceutical Product Development (PPD), LLC.

9.1.3 Measurement of Circulating Cytokines in Human Plasma

Multiplex cytokine measurements will be carried out in isolated human plasma samples from patients before and after therapy using the Meso Scale Discovery (MSD) multiplex cytokine assay and electrochemiluminescence platform. Specifically, we will evaluate up to 10 pro-inflammatory and Th1/Th2 immune mediators (IFN-γ, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α) using the validated MSD V-Plex Human Pro-Inflammatory Cytokine Panel. Quality control samples with pre-defined levels of each cytokine will be used to verify assay performance. The Immune Monitoring Facility at MSKCC is equipped with the MSD QuickPlex SQ 120 Imager for detection and quantitation of ECL signals from this assay, and has extensive experience running cytokine immunoassays in human biological fluid samples.

9.1.3.1 Collection of Specimen(s)

Plasma used for this assay will be obtained from the plasma supernatant of the PBMC density gradient spins, from section 9.1.4.1 below.

9.1.3.2 Handling of Specimens(s)

The samples should be labeled with the PBTC Accession number and the Course, Day information for each sample.

9.1.3.3 Shipping of Specimens

The sample collection and shipping dates must be documented in the eCRF. All samples should be forwarded via FedEx, Monday through Thursday, by completing the internet form at http://www.fedex.com/us/ and requesting FedEx to email the laboratory. Fed Ex account information for the shipment of samples can be obtained on the PBTC website. Weekend deliveries are not permitted. Sites should contact the PBTC CRB of shipment. Biologic specimens should be shipped on dry ice, along with a completed Biologic Specimen Transmittal Form located on the PBTC-051 webpage to PBTC CRB as described in section 9.1.1.2.

9.1.3.4 Site Performing Correlative Study

Immune Monitoring Facility, Memorial Sloan Kettering Cancer Center

9.1.4 Characterization of T Cell Activation/Exhaustion Phenotype in Human PBMC

Multiparameter flow cytometry will be utilized to characterize T cell subset frequencies and the expression of a number of surface and intracellular markers of T cell activation and exhaustion. Specifically, a validated 11-color flow panel will be used to quantitate live, conventional and regulatory T cells as defined by CD3, CD4, CD8, and FoxP3 markers. Gated cell subsets will then be evaluated for expression of proliferative/costimulatory markers including Ki-67 and ICOS, as well as T cell exhaustion/inhibitory molecules such as CTLA-4, PD-1, TIM-3, and LAG-3. Marker positivity will be defined using the appropriate gating controls. The Immune Monitoring Facility at MSKCC is equipped with the BD Fortessa 4-laser flow cytometer for acquisition and analysis of cells stained with the flow panels in the core.

9.1.4.1 Collection of PBMC and Plasma Specimen(s) (for T cell phenotyping and Cytokine analysis respectively)

The blood samples (minimal 16 mL, ideal 32 mL) will be collected pre-treatment and 1-week post-the first dose of APX005M. Additional blood samples should be collected at 2 weeks, 3 weeks, 6 weeks, and 9 weeks post-treatment if feasible.

Collection and Processing of PBMC and Plasma Using *CPT tube*

- Peripheral blood should be collected in a BD Vacutainer CPT™ Cell Preparation Tube with Sodium Heparin. 8 mL CPT tubes can be obtained from Fisher Scientific (Cat# 14-959-51D) or Becton-Dickinson (BD No. 362753). The 8 mL tubes have a 6 mL minimum draw. Samples should ideally be processed within 2-4 hours of collection. In the event of a delay in processing, samples can be left at ambient temperature for up to 24 hours before processing preferably with gentle rotation to keep the anticoagulant in the tube mixed with the blood.
- Centrifuge the CPT™ tube at 1500 x g for 30 minutes at room temperature (20° C to 25° C). DO NOT APPLY THE BRAKE ON THE CENTRIFUGE. Set brake to 0 ("slow mode").
- It may be necessary to spin the tube 10-15 min longer to ensure that all of the red blood cell components have been separated from the plasma layer through the polyester gel barrier if separation does not occur during initial spin.
- The tube should be removed immediately from the centrifuge. The white mononuclear cell layer and yellow plasma lie above the polyester gel plug.
- Using a sterile pipette, remove as much of the plasma component (upper half of the CPT tube) without disturbing the mononuclear cell layer. Save plasma supernatant for cytokine analyses described in section 9.1.3 by distributing 0.5 mL aliquots into 1 mL cryovials and freezing at -20°C to -80°C.
- Transfer the mononuclear cell layer to a labeled 15-mL conical centrifuge tube and add sufficient sterile room temperature magnesium or calcium-free phosphate buffered saline (PBS) containing 10% heat-inactivated fetal bovine serum (FBS) to fill the conical tube to 15 ml and recap.
- Centrifuge at 450 x g for 10 minutes in a refrigerated centrifuge (4°C). Use acceleration 9, with brakes on ("fast mode").
- Remove supernatant, being careful not to aspirate the cellular pellet at the bottom of the tube.
- Resuspend cells into a total of 15 mL fresh PBS/10% FBS wash buffer and centrifuge again at 300 x g for 10 min at 4°C. Use acceleration 9, brakes on ("fast mode").
- Resuspend cells in 5-10 mL fresh wash buffer and place on ice.
- Remove 10 µl of the cell suspension and dilute in appropriate buffer for counting cells using a hemacytometer or automated cell counting instrument.
- Count cells, taking into account dilution factor, and record total yield and viability.
- Centrifuge cell sample at 250 x g 4°C, brakes on, for 10 minutes.
- Remove supernatant and resuspend cells at 6-8 x 10^6 viable cells/mL in ice-cold cell freezing media (FBS containing 10% DMSO) and aliquot 1 mL into labeled cryovials for each sample.
    - NOTE: For a total cell count below 6 x 10^6 viable cells, resuspend sample in a

minimum of 0.5 ml in 1 cryovial.

- Place cryovials in a Mr. Frosty or CoolCell -1°C/min controlled rate freezing container and place cooling container at -80°C for at least 24 hrs for controlled rate freezing.
- Transfer cells in cryovials to a liquid nitrogen freezer within a week of the initial -80°C freezing.

9.1.4.2 Handling of Specimens(s)

The samples should be labeled with the PBTC Accession number, sample type, date of collection and study ID for each sample.

9.1.4.3 Shipping of Specimens

The sample collection and shipping dates must be documented in the eCRF. All samples should be forwarded via FedEx, Monday through Thursday, by completing the internet form at http://www.fedex.com/us/ and requesting FedEx to email the laboratory. Fed Ex account information for the shipment of samples can be obtained on the PBTC website. Weekend deliveries are not permitted. Sites should contact the PBTC CRB for shipment, and specimens will be shipped to PBTC CRB. Biologic specimens should be shipped on dry ice, along with a completed Biologic Specimen Transmittal Form located on the PBTC-051 webpage to PBTC CRB as described in section 9.1.1.2.

9.1.4.4 Site Performing Correlative Study

Immune Monitoring Facility, Memorial Sloan Kettering Cancer Center

9.1.5 Tumor mutational burden assessment

Pre-trial frozen or FFPE tumor tissue (10-20 unstained slides) and PBMC (from 5 mL blood) will be submitted. We will perform whole exome sequencing on matched tumor and normal PBMC pairs. We will also perform RNAseq and TCR sequencing. We will analyze this data to determine how mutational burden, spectrum of neoantigens, and TCR repertoires influence patient response. We have pioneered these approaches for immunotherapeutics and will apply the expertise to dissecting the genomic and immunologic correlates of response versus resistance.

For DIPG patients without tumor samples, we will perform peripheral TCRseq analysis, which just requires PBMCs.

9.1.5.1 Collection of Specimen(s)

Frozen or FFPE samples will be obtained for tumor genomics analysis. Tumor samples should be collected and delivered to PBTC CRB. PBMCs should be collected as described in section 9.1.4.1 above.

9.1.5.2 Handling of Specimens(s)

The pre-treatment tumor tissue and PBMC should be labeled with the PBTC Accession number, sample type, date of collection and study ID.

9.1.5.3 Shipping of Specimen(s)

The sample collection and shipping dates must be documented in the eCRF. All samples should be forwarded via FedEx, Monday through Thursday, by completing the internet form at http://www.fedex.com/us/ and requesting FedEx to email the laboratory. Fed Ex account information for the shipment of samples can be obtained on the PBTC website. Weekend deliveries are not permitted. Sites should contact the PBTC CRB for shipment. Biologic specimens should be shipped in dry ice (for frozen tumor samples) or at room temperature (for FFPE blocks), along with a completed Biologic Specimen Transmittal Form located on the PBTC-051 webpage to PBTC CRB as described in section 9.1.1.2.

9.1.5.4 Site Performing Correlative Study

Immunogenomics and Precision Oncology Platform, Memorial Sloan Kettering Cancer Center.

9.2 Neuroimaging Studies

Patients will have MRI Brain with and without contrast performed within 3 weeks prior to enrollment, at the end of course 2, every 3 courses through course 14, and then every 4 courses till the end of therapy, and at the time of disease progression or completion of treatment. MRI Spine should be performed prior to therapy and at the same time points as standard MRI Brain, if clinically indicated. Tumor response will be determined for all subjects by comparison of the product of 2-dimensional measures of the tumor on the baseline and follow-up MRI scans using T-2 and/or FLAIR sequences. Due to concern regarding immune-related pseudoprogression, in the absence of clinical deterioration, any initial assessment of progressive disease (PD) will be confirmed by a repeat evaluation at the next tumor assessment time point, but no sooner than 4 weeks later.

Standard MR imaging will include Sagittal T1 MPRAGE, axial T2 FLAIR, axial T2, and post gadolinium sagittal T1 MPRAGE (with reconstructions). The standard MR parameters are listed on the PBTC NIC web page located at http://www.childrenshospital.org/research/centers-departmental-programs/pediatric-brain-tumor-consortium-neuroimaging-center under Neuroimaging Studies/ Specific MR Imaging Sequences- Open PBTC Protocols. There is also a link to the same PBTC-NIC webpage from the PBTC-051 protocol study page.

Volumetric analyses will be done at the Neuroimaging Center (NIC, Children's Hospital Boston) via the Vitrea (Vitrea™) workstation, from the axial FLAIR and T1-weighted post-contrast brain images.

9.2.1 Image Transfer

For those patients showing a response, the MRI scan from that date, the confirmation scan obtained approximately 3 courses later, if available and the corresponding baseline scan should be uploaded to the PBTC Neuroimaging Center (NIC) for central review.

All patient specific data are stripped from the images and replaced with PBTC Accession numbers prior to transmitting the images to the NIC. All image data transfer is accomplished using PGP (pretty-good-privacy) 128-bit encryption which meets industry standard for secure communication.

9.2.2 Neuroimaging Review

Local review of MR imaging studies at each site and central review of the MR imaging studies will be conducted through the PBTC Neuroimaging Center (NIC). NIC review will include assessment of response to therapy (as feasible). The director and one neuroradiologist of NIC will review the imaging studies at study completion. If the local and central review are not in agreement the NIC neuroradiologist will confer with the participating site to determine why there is a discrepancy via conference call.

10 STUDY CALENDAR

Data is to be submitted according to the Data submission timelines located on the PBTC-051 webpage.

	Pre-therapy	Course 1-2	Courses 3-Course 36	Completion/ Off Treatment
Physical Assessments				
Medical history	X	Weekly	X[A]	X
Physical exam /height/weight	X	Weekly	X[A]	X
Vital signs	X	Weekly	X[A]	X
Pulse oximetry	X	Weekly	X[A]	X
Performance status	X	Weekly	X[A]	X
Neurologic exam	X	Weekly	X[A]	X
Laboratory Evaluations				
CBC [B] (WBC, HgB, Platelets, ANC, ALC)	X	Weekly	X[A]	X
Serum Chemistry [B] (Sodium, Potassium, Bicarbonate, Chloride, Calcium, BUN, Creatinine, Glucose, Phosphorous, Magnesium, Albumin, Total Protein)	X	Weekly	X[A]	X
SGPT(ALT), SGOT(AST), Total Bilirubin [B]	X	Weekly	X[A]	X
Serum or Urine pregnancy test[C] (for females of childbearing potential)	X	X[A]	X[A]	
CD4 cell count [J]	X			
B cell count (CD19 cells in blood)	X	X[H]	X[H]	X
Serum Immunoglobulin G (IgG)	X	X[A]	X[A]	X
Coagulation (PT, INR, aPTT) [D]	X			
ECG (QTc)	X			
Echocardiogram or MUGA scan	X			X
CSF Cytology (if clinically indicated)[G]	X[G]	X[G]		
Imaging Assessments				
Brain MRI (standard) [E]	X	X	X	X
Spine MRI (if clinically indicated)[I]	X[I]	X[I]	X[I]	X[I]
Correlative Studies[F]				
Pharmacokinetic Studies[F]	X	X	X	
Anti-Drug Antibody Assay[F]	X	X	X	X
Circulating Cytokines[F]	X	X	X	
T cell phenotyping[F]	X	X	X	

Tumor Mutational Burden Assessment[F] (PBMC and tumor tissue) (Whole Exome Sequencing)	**X**			

A. To be completed prior to each course.
B. To be done more frequently throughout the duration of treatment if required to monitor toxicities.
C. Within 72 hours prior to starting treatment, if eligibility pregnancy test is within 72 hours, no need to repeat prior to Course 1.
D. Coagulation tests include PT, INR, and aPTT; INR is only required at screening and as clinically indicated thereafter.
E. Patients will have MRI Brain with and without contrast performed within 3 weeks prior to enrollment, at the end of course 2, every 3 courses through course 14, and then every 4 courses till the end of therapy, and at the time of disease progression or completion of treatment.
F. All the correlative studies are required. See section 9.1 for collection schedule and sample requirements.
G. Cerebrospinal fluid assessment via lumbar puncture or Ommaya tap for patients with clinical concern for disseminated disease per the discretion of the treating physician.
H. B cell count will be tested pre-therapy, at the end of course 2, every 3 courses through course 14, and then every 4 courses till the end of therapy, and at the time of disease progression or completion of treatment.
I. Spine MRI should be performed prior to therapy and at the same time points as standard Brain MRI, if clinically indicated.
J. CD4 cell count should be tested pre-therapy for patients with history of autologous bone marrow/stem cell transplant.

11 MEASUREMENT OF EFFECT

Although the clinical benefit of APX005M has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should have MRI scan within 3 weeks prior to enrollment, at the end of course 2, every 3 courses through course 14, and then every 4 courses till the end of therapy, and at the time of disease progression or completion of treatment. In addition to a baseline scan, confirmatory scans will also be obtained 9 weeks following initial documentation of an objective response.

11.1 Therapeutic Effects

11.1.1 Definitions

- Evaluable for Toxicity

Patients who receive at least 1 dose of APX005M and are removed from treatment for toxicity during the dose-finding period (first 6 weeks of treatment) are evaluable for estimating the MTD.

Patients who have completed all therapy during the dose-finding period but who failed to comply with all the specified clinical and laboratory monitoring requirements during the DLT observation period may be considered inevaluable for estimating the MTD and replaced.

Patients who receive less than two doses of the protocol specified therapy and who go off treatment for reasons other than toxicity (e.g., progressive disease, withdrawal of consent, etc.) during the dose finding period will be considered inevaluable for estimating the MTD and will be replaced.

- Evaluable for Objective Response

Only those patients who have measurable disease present at baseline, have received at least one dose of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the first scheduled MRI will also be considered evaluable.)

11.2 Disease Parameters

In order to completely document the assessment of response, the measurements of the longest tumor dimension, and its perpendicular, of all target lesions upon which the assessments of tumor response are based should be explicitly noted in the radiology report for the baseline and all subsequent follow-up exams. Reports for the follow-up exams should reiterate the measurements obtained at baseline for each target lesion. Non-target lesions or newly occurring lesions should also be enumerated in these reports, and changes in non-target lesions should be described. 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.

Tumor response criteria are determined by changes in size using the longest tumor dimension, and it's perpendicular. FLAIR, T2 or post contrast T1 weighted images may be used - whichever gives the best estimate of tumor size.

Since many tumors contain non-enhancing components (or, in some cases, the tumor may not enhance at all), both the enhancing and the non-enhancing components must be evaluated – on post contrast T1 weighted images and on FLAIR/T2 weighted images respectively. Increase in enhancement on T1 weighted images without accompanying increase in disease bulk on T2 or FLAIR images is not considered tumor progression. In return, enlarging areas of non-enhancing tumor (defined as mass effect/tissue thickening) are evidence of tumor progression. Conversely, decrease in enhancing tumor component without decrease in overall FLAIR/T2 extent may represent change in tumor permeability (commonly observed with antiangiogenic therapies) rather than represent tumor response.

11.2.1 Method

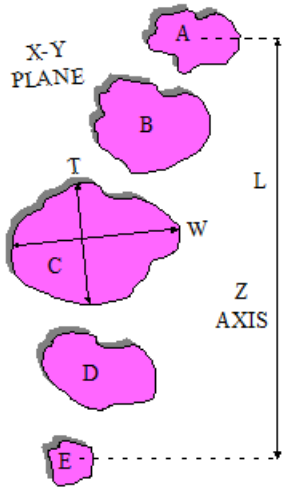
The following section describes the methodology.

- For MRI imaging (preferred), the longest measurement of the tumor (or width, W) should be determined. It can be measured from the axial plane or the plane in which the tumor is best seen or measured, provided the same plane is used in follow ups. Longest diameter of target lesion(s) should be selected in the axial plane only for CT.

- The measurement (transverse (T)) perpendicular to the width in the selected plane should be determined. NOTE: A measurable lesion should have a minimal transverse measurement that is at least twice the combined thickness of the image slice and the inter-slice gap. For example, with a 4 mm slice and a 0.4 mm gap, minimal measurable lesion diameter is 8.8 mm. Smaller lesions would not be measurable for study purpose.

- The cystic or necrotic components of a tumor are not considered in tumor measurements. Therefore only the solid component of cystic/necrotic tumors should be measured. If cysts/necrosis composes the majority of the lesion, the lesion may not be “measurable”.
  Options:
  - if the cyst/necrosis is eccentric, the W and T of the solid portion should be measured, the cyst/necrosis excluded from measurement
  - if the cyst/necrosis is central but represents a small portion of the tumor (< 25%), disregard and measure the whole lesion
  - if the cyst/necrosis is central but represents a large portion of the tumor, identify a solid aspect of the mass that can be reproducibly measured

- Leptomeningeal tumor spread is usually not a target lesion, and usually cannot be measured accurately. Presence and location of leptomeningeal tumor spread should be noted, and change in extent/thickness assessed on follow up studies.



COG GUIDELINE: TUMOR SIZE MEASUREMENT BASED ON CROSS-SECTIONAL IMAGING

- A, B, C, D, & E are contiguous parallel slices in the X-Y plane (usually axial) showing the tumor
- W and T are the maximal perpendicular diameters on the slice (C in this example) showing the largest surface area
- Tumor length in the Z-axis (L) (perpendicular to X-Y plane) can be obtained either by the [a] (difference in table position of the first and last slices showing the tumor + one slice thickness), or [b] the product of (slice thickness + gap) and the number of slices showing the tumor

11.2.2 Overall Response Assessment

The overall response assessment takes into account response in both target and non-target lesion, and the appearance of new lesions, 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, and new lesions in the preceding columns.

Overall Response Assessment

Target Lesions	New Lesions*	Overall Response
CR	No	**CR**
PR	No	**PR**
SD	No	**SD**
PD	Yes or No	**PD**
Any	Yes	**PD**
CR – Complete Response; PD – Progressive Disease; PR – Partial Response; IR – Incomplete Response; SD – Stable Disease *If CSF cytology becomes positive, it will be considered a new lesion and progressive disease.		

11.2.3 Selection of Target and Non-target Lesions

- For most CNS tumors, only one lesion/mass is present and therefore is considered a "target" for measurement/follow up to assess for tumor progression/response.
- If multiple measurable lesions are present, a minimum of the 2 largest lesions should be measured; a maximum of 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 tumor to decrease the partial volume effect (e.g., 8 mm lesion for a 4 mm slice).

11.3 Tumor Response Criteria

11.3.1 Complete Response (CR)

Complete disappearance on MR of all evaluable tumor and mass effect, on a stable or decreasing dose of corticosteroids (or receiving only adrenal replacement doses), accompanied by a stable or improving neurologic examination, and maintained for at least 9 weeks. If CSF was positive, it must be negative.

11.3.2 Partial Response (PR)

Greater than or equal to 50% reduction in tumor size by bi-dimensional measurement, as compared with the baseline measurements, on a stable or decreasing dose of corticosteroids, accompanied by a stable or improving neurologic examination, and maintained for at least 9 weeks.

11.3.3 Stable Disease (SD)

Neurologic exam is at least stable and maintenance corticosteroid dose not increased, and MR/CT imaging meets neither the criteria for PR nor the criteria for Progressive Disease.

11.3.4 Progressive Disease (PD)

Progressive Disease (PD): Progressive neurologic abnormalities or worsening neurologic status not explained by causes unrelated to tumor progression (e.g., anticonvulsant or corticosteroid toxicity wean, electrolyte disturbances, sepsis, hyperglycemia, etc.), OR a greater than 25% increase in the bi-dimensional measurement, taking as a reference the smallest disease measurement recorded since the start of protocol therapy, OR the appearance of a new tumor lesion.

Increasing doses of corticosteroids required to maintain stable neurological status should be strongly considered as a sign of clinical progression unless in the context of recent wean or transient neurologic change.

* Consideration for possible pseudo-progression

For patients showing possible radiographic evidence of tumor progression on MRI during the first 6 months after initiation of APX005M (on required MRI studies performed at the end of Course 2, 5, 8), the treating physician will have the option of allowing the patient to remain on study, continuing protocol therapy, and repeating disease reassessment in 4-6 weeks. Provided that the patient does not show clinical deterioration consistent with tumor progression, has been on a stable or declining dose of steroids, and the subsequent MRI demonstrates tumor regression or stable disease, then the patient will remain on study and continue protocol therapy, and the frequency of subsequent MRI will revert to pre-specified intervals. If the repeat MRI after 4-6 weeks shows disease progression, rather than pseudo-progression, then the time of progression will be the date of the initial MRI, not the follow up scan. The addition of steroids is allowed, at the time of suspected pseudo-progression, as long as the patient can be maintained on a decreasing dose.

11.3.5 Progression-free Survival

Interval of time between date of initiation of protocol treatment and minimum date of documentation of PD, death due to any cause, or date of last follow-up.

11.3.6 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 CR is 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: 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.

12 STUDY OVERSIGHT AND DATA REPORTING/ REGULATORY REQUIREMENTS/ CONFIDENTIALITY

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7 (Adverse Events: List and Reporting Requirements).

12.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through Medidata Rave.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the Pediatric Brain Tumor Consortium's (PBTC) data safety monitoring plan.

12.2 Data Reporting

Data collection for this study will be done exclusively through the Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP IAM account (check at https://ctepcore.nci.nih.gov/iam) and the appropriate Rave role (Rave CRA, Read-Only, CRA (Lab Admin, SLA or Site Investigator) on either the LPO or participating organization roster at the enrolling site. To the hold Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave. If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at [redacted] or by e-mail at ctsucontact@westat.com.

Users may also contact OBDMC to get help with study specific issues including clinical forms, data entry, data query, data sign-offs and/or uploading of regulatory and other required documents. For complete OBDMC contact details, click on the OBDMC Contact Information link that is available in the Members' Area of the PBTC website (http://www.pbtc.org/).

12.2.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. A protocol and subject-specific CDUS "Abbreviated" data set will be submitted electronically to CTEP on a quarterly basis via CDUS OPEN (Oncology Patient Enrollment Network). Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP website (http://ctep.cancer.gov/reporting/cdus.html).

12.2.2 Responsibility for Data Submission

The OBDMC for the PBTC is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.3 CTEP Multicenter Guidelines

N/A

12.4 Collaborative Agreements Language

N/A

12.5 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study and the data will be released to an unauthorized third party without the prior written approval of the Pediatric Brain Tumor Consortium (PBTC).

The PBTC protocol coordinators, other authorized representatives of the sponsor, regulatory representatives, PBTC auditors, representatives of the IRB or the pharmaceutical collaborator supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

Source documents which are the original records of clinical findings, observations or activities in a clinical trial are to be maintained at each participating site. Sites must upload all source documentation to the PBTC via the RAVE database. In the event the patient experiences unexpected events, additional source documentation may be requested to complete the event review. These documents may include but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda and radiographic images.

Study participant study related data, which is for purposes of statistical analysis and scientific reporting will be transmitted to the Pediatric Brain Tumor Consortium electronically via the RAVE database. This will not include the participant's contact or identifying information. Rather, research participants and their research data will be identified by a unique study identification number assigned at the time of screening or registration. The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and institutional regulations.

The study data entry and study management systems used by clinical sites and by the PBTC will be secured and password protected. At the end of the study, all study data is maintained on a secure server.

After the study is completed, the data collected will be maintained on a server and may be used by other investigators, including those outside the study. With the participant's approval and as approved by local IRBs, biological samples labeled only with the participant's protocol specific identification number will be stored at the PBTC Central Review and Biorepository and could be made available to other investigators for future unspecified research. Investigators conducting future studies will not have access to the key for stored data collected while the participant is on study. Clinical data will be de-identified before it is shared with other investigators.

If the participant agrees to submit a repository sample, those samples contain genetic information that may be used for research related to brain tumors and their treatment. They may also be used to develop tests/assays to improve diagnosis and treatment of these diseases in the future. Genetic research may consist of the analysis of one or more genes or the analysis of genetic markers throughout the genome.

13 STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

13.1.1 Stratification Factors

Stratum 1: Recurrent or Refractory Patients

Patients with a histologically confirmed diagnosis of a primary malignant CNS tumor that is recurrent, progressive, or refractory.

Stratum 2: Newly diagnosed DIPG - previously this stratum was on hold until the pediatric RP2D was established in Stratum 1 patients. Stratum 1 was closed to accrual on May 14, 2020 and dose level 3 (0.6 mg/kg APX005M) was declared to be the RP2D in Stratum 1. This stratum is now open to accrual.

Patients with diffuse intrinsic pontine gliomas (DIPGs) will be eligible 6 to 14 weeks post-completion of radiation therapy if without evidence of progression.

13.1.2 Dose Finding for Stratum 1

This is a safety and dose finding study of APX005M in pediatric patients with malignant brain tumors. The starting dose level for this study will be dose level 1 (0.1 mg/kg) with possible escalation to 2 additional dose levels barring safety concerns. The DLT observation period will be the first 2 cycles (42 days). Given the relatively limited safety data available with this agent and none in pediatrics, we have chosen to use the 3+3 design for dose escalation due to its conservative nature with respect to cohort sizes. For the 3+3 designs, dose escalations are planned in cohorts of three patients. No intra-patient escalation will be allowed and dose escalation will not be considered until toxicity information is available from at least 3 evaluable patients at the current dose level. Based on this approach, the MTD will be empirically defined as the highest dose level at which six patients have been treated with at most one patient experiencing a DLT and the next higher dose level has been determined to be too toxic. If the lowest dose level studied is too toxic or the highest dose level studied is considered safe, the estimate of the MTD will not be available. In the event that dose level 3 is deemed to be too toxic following escalation from dose level 2, dose level 2.5 will be studied as an intermediate level.

If all the dose levels are investigated with acceptable toxicity, consideration will be given to investigating higher dose levels based on discussions between the study committee and the sponsor. If a decision is made to not study higher doses and six patients have been treated safely at the highest dose level, then the highest dose level may be recommended for further study in Phase II trials.

The recommended phase II dose (RP2D) will be based on the MTD and the totality of the safety/efficacy data. Once the MTD or a RP2D is identified, the total number of patients treated at the MTD/RP2D will be increased to 12 to further define the toxicity profile. In the event that excessive toxicities are observed in these additional patients, the MTD/RP2D may be revised and de-escalation to a lower dose level may occur.

In the event of a single fatal toxicity, the OBDMC will suspend enrollment, pending discussion with the study chair, the PBTC Toxicity Monitoring Committee, the PBTC DSMB and others as necessary.

13.1.3 Safety Study for Stratum 2

Barring excessive toxicity, we expect to enroll approximately 12 evaluable DIPG patients in Stratum 2 after pediatric RP2D has established in Stratum 1 to assess the safety of this agent in patients with non-progressive DIPG post radiation therapy. The starting dose for the DIPG cohort

will be one dose level below the RP2D determined in non-DIPG patients where 3 patients will be enrolled. If no DLTs are observed then we will escalate to the RP2D where we will treat 6 DIPG patients simultaneously. If 1 DLT is observed in 3 patients then we will enroll 3 additional patients at the starting dose level. If 2 or more DLTs are observed at the starting dose level then de-escalation to a lower dose level will be considered and a similar approach will be repeated. If no more than 1/6 patients experience a DLT at the starting dose level then we will escalate to the RP2D established in Stratum 1 and open 6 slots simultaneously to evaluate the safety of this dose in DIPG patients. In the event that we have to de-escalate to a lower dose level, we will require that 6 patients are treated with no more than 1 DLT at the dose that is determined to be the RP2D in this stratum.

13.2 Sample Size/Accrual Rate

13.2.1 Projected Accrual Rates and Study Duration

PBTC experience with prior protocols for the patient population targeted by this trial, demonstrates that accrual of 1-2 patients per month is feasible. Thus, when all PBTC institutions have IRB approval, we expect an annual accrual of approximately 15 to 25 patients. Based on these estimates we expect the trial to be completed within 1.5 -2 years with a maximum accrual of 45 evaluable patients, including a few patients who may be declared inevaluable for MTD estimation.

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	2	2	0	0	**4**
Native Hawaiian or Other Pacific Islander	0	0	0	1	**1**
Black or African American	3	3	2	2	**10**
White	8	8	4	4	**24**
More Than One Race	2	2	1	1	**6**
Total	**15**	**15**	**7**	**8**	**45**

13.3 Analysis of Primary/Secondary endpoints

We will summarize the toxicity data by dose level and attribution. Number of responses as well as PFS and OS (in DIPG patients) observed on the trial will be reported descriptively. The pharmacokinetics and anti-drug antibody incidence will be analyzed with standard methodologies. We will also provide summary statistics regarding the immunologic markers that are proposed to be studied as part of this trial. Given the small sample size as well as the heterogeneous nature of patients who will enroll on this trial all of these analyses will be exploratory.

13.3.1 Statistical Analysis of Pharmacokinetics

Plasma drug concentrations and pharmacokinetic parameters will be presented in tabular and graphical form. Pharmacokinetic parameters of interest, such as apparent volume of the central compartment (Vc/F), elimination rate constant (Ke), half-life ($t_{1/2}$), apparent clearance (CL/F), and area under the plasma concentration time curve (AUC) will be estimated using compartmental methods. Dose proportionality in pharmacokinetic parameters will be investigated by performing one-way analysis of variance (ANOVA) on dose-normalized parameters.

In addition to estimating individual pharmacokinetic parameters, we will also estimate the population parameters using nonlinear mixed effects modeling methods (NONMEM). This method estimates the population parameters and both the inter- and intra-subject variability. Once the population parameters and corresponding covariance matrix are estimated, individual estimates can be obtained using post hoc analysis.

REFERENCES

1. Merchant MS, Baird K, Wexler LH, Rodriguez-Galindo C, Mackall C. Ipilimumab: First results of a phase I trial in pediatric patients with advanced solid tumors: American Society of Clinical Oncology; 2012.

2. Margolin K, Ernstoff MS, Hamid O, et al. Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. *The lancet oncology.* 2012;13(5):459-465.

3. Sampson JH, Vlahovic G, Sahebjam S, et al. Preliminary safety and activity of nivolumab and its combination with ipilimumab in recurrent glioblastoma (GBM): CHECKMATE-143: American Society of Clinical Oncology; 2015.

4. Reardon D, Sampson J, Sahebjam S, et al. Safety and activity of nivolumab (nivo) monotherapy and nivo in combination with ipilimumab (ipi) in recurrent glioblastoma (GBM): Updated results from checkmate-143. *J Clin Oncol.* 2016;34.

5. Vonderheide RH, Flaherty KT, Khalil M, et al. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. *Journal of Clinical Oncology.* 2007;25(7):876-883.

6. Vonderheide RH, Glennie MJ. Agonistic CD40 antibodies and cancer therapy: AACR; 2013.

7. Barr T, Heath A. Functional activity of CD40 antibodies correlates to the position of binding relative to CD154. *Immunology.* 2001;102(1):39-43.

8. Vega EA, Graner MW, Sampson JH. Combating immunosuppression in glioma. 2008.

9. Chonan M, Saito R, Shoji T, et al. CD40/CD40L expression correlates with the survival of patients with glioblastomas and an augmentation in CD40 signaling enhances the efficacy of vaccinations against glioma models. *Neuro-oncology.* 2015;17(11):1453-1462.

10. Derouazi M, Di Berardino-Besson W, Belnoue E, et al. Novel Cell-Penetrating Peptide-Based Vaccine Induces Robust CD4+ and CD8+ T Cell–Mediated Antitumor Immunity. *Cancer research.* 2015;75(15):3020-3031.

11. Kosaka A, Ohkuri T, Okada H. Combination of an agonistic anti-CD40 monoclonal antibody and the COX-2 inhibitor celecoxib induces anti-glioma effects by promotion of type-1 immunity in myeloid cells and T-cells. *Cancer Immunology, Immunotherapy.* 2014;63(8):847-857.

12. Zhang Q, Wang C, Liu Z, et al. Notch signal suppresses Toll-like receptor-triggered inflammatory responses in macrophages by inhibiting extracellular signal-regulated kinase 1/2-mediated nuclear factor κB activation. *Journal of Biological Chemistry.* 2012;287(9):6208-6217.

13. Hess S, Engelmann H. A novel function of CD40: induction of cell death in transformed cells. *Journal of Experimental Medicine.* 1996;183(1):159-167.

APPENDIX I: Performance Status Scale

The Karnofsky Scale is designed for subjects > 16 years of age, and the Lansky Scale is designed for subjects ≤ 16 years old. Use the table below to determine the score (10-100) that best represents the recipient's activity status at the requested time point.

Karnofsky Scale (recipient age > 16 years)		Lansky Scale (recipient age ≤16 years)	
Able to carry on normal activity; no special care is needed		**Able to carry on normal activity; no special care is needed**	
100	Normal, no complaints, no evidence of disease	**100**	Fully active
90	Able to carry on normal activity	**90**	Minor restriction in physically strenuous play
80	Normal activity with effort	**80**	Restricted in strenuous play, tires more easily, otherwise active
Unable to work, able to live at home cares for most personal needs, a varying amount of assistance is needed		**Mild to moderate restriction**	
70	Cares for self, unable to carry on normal activity or to do active work	**70**	Both greater restrictions of, and less time spent in active play
60	Requires occasional assistance but is able to care for most needs	**60**	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Requires considerable assistance and frequent medical care	**50**	Considerable assistance required for any active play, fully able to engage in quiet play
Unable to care for self, requires equivalent of institutional or hospital care, disease may be progressing rapidly		**Moderate to severe restriction**	
40	Disabled, requires special care and assistance	**40**	Able to initiate quite activities
30	Severely disabled, hospitalization indicated, although death not imminent	**30**	Needs considerable assistance for quiet activity
20	Very sick, hospitalization necessary	**20**	Limited to very passive activity initiated by others (e.g., TV)
10	Moribund, fatal process progressing rapidly	**10**	Completely disabled, not even passive play