Effect of rhIL-7-hyFc on increasing lymphocyte counts in patients with newly diagnosed non-severe lymphopenic gliomas following radiation and temozolomide

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Protocol Revision History

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Amendment #7 Version	**03/04/2020**
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Principal Investigator Signature Page

Principal Investigator (printed):

Name of Institution:

PI Signature *Date*

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.

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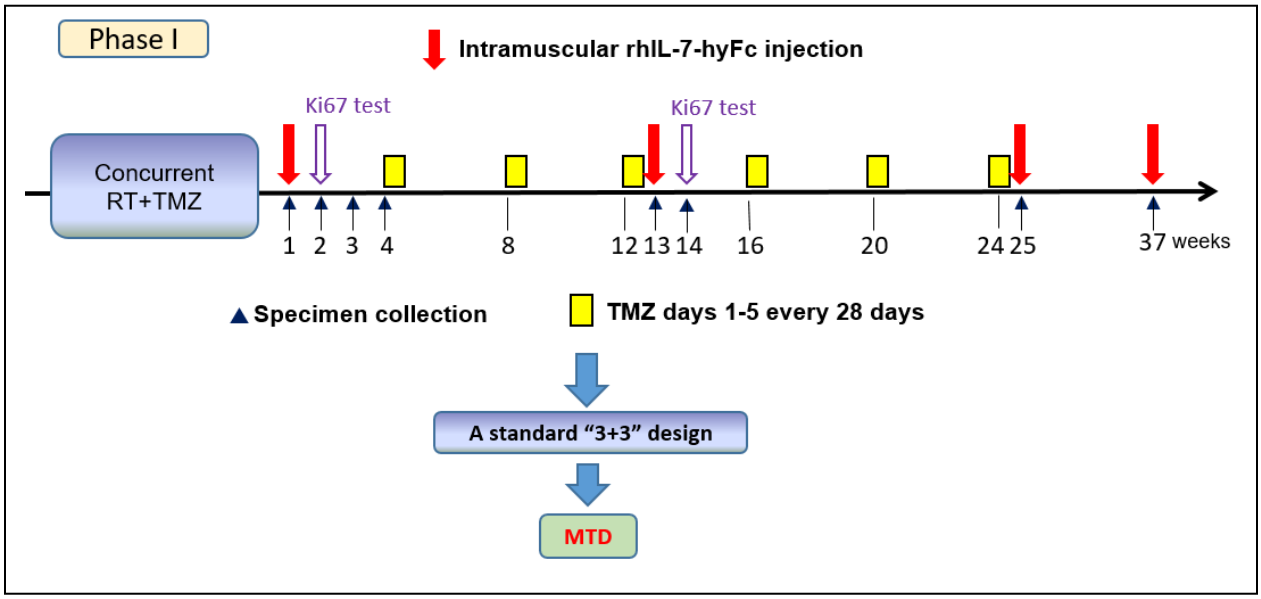
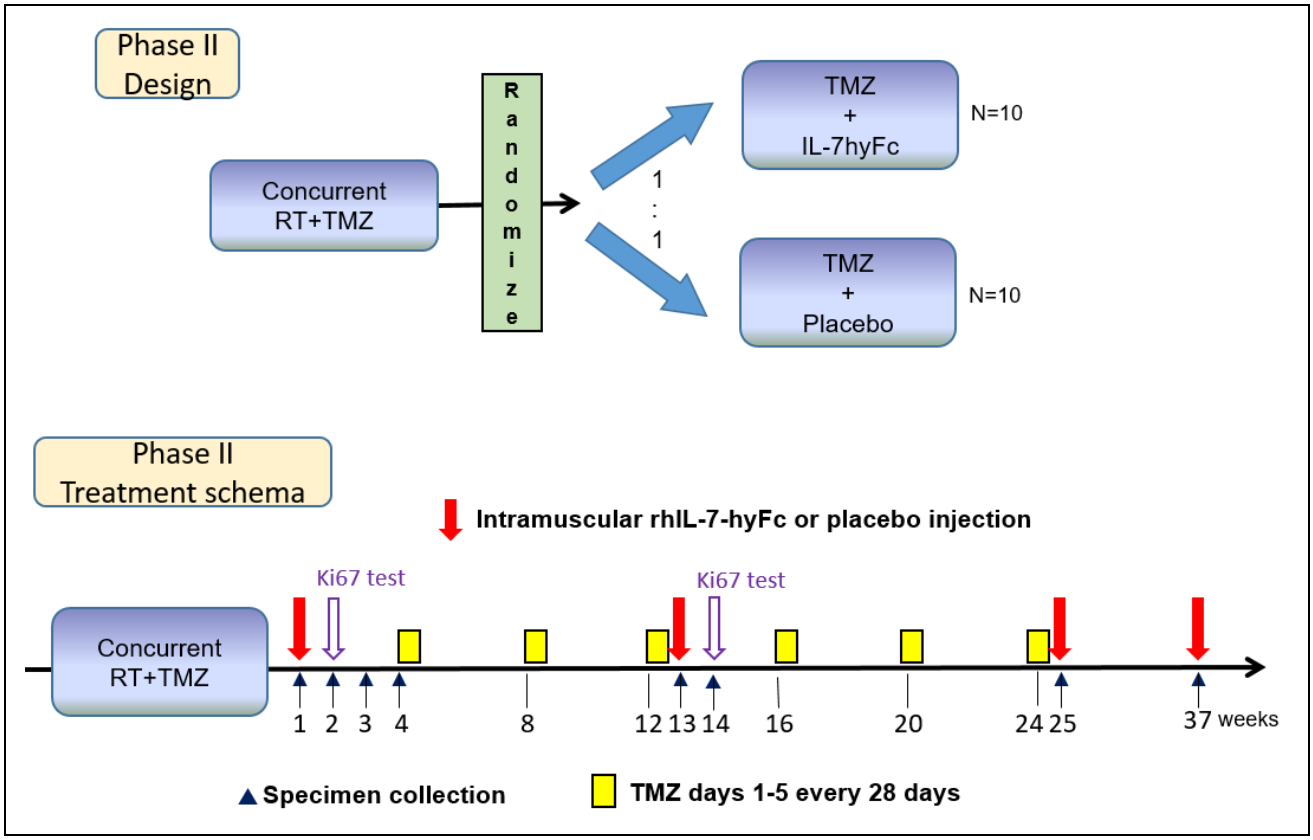
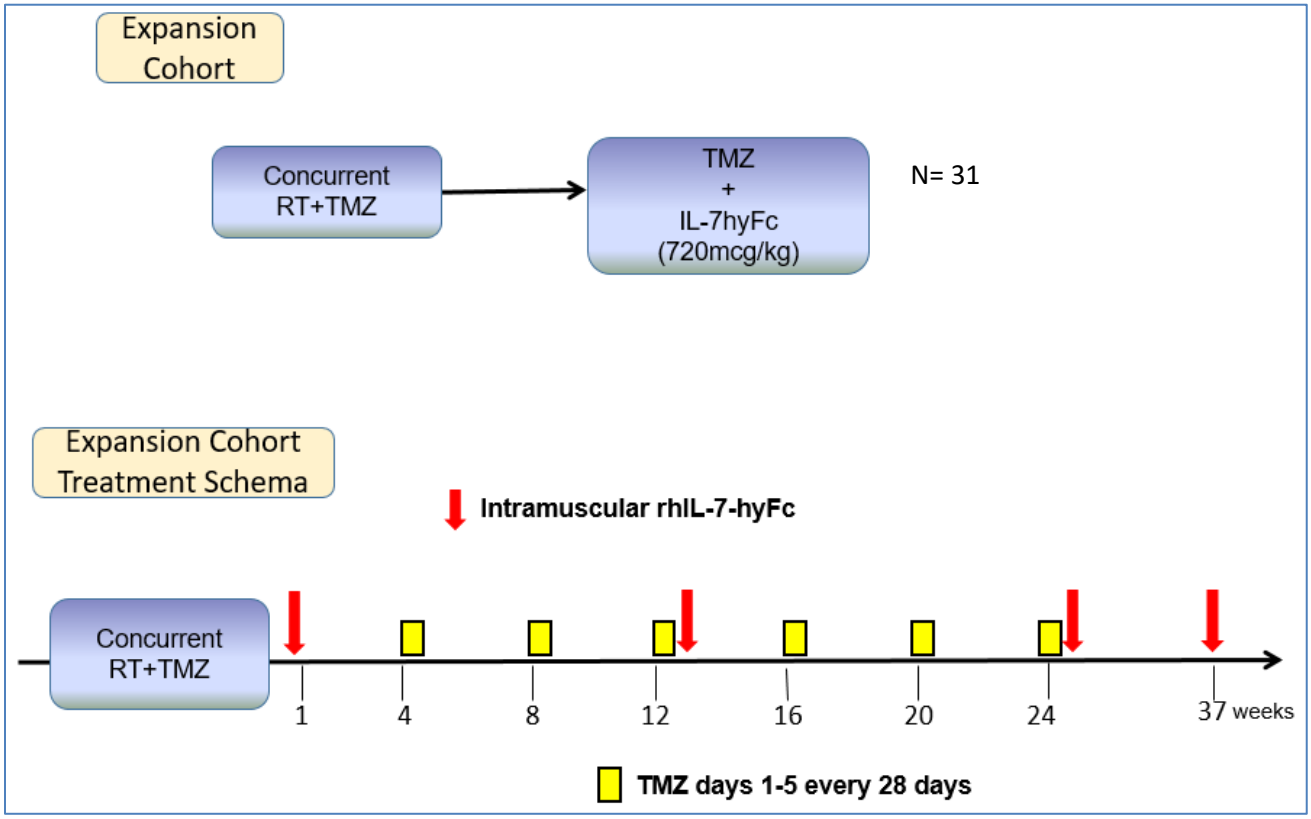
SCHEMA



Dose Level	rhIL-7-hyFc Dose
1	60 mcg/kg
2	120 mcg/kg
3	240 mcg/kg
4	540 mcg/kg
5	720 mcg/kg
6	960 mcg/kg


Phase II Design
Concurrent RT+TMZ
Randomize
1 : 1
TMZ + IL-7hyFc
N=10
TMZ + Placebo
N=10
Phase II Treatment schema
Intramuscular rhIL-7-hyFc or placebo injection
Ki67 test
Ki67 test
Concurrent RT+TMZ
1 2 3 4 8 12 13 14 16 20 24 25 37 weeks
▲Specimen collection
TMZ days 1-5 every 28 days


Expansion Cohort
Concurrent RT+TMZ
TMZ + IL-7hyFc (720mcg/kg)
N= 31
Expansion Cohort Treatment Schema
Intramuscular rhIL-7-hyFc
Concurrent RT+TMZ
1 4 8 12 16 20 24 37 weeks
TMZ days 1-5 every 28 days

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1 BACKGROUND AND RATIONALE

1.1 Treatment-related lymphopenia and high grade gliomas

Patients with glioblastoma (GBM) routinely receive radiation (RT), temozolomide (TMZ), and glucocorticoids. However, these treatments cause severe (grade III-IV) and long lasting lymphopenia in 40% of patients. This treatment-related lymphopenia (TRL) (**Fig. 1**) is associated with shorter survival (Grossman, 2011) (**Fig. 2**). Any grade of lymphopenia was seen in up to ~75% of patients who were treated with RT and TMZ. The survival time is directly associated with the levels of CD4 and total lymphocyte counts. These data were derived from a prospective multi-center study with ~100 patients enrolled and it provides strong scientific rationale for this study. A similar relationship between TRL and shorter survival also exists in patients with newly diagnosed pancreatic, non-small cell lung cancer, and head and neck cancer (Grossman, 2015). Although data were obtained from retrospective analyses, these studies showed strikingly similar results. Thus these prior retrospective studies provide a strong scientific rationale for designing active interventions to enhance lymphocyte recovery or minimize TRL in an effort to improve outcomes for patients receiving chemo-radiotherapy for GBM and other cancers. Recently, we reported that nearly all lymphocytes and lymphocyte subsets were significantly depressed after RT + TMZ, and remained at low levels for more than one year (Campian, 2017). Data from computer modeling studies suggest that a standard course of partial brain radiation is potentially lethal to over 95% of circulating lymphocytes (Yovino 2013). Recent data suggest that inadvertent radiation exposure to circulating lymphocytes passing through a treatment field could play a key role in TRL development in pancreatic cancer (Tang 2014; Wild 2013).

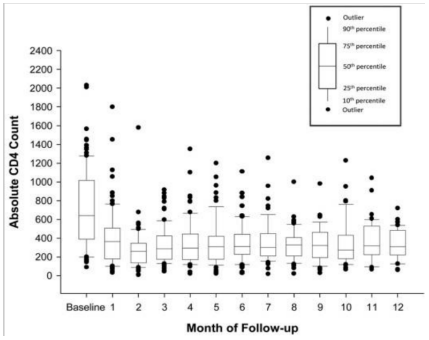


Fig. 1. **CD4 Count Trend over Time.** The median CD4 counts prior to chemoradiation were 664 cells/mm^3. CD4 counts dropped significantly after 2 months. 73% had CD4 counts <300 cells/mm^{3}40% had CD4 counts <200 cells/mm^3. CD4 counts remained persistently low during the 12 months of

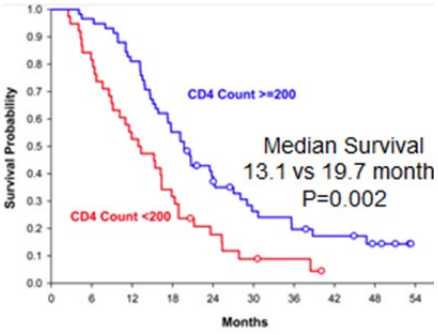


Fig. 2. **Overall survival by CD4 counts at 2 months.** Survival in patients with CD4 counts <200 cells/mm^3 at 2 months was shorter than those with higher CD4 counts.

It remains to be determined if the immune system can be restored after TRL-induced damage. Correction of TRL through an immunotherapeutic approach would potentially represent a major advance for the treatment of patients with GBM. However, the best way for restoring immune status has not been established. Here, we explore a novel approach to prevent or mitigate TRL in GBM in order to improve survival in these cancer patients.

1.2 Interleukin-7 and immune reconstitution

Interleukin-7 (IL-7), a cytokine secreted by stromal cells in the bone marrow and thymus

is required for human thymocyte development and for maintenance of mature peripheral lymphocyte homeostasis. In addition it is also important in both B cell and NK cell proliferation and survival. IL-7 plasma levels are inversely correlated with peripheral T CD4 cell counts. Although IL-7 is limited under normal conditions, it accumulates during lymphopenic conditions (Sportès 2008). IL-7 administration produces “new T cells”, and in preclinical studies IL-7 therapy significantly restored T cell immune function in mice (Mackall 1997) and primates (Beq 2006; Fry 2003; Storek 2003). Recent clinical trials using recombinant human interleukin (rhIL-7) have demonstrated its potential to expand and protect CD4 and CD8 T-cells (Mackall 2011). To date, rhIL-7 has been studied in multiple solid tumors with good tolerance and limited toxicity (Mackall 2011). The most common side effects reported for rhIL-7 therapy are low-grade fever, malaise, transient increases in liver enzyme levels, erythema and induration at the site of administration. Because IL-7 is a modest activator of effector T cells, which quickly internalize their IL-7 receptors after binding (Sportès 2008), rhIL-7 does not induce cytokine storm syndrome. In addition, rhIL-7 preferentially expands naïve and memory T cells with a significant broadening of circulating T cell receptor (TCR) repertoire diversity (Sportès 2008). In vivo half-life of rhIL-7 has been noted to be very short as 6.5-9.8 hours limiting its clinical use (Sportès 2010). In addition to its short in vivo half-life the availability of rhIL-7 has been limited to negligible for the past decade. The French pharmaceutical company Cytheris (CYT107) has been unable to produce sufficient rhIL-7 for clinical trials and in fact went out of business in 2013. CYT107 was then taken by a US Biotech Revimmune Inc in 2014. However, the company has not been able to provide sufficient CYT107 due to financial difficulties. This has resulted in a relative and, at times, complete lack of rhIL-7 for clinical trials. There is a lack of clinical grade rhIL-7, coupled with the absence of any pharmaceutical companies (up until now) interested in the clinical development of this cytokine. In addition, the very short half-life of the Cytheris rhIL-7 made it challenging to administer in out-patient settings. *These provocative data underscore the need for alternative sources of more active and longer-acting IL-7 for human clinical trials*. In this study, we will be the first in the United States to test a novel long acting recombinant human IL-7 analogue, rhIL-7-hyFc (half-life is 48-92 hours) from NeoImmmuneTech Inc. (NIT) for the treatment of patients with high grade glioma (HGG).

1.3 IL-7 in GBM

We recently reported that the levels of IL-7 were inappropriately low in patients with GBM treated with chemoradiation who developed lymphopenia (Campian 2017). Similar results were reported in another study (Ellsworth, 2014). These findings suggest that IL-7 did not increase appropriately to lymphopenia in these groups of patients. This finding raises the question of whether administration of exogenous IL-7 could improve CD4 counts and immune reconstitution in this patient population.

1.4 rhIL-7-hyFc

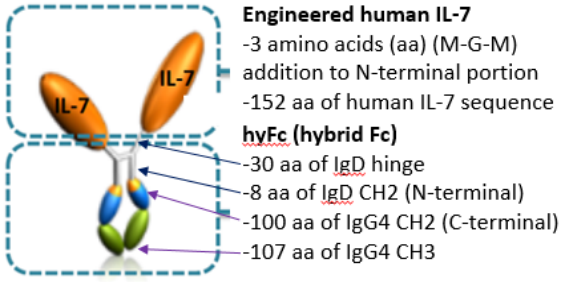


Fig. 3. IL-7hyFc protein structure

rhIL-7-hyFc is a long-acting cytokine consisting of recombinant human IL-7 fused to a hybrid Fc (hyFc) region of IgG (**Fig. 3**). A first-in-human clinical trial of rhIL-7-hyFc (Protocol No. GX-I7-HV-001) was performed as a single ascending dose escalation study in healthy normal volunteers by NIT and Genexine. rhIL-7-hyFc was well tolerated in all patients without any serious adverse events. Subcutaneous administration of increasing doses of rhIL-7-hyFc led to a dramatic increase in the proliferation markers of T cells and percentage of effector memory and central memory T cells within one week, returning to baseline in approximately three weeks. In addition, the increasing doses of rhIL-7-hyFc were associated with more rapid and robust increases in total lymphocyte counts and Tcm and Tem cell subsets. Currently, a Phase I study in solid tumors is ongoing in South Korean (Protocol No.: GX-17-CA-003). They have shown that rhIL-7-hyFc was safely administered at 60 mcg/kg, 120 mcg/kg, 240 mcg/kg, 480 mcg/kg, 720 mcg/kg, 960 mcg/kg and 1200 mcg/kg dose levels without DLTs. In Study GX-I7-CA-004 in GBM, no DLTs were observed at 840 mcg/kg. Please refer to Appendix B for more detailed safety data.

Risks associated with taking rhIL-7-hyFc based on clinical experience include skin toxicity, liver toxicity, headaches, and enlarged lymph nodes.

1.5 Study rationale

Here we have developed a phase I/II clinical trial to evaluate the effect of rhIL-7-hyFc on lymphocyte counts in patients with HGG.

A phase I study will test whether rhIL-7-hyFc can be safely administered to patients with HGG. Six doses of rhIL-7-hyFc will be tested using a mix of Accelerated Phase and standard 3+3 dose-escalation design. The phase II portion to test effect of rhIL-7-hyFc on lymphocyte counts will use placebo-controlled randomization in HGG patients whose treatment include the standard RT and TMZ.

In order to better understand the impact and benefit of rhIL-7-hyFc administration, we will collect peripheral blood and tumor tissue specimens (when possible) before and after rhIL-7-hyFc treatment in all patients. Peripheral blood mononuclear cells (PBMC) will be collected and cryopreserved for analysis. Lymphocyte counts and T cell subsets will be evaluated by Fluorescence Activated Cell Sorting (FACS). T cell repertoire diversity will be characterized using PCR based deep sequencing by ImmunoSEQ assays (Adaptive Biotechnologies, Seattle, WA). Immunogenicity such as the occurrence of anti-drug antibodies (ADA) and neutralizing ADA will be monitored in collaboration with NIT.

1.6 Protocol Amendment #3

1.6.1 Synopsis

In this amendment, the dose escalation schema was updated to remove dose level

360 mcg/kg and update the maximum dose level to 960 mcg/kg.

1.6.2 Rationale

Currently, a Phase 1b trial of single-agent NT-I7 (rhIL-7-hyFc) is being conducted in patients with advanced solid tumors in Korea (Study No. GX-I7-CA-003) utilizing a 3+3 dose escalation approach to determine the Recommended Phase 2 Dose (RP2D). The dose level ranges for this study include 60 μg/kg, 120 μg/kg, 240 μg/kg, 480 μg/kg, 720 μg/kg, 960 μg/kg and 1200 μg/kg given intramuscularly (IM) in multiple doses. Thus far, the safety profile of NT-I7 in the study has been very similar to the safety profile observed in the healthy volunteers study. There have been no dose limiting toxicities (DLTs). The most common treatment-related adverse events observed to date were injection-site reactions, which were mild to moderate in severity and resolved within a few days, usually without treatment intervention.

1.7 Protocol Amendment #4

1.7.1 Synopsis

In this amendment, an additional 3 patients will be enrolled to the dose level 4, 540 mcg/kg.

1.7.2 Rationale

Due to the AEs seen in the 3 patients enrolled at dose level 4 (540 mcg/kg), 3 additional patients will be added to that dose level. One out of these patients experienced Grade 2 AEs and all 3 of these patients experienced Grade 1 AEs. The additional 3 patients will be monitored for toxicity.

In addition, it has been observed in this study and others that patients have received a relatively high total dose due to high body weight. With this observation, NeoImmuneTech (NIT) has released a pharmacy manual with revised dose calculation instructions for obese patients. rhIL-7-hyFc dosing is determined by actual body weight for non-obese patients; for obese patients (BMI ≥ 30kg/m²), dosing will be determined by adjusted body weight (see below formula). Weight must be re-assessed prior to each rhIL-7-hyFc dosing; if the patient's weight changes +/- 10% from the baseline weight measurement, rhIL-7-hyFc dose should be based on the new weight measurement.

BMI= (Weight in Kg)/(Height in meters)²

For obese patients (BMI ≥ 30kg/m²), proceed with the following steps:

1. Determine Ideal Weight (1 kg = 2.2 lbs):

Males: 50 kg + 2.3 kg x (inch over 5 feet)
Females: 45.5 kg + 2.3 kg x (inch over 5 feet)
(Patients less than 5 feet: subtract 2.3 kg/inch under 5 feet)

2. Determine Adjusted Body Weight:

Ideal Weight + 0.4 x (actual weight – ideal weight) = Adjusted Body Weight

This change will be reflected in the next pharmacy manual when revised.

1.8 Protocol Amendment #11

1.8.1 Synopsis

This amendment adds an expansion cohort of 31 patients to the experimental arm (TMZ + rhIL-7-hyFc).

Amendment 11 added an additional 31 patients to the rhIL-7hyFc treatment arm as an expansion cohort to evaluate the PFS in patients with IDH wildtype and MGMT promoter unmethylated GBM. In the expansion cohort, labs will be drawn per SOC and no research specimens will be collected. As of Amendment 14, enrollment into the expansion cohort may begin prior to completion of the phase II enrollment.

1.8.2 Rationale

Nineteen patients were enrolled to the phase I portion of the study. Among those, 12 patients were IDH wildtype with MGMT promoter unmethylated GBM. Their mPFS to date (as of 7/29/2021) is 11.53 months (range 5.13- 14.8+). The reported mPFS is 5.4 months for unfavorable molecular profile and 7.3 months for favorable molecular profile in MGMT promoter unmethylated GBM (reference: Gilbert MR, 2014 NEJM, PMCID: PMC4201043). This preliminary efficacy data suggests a potential improvement in mPFS with the study treatment.

The purpose of this amendment is to test whether there is a significant improvement in mPFS.

2 STUDY OBJECTIVES

2.1 Primary objectives

1. Phase I: To evaluate the safety, tolerability, and to determine the maximal tolerated dose (MTD) of rhIL-7-hyFc in patients with glioma.
2. Randomized Phase II: To test the effect of rhIL-7-hyFc on absolute lymphocyte count (ALC) compared to control.
3. Phase II Expansion Cohort: To determine the mPFS of patients with gliomas that are IDH1 wildtype, as defined by negative immunohistochemistry using an R132H-specific antibody, and MGMT promotor unmethylated who receive rhIL-7-hyFc.

2.2 Secondary objective

To evaluate anti-drug antibodies (phase I and randomized phase II only)

2.3 Exploratory objectives

These objectives apply to the phase I and randomized phase II portions of this study only.

1. To evaluate the impact of ADA on ALC level
2. To evaluate the effect of concurrent dexamethasone
3. To evaluate the serial T cell lymphocyte subtypes over time
4. To evaluate the serial cytokine levels
5. To evaluate the impact of adjuvant temozolomide on rhIL-7-hyFc effects on ALC
6. To monitor overall response rate (ORR), progression free survival (PFS) and overall survival
7. To explore the impact on tumor infiltrating lymphocytes (TIL) after rhIL-7-hyFc treatment (When tumor tissue specimens are available).
8. Functional analysis of T cells before and after rhIL-7-hyFc treatment

3 PATIENT ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

1. WHO grade III, grade IV, and high risk grade II gliomas that require RT and TMZ treatment.
2. Phase 2 Expansion Cohort ONLY: Must be IDH1 wildtype, as defined by negative immunohistochemistry using an R132H-specific antibody, and MGMT promoter unmethylated glioblastoma multiforme (WHO grade IV).
3. Post-operative treatment must have included radiation and TMZ. Prior Gliadel Wafers are allowed. Glucocorticoid therapy is allowed. TTF device is allowed.
4. Adequate organ and marrow function defined as follows:
   a. Absolute neutrophil count ≥ 1,000/mcL
   b. Platelets ≥ 75,000/mcL
   c. Hemoglobin ≥ 8 g/dL
   d. Total bilirubin ≤ 3.0 x institutional upper limit of normal
   e. AST (SGOT)/ALT (SGPT) ≤ 3.0 × institutional upper limit of normal
5. ALC ≥ 600/mcL (required for phase I and randomized phase II only)
6. Karnofsky Performance Status (KPS) ≥ 60% (i.e. the patient must be able to care for himself/herself with occasional help from others).
7. Able to provide written informed consent (or consent from a legally authorized representative).
8. Women of childbearing potential must have a negative serum pregnancy test prior to study entry (within 14 days). Patients must be willing to be on adequate contraception during treatment as defined in Section 5.5.
9. ≥ 18 years of age.

3.2 Exclusion Criteria

1. Receiving any other investigational agents which may affect patient's lymphocyte counts.
2. Pregnant women are excluded from this study because rhIL-7-hyFc has not been evaluated regarding its potential for teratogenic or abortifacients effects. There is a potential risk for adverse events in nursing infants secondary to treatment of the

mother with the study drug, breastfeeding should be discontinued if the mother is treated with rhIL-7-hyFc.

3. Has an active viral infection requiring systemic treatment at screening.
4. Has active autoimmune disease or syndrome (i.e. moderate or severe rheumatoid arthritis, moderate or severe psoriasis, multiple sclerosis, myasthenia gravis, Guillain-Barre syndrome, systemic lupus erythematosis, scleroderma, ulcerative colitis, Crohn's disease, autoimmune hepatitis, granulomatosis with polyangiitis, etc.,) that requires systemic treatment at the time of screening. Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment. Subjects are permitted to enroll if they have vitiligo, resolved childhood asthma/atopy, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger.
5. Receipt of live attenuated vaccine within 30 days before the first dose of study treatment. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, Bacillus Calmette-Guérin (BCG), Zoster, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g. FluMist) are live attenuated vaccines and are not allowed.
6. Has clinically significant cardiac enzymes ([TnI or TnT] *or* CK)
7. Patients with a clinically significant EKG on screening triggering a echocardiogram which is also clinically significant

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center OnCore database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and date of birth (DOB)
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center OnCore database at Washington University.

4.3 Assignment of UPN

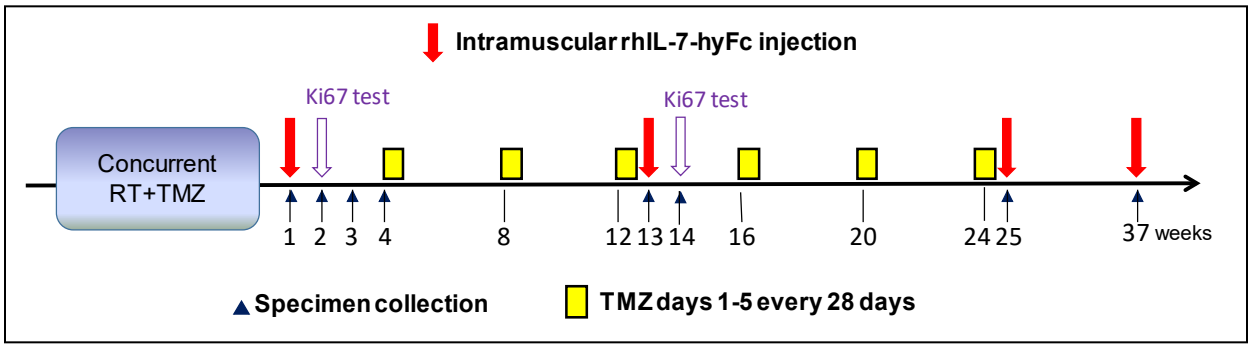
Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

4.4 Randomization

A total of 20 patients during phase II will be randomized 1:1 to receive either placebo or rhIL-7hyFc at the dose determined during phase I. Patients will be stratified by concomitant use of steroids (Yes/No). This study is double-blinded (participant, and physician/study coordinator are all blinded; pharmacist and study statistician are not blinded). Approximately equal number of patients will be assigned to each treatment group. The randomization will be performed in OnCore. Randomization will occur on eligible patients only and is auto generated after the eligibility is confirmed, the patient has an on-study date, and the strata steroid use (Yes/No) is confirmed; which will return either an 'A' or 'B' assignment. Only the pharmacist and statistician will know the treatment corresponding to each assignment.

Patients enrolled to the phase II expansion cohort will not be randomized.

5 RESEARCH PLAN



The Phase I part will begin with an Accelerated Phase with 1 patient per cohort at the first 2 doses (60 mcg/kg and 120 mcg/kg), followed by a standard 3+3 design on the remaining four dose levels (see Table in Section 5.2), each with a cohort of 3 independent patients each time and with a maximum total of 6 patients. The dose escalation rules are summarized in Section 5.3.3. Patients enrolled in dose level 1 and dose level 2 (60 mcg/kg and 120 mcg/kg) will have the option to increase their dose to 240 mcg/kg if no DLTs are observed at the 240 mcg/kg dose level and they are still receiving treatment on study.

The maximum tolerated dose (MTD) if no DLT at the highest dose level will be reviewed together with ALC and PK/PD data to determine a recommended phase 2 dose (RP2D) for use in the Phase II study. In the Phase II part of the study, 20 eligible patients will be randomized at a 1:1 ratio to receive either rhIL-7-hyFc or placebo. The MTD was determined to be 720 mcg/kg.

CBC with differential (including ALC) and CD4 counts will be tested during RT and TMZ treatment as standard of care (SOC). Research blood will be collected prior to the initiation of the study treatment and at the selected time points for patients enrolled in phase I and randomized phase II. No research blood will be collected for the expansion cohort.

If ADA is observed in week 45, ADA levels need to be monitored every 2 months until it decreases to the basal level. The monitoring of ADA levels should not interrupt rhIL-7-hyFc administrations if patients do not experience DLTs.

An additional 31 patients will be enrolled in a non-randomized fashion as an expansion cohort to receive 720 mcg/kg rhIL-7-hyfc. These patients must have GBM that is IDH1 wildtype confirmed by negative immunohistochemistry using an IDH1 mutant-specific antibody, and MGMT promoter unmethylated. These patients will have labs drawn as per SOC with no research collections. Enrollment into the expansion cohort may begin prior to completion of the phase II enrollment.

5.1 Premedications

Benadryl 25-50 mg oral can be given 30-60 min prior to rhIL-7-hyFc injection as pre-medication to prevent skin reactions. This step is optional per patient and treating physician's preference.

5.2 Agent Administration

Per standard treatment, patients will receive concurrent RT + TMZ followed by adjuvant

TMZ on Days 1-5 of a 28-day cycle for a total of 6 cycles. rhIL-7hyFc / placebo will be given by intramuscular injection starting at the end of RT + TMZ (within 14 days after last day of RT + TMZ). The second injection will be administered 3-5 days after the last dose of cycle 3 TMZ treatment (~week 13 in treatment schema). The third injection will be given 3-5 days after the last dose of cycle 6 TMZ treatment (~week 25 in treatment schema). Note that the second and third injections should be administered once between Day 3 through 5 following the last dose of TMZ to achieve the strongest response (meaning that if TMZ is delayed, the rhIL-7-hyFc/placebo injections will be delayed as well). The fourth injection (last injection in the study) will be given after completion of monthly TMZ (~Week 37 as shown in Treatment Schema). A total of 4 doses of rhIL-7-hyFc injections are planned.

5.3 Dose Escalation Schema (Phase I)

Dose Level	rhIL-7-hyFc Dose (mcg/kg)
1	60
2	120
3	240
4	540
5	720
6	960

Dose escalation will not occur until all patients in the cohort are 30 days out from their first dose of rhIL-7-hyFc and the Principal Investigator has been able to review all toxicities.

5.4 Definition of MTD, DLT, and Dose Escalation Criteria

5.4.1 Definition of Maximum Tolerated Dose (MTD)

The maximum tolerated dose (MTD) is defined as the dose of rhIL-7-hyFc that yields a dose limiting toxicity rate less than 33%. If an MTD is not reached, the highest administered dose (960 mcg/kg) will be the highest dose to test for safety.

5.4.2 Dose Limiting Toxicities (DLTs)

DLT will be defined as ≥ grade 3 AEs that occur within 30 days from the treatment start possibly related to the drug regimen, with severity graded according to the Common Terminology Criteria for Adverse Events (CTCAE) 5.0.

DLT definition will have the following exceptions:

- Grade 3 dermatologic AEs, including injection site reaction, that resolves to Grade ≤ 2 in ≤ 3 days with supportive care
- transient Grade 3 influenza-like symptoms or pyrexia controlled with medical management
- Grade 3 fatigue that resolves to Grade ≤ 2 in ≤ 3 days

- Grade 3 neurologic toxicity responding within ≤ 7 days to steroids, anticonvulsants, or electrolyte correction
- Grade 3 laboratory abnormalities that are asymptomatic and considered by the investigator not to be clinically significant

The DLT evaluation period is the first 30 days of therapy. The target DLT rate is <33%.

5.4.3 Dose Escalation Criteria

The starting dose level will be 60 mcg/kg, with one patient enrolled. If no DLT is observed at the 60 mcg/kg dose level, a new patient will be enrolled to dose level 2 (120 mcg/kg). If no DLT is observed at that dose level, the study will proceed to the conventional 3+3 design and enroll 3 patients to dose level 3. If any DLTs are observed in the single patient enrolled in either dose level 1 or dose level 2, the accelerated phase will convert to a conventional 3+3 design at the dose level where the DLT was observed and remain so for all the dose levels above. If excess DLTs (≥ 2 out of a total of 6 patients at a given dose level) are observed at dose level 1, the study will halt.

Dose escalations will proceed as follows:

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next higher dose level.
≥2	Dose escalation will be stopped. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. If 0 of these 3 patients experience DLT, proceed to the next dose level. If 1 or more of this group suffer DLT, then dose escalation is stopped. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≥2 out of 6	Dose escalation will stop and the prior dose level will be considered the MTD. This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

5.5 Phase II and Expansion Cohort

Patients will be randomized to either rhIL-7-hyFc or placebo on a 1:1 basis in the phase II portion of the study. Dosing will be administered as described in Section 5.2 at a dose of 720 mcg/kg.

As part of an expansion cohort, an additional 31 patients with IDH1 wildtype confirmed by negative immunohistochemistry using an IDH1 mutant-specific antibody, and MGMT promoter unmethylated GBM will be enrolled, also at the 720 mcg/kg dose level. These patients will not be randomized; they will all receive rhIL-7-hyFc and will also be treated as described in Section 5.2. Enrollment into the expansion cohort may begin prior to completion of the phase II enrollment.

5.6 Toxicity, Response, and DLT Evaluations

All patients who receive any study treatment are evaluable for toxicity. Patients are evaluated from first receiving study treatment until a 30-day follow up after the conclusion of treatment or death.

All patients are evaluable for disease response unless they discontinue treatment prior to having any disease assessment.

A patient is evaluable for DLT assessment only during the first 30 days after the first dose of rhIL-7-hyFc treatment and only if enrolled in phase I of the study.

Only patients enrolled to phase I or the randomized phase II portions of the study are evaluable for the secondary and exploratory objectives.

5.7 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative pregnancy test within 14 days prior to the first dose of the study agent. Patients must be willing to be on adequate contraception during treatment.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 90 days following the last dose of the study agent.

If a patient is suspected of being pregnant, the study agent should be immediately discontinued. In addition, a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within 90 days after the last dose of the study agent, the investigator must be notified in order to facilitate outcome follow-up.

5.8 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy will be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue for up to 4 doses of rhIL-7-hyFc or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or that rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unable to receive further treatment in the judgment of the investigator
- Confirmed pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.9 Duration of Follow-up

Patients will only be off study at the time of death. All patients will be assessed for survival by phone call, clinic visit, or medical records (e.g. physician notes/laboratory results of clinic or hospital visit) at 2 years and 5 years following the end of study treatment.

6 DOSE DELAYS / DOSE MODIFICATIONS

6.1 Temozolomide Dose Modifications

TMZ dose delays or modifications will be per SOC at the treating physician's discretion.

6.2 rhIL-7-hyFc Dose Modifications

Dose of rhIL-7-hyFc	Dose Level -1	Dose Level -2	Dose Level -3
	1st Reduction of rhIL-7-hyFc	2nd Reduction of rhIL-7-hyFc	3rd Reduction of rhIL-7-hyFc
starting dose per protocol	50% of the starting dose	25% of the starting dose	discontinue study drug

Discontinuation Rules and Procedures for rhIL-7-hyFc-Related Adverse Events

Toxicity	Hold Treatment for Grade	Guidance for Restarting	Guidance for Permanent Discontinuation	Supportive Care Guidelines
Immune medicated Myocarditis	2 - 4	**NA**	• Permanently discontinue	• Initiate treatment as per institutional guidelines and consider

Toxicity	Hold Treatment for Grade	Guidance for Restarting	Guidance for Permanent Discontinuation	Supportive Care Guidelines
				antiarrhythmic drugs, temporary pacemaker, ECMO, or VAD as appropriate.
AST/ALT or bilirubin increase/ immune-related hepatitis	2	**Monitor frequently** **Related:** reduce by 1 dose level **Unrelated:** same dose level	• AST or ALT > 5 × ULN or total bilirubin > 3 × ULN • Toxicity fails to resolve within 12 weeks of last dose of rhIL-7-hyFc • Permanently discontinue for liver enzyme elevations meeting criteria for Hy's Law	• Monitor LFT results frequently (consider weekly) • Treat with IV or oral corticosteroids
	3/4	N/A	• Permanently discontinue	• Treat with IV corticosteroids for 24 to 48 hours. When symptoms resolve to ≤ Grade 1, taper steroids over no less than 4 weeks
Rash/dermatitis	2	Consider interrupting for Grade 2 for > 1 week		• Start topical emollients, mild-strength corticosteroid cream
	3	Toxicity resolves to Grade 0-1 **Related:** reduce by 1 dose level **Unrelated:** same dose level	• Toxicity fails to resolve within 12 weeks of last dose or inability to reduce corticosteroids to ≤ 10 mg of prednisone or equivalent per day within 12 weeks	• Start topical emollients, antihistamines, and high-strength corticosteroid cream • Consider to administer oral corticosteroids
Rash/dermatitis	4	N/A	• Permanently discontinue	• Treat with IV steroids followed by high-dose oral steroids. When symptoms resolve to ≤ Grade 1/2, taper steroids over no less than 4 weeks
Other AEs (except lymphopenia) not otherwise listed	2/3	Toxicity resolves to Grade 0-1 **Related:** reduce by 1 dose level **Unrelated:** same dose	• Toxicity fails to resolve within 12 weeks • Any Grade 3 toxicity that recurs	• Corticosteroids may be indicated

Toxicity	Hold Treatment for Grade	Guidance for Restarting	Guidance for Permanent Discontinuation	Supportive Care Guidelines
	4	N/A	• Permanently discontinue	• Corticosteroids may be indicated

7 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to Appendix C for definitions and Appendix D for a grid of reporting timelines.

Adverse events will be tracked from start of treatment through 30 days after the end of study treatment. All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

- Baseline adverse events, which shall be recorded on the medical history CRF
- Clinical or laboratory adverse events that are grade 1
- Lymphopenia

Refer to the data submission schedule in Section 11 for instructions on the collection of AEs in the EDC.

Reporting requirements for Washington University study team may be found in Section 7.1. Reporting requirements for secondary site study teams participating in Washington University-coordinated research may be found in Section 7.2.

7.1 Sponsor-Investigator Reporting Requirements

7.1.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

7.1.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Washington University Sponsor-Investigator is required to notify the QASMC of any unanticipated problems involving risks to participants or others occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 calendar days** of receipt of IRB acknowledgment via email to qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

For events that occur at secondary sites, the Washington University Sponsor-Investigator is required to notify the QASMC within 10 days of Washington University notification via email to qasmc@wustl.edu. Submission to QASMC must include either the myIRB form and supporting documentation or (if not submitted to myIRB) the date of occurrence, description of the event, whether the event is described in the currently IRB approved materials, the event outcome, determination of relatedness, whether currently enrolled participants will be notified, and whether the informed consent document and/or any study procedures will be modified as a result of this event.

7.1.3 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the Washington University Principal investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix C for definitions) no later than **7 calendar days** after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix C) no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix C) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
    - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
    - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
    - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting
- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within **15 calendar days** after it is determined that the information qualifies for reporting

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Siteman Cancer Center Protocol Development team of each

potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Siteman Cancer Center Protocol Development team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

7.1.4 Reporting to NeoImmuneTech, Inc.

All events being submitted to the FDA should be submitted concurrently to NeoImmuneTech by emailing them to Floryn Ajuzie, Clinical Trials Manager, at fajuzie@neoimmunetech.com and safety107@neoimmunetech.com.

7.1.5 Reporting to Secondary Sites

The Washington University Sponsor-Investigator (or designee) will notify the research team at each secondary site of all unanticipated problems involving risks to participants or others that have occurred at other sites within 10 working days of the occurrence of the event or notification of the Sponsor-Investigator (or designee) of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable. Refer to Section 16.0 (Multicenter Management) for more information.

7.2 Secondary Site Reporting Requirements

The research team at each secondary site is required to promptly notify the Washington University Sponsor-Investigator and designee of all serious adverse events (refer to Appendix C, Section D) within 1 working day of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using either an FDA Form 3500a (MedWatch) and Washington University's cover sheet (Appendix E)). A formal written report must be sent to the Washington University Sponsor-Investigator and designee and research coordinator within 4 calendar days (for fatal or life-threatening suspected adverse reactions) or 11 calendar days (for serious unexpected adverse reactions) of the occurrence of the event or notification of the secondary site's PI of the event.
The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines. The research team at Washington University is responsible for reporting all applicable events to the FDA and NeoImmuneTech as needed.
Washington University pre-approval of all protocol exceptions must be obtained prior to implementing the change. Local IRB approval must be obtained as per local guidelines.

Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

7.3 Exceptions

Events that do not require expedited reporting as described in Section 7.1 include:

- Planned hospitalizations
- Hospitalizations < 24 hours
- Respite care
- Events related to disease progression

Events that do not require expedited reporting must still be captured in the EDC.

8 PHARMACEUTICAL INFORMATION

8.1 rhIL-7-hyFc

8.1.1 Description of Drug Substance

rhIL-7-hyFc protein is produced by inserting the gene expressing rhrhIL-7-hyFc into the eukaryotic expression vector pAD15 at the Multiple Cloning Site. The Chinese Hamster Ovary (CHO) cell line DG44 is used to produce rhIL-7-hyFc.

rhIL-7-hyFc has a molecular weight of 104 KDa and is composed of 400 amino acids with 155 amino acids for IL-7 and 30 for the IgD hinge, 8 for IgD the CH2 domain, and 207 for the IgG4 region. rhIL-7-hyFc contains 11 disulfide bonds, 1 O-glycosylation, and 3 N-glycosylation sites.

8.1.2 Chemical and Structural Formula

Chemical Formula: $C_{4012}H_{6372}N_{1104}O_{1238}S_{42}$

Structural Formula: rhIL-7-hyFc is a fusion protein comprising human IL-7 fused to the human immunoglobulin D (IgD) hinge region. This in turn is fused to the N-terminal region of CH2 from IgD and two key regions of the antibody immunoglobulin G4 (IgG4): C-terminal region of CH2, and the entire CH3 region.

8.1.3 Pharmaceutical Properties

rhIL-7-hyFc is supplied in a sterile, preservative-free liquid form in a single-use vial. One vial (1.1mL) contains 25 mg per 1 mL of the active ingredient of the finished drug product. The purity of the active ingredient must be 89.0% or higher based on size-exclusion ultra-high-performance liquid chromatography (SE-UHPLC) testing and 90.0% or higher based on reverse-phase high-performance liquid chromatography (RP-HPLC) testing. The rhIL-7-hyFc finished drug product should be a colorless, clear solution and should not contain any particulate matter that can be observed visually.

8.1.4 Dosage Form

In addition to the active ingredient rhIL-7-hyFc, each vial contains sucrose, D-sorbitol, tri-sodium citrate dehydrates, citric acid monohydrate, and Polysorbate 80 as a stabilizer and buffer. These ingredients meet the specification criteria of the European pharmacopeia (Ph. EUR).

rhIL-7-hyFc is supplied in a 1.1 mL vial package at a concentration of 25 mg protein/mL. The finished drug product solution contained in the vial is a liquid injection dosage form at pH 5.0±0.5 and a colorless, clear solution. There should not be any floating particulates under gross observation.

rhIL-7-hyFc can be diluted with its diluent for intramuscular administration but should not be mixed with other drugs or solutions. Guidelines for dilution of rhIL-7-hyFc solution by hospital pharmacy are described in IB.

8.1.5 Storage and Handling

Vials that contain rhIL-7-hyFc must be kept refrigerated at 2~8℃. The chemical and physical stability of the rhIL-7-hyFc finished drug product are being evaluated in an ongoing stability study at 2~8℃. It is recommended that vials are protected from direct light until the time of use.

8.1.6 Route of Administration

Intramuscular injection. Do not shake vials before injection. rhIL-7-hyFc should be administered slowly in the intramuscular space. A vial is restricted to one patient and to one day of treatment.

8.2 rhIL-7hyFc Diluent (Will be used as Placebo in Phase II)

8.2.1 Pharmaceutical Properties

rhIL-7hyFc Diluent is supplied in a sterile, preservative-free liquid form, filled in the amount of 1.0 mL in a single-use, 2 mL glass vial. One vial contains the same ingredients with no active ingredient as the rhIL-7hyFc Drug Product. The rhIL-7-hyFc Diluent finished drug product should be a colorless, clear solution and should not contain any particulate matter that can be observed visually.

8.2.2 Dosage Form

Each vial contains sucrose, D-sorbitol, tri-sodium citrate dehydrates, citric acid monohydrate, and Polysorbate 80 as a stabilizer and buffer. These ingredients meet the specification criteria of the European pharmacopeia (Ph. EUR).

Composition of rhIL-7-hyFc Diluent

Component	Reference to standards	Function	Quantity per mL
Tri-Sodium citrate dihydrate	Ph.Eur.	pH adjusting agent	3.68 mg

Citric acid monohydrate	Ph.Eur.	pH adjusting agent	1.58 mg
Sucrose	Ph.Eur.	Osmolality modifying agent	50.00 mg
D-Sorbitol	Ph.Eur.	Osmolality modifying agent	15.00 mg
Polysorbate 80	Ph.Eur.	Surfactant	0.5 mg
Water for injection	Ph.Eur.	Solvent	q.s. 1 mL

rhIL-7-hyFc Diluent is supplied in a 2 mL vial package filled with 1 mL. The finished drug product solution contained in the vial is a liquid injection dosage form at pH 5.0±0.5, osmolality is 300±40 mOsm/kg, and a colorless, clear solution. There should not be any floating particulates under gross observation.

8.2.3 Storage and Handling

Vials must be kept refrigerated at 2~8℃. The chemical and physical stability of the rhIL-7-hyFc Diluent finished drug product are being evaluated in an ongoing stability study at 2~8℃. It is recommended that vials be protected from direct light until the time of use.

8.2.4 Route of Administration

Intramuscular injection. Do not shake vials before injection. rhIL-7-hyFc Diluent should be administered slowly in the intramuscular space. A vial is restricted to one patient and to one day of treatment.

9 CORRELATIVE STUDIES

NOTE: only patients enrolled to phase I and the randomized portion of phase II will have blood drawn for the correlative studies. Patients enrolled to the expansion cohort will not have research blood drawn.

9.1 T lymphocyte subtypes and other PBMC subsets analysis

The effect of rhIL-7-hyFc on immune cell phenotype and function in HGG patients is unknown. T lymphocyte absolute number and phenotype (i.e., CD4+ and CD8+ T cell naïve, effector memory, and central memory subsets, CD4+ Treg and activated CD4+ and CD8+ T cells) will be assessed using a 21-color, whole blood PBMC immunophenotyping assay as reported by the DiPersio laboratory (Staser 2017). The frequency and percentage of these and other PBMC subsets (i.e., T cells, NK cells, NKT cells, B cells, monocytes, dendritic cell subsets, and MDSC subsets) will also be investigated. The absolute change in each parameter, as well as variance in each parameter over time for each patient (mean, median, and SE/SD), will be evaluated. In addition, co-stimulatory and co-inhibitory receptors (PD-1, PD-L1, ICOS, TIM3, and LAG3) and T cell trafficking molecules (CXCR3, CCR5, LFA-1 and VLA-4) will be analyzed on cryopreserved PBMC. Cell proliferation Ki67 will be tested at the selected time shown in Treatment Schema.

Additional markers delineating T cell exhaustion and activation, or other parameters may be analyzed on cryopreserved PBMC.

9.2 T cell repertoire diversity

Because IL-7 receptor expression is high on resting, naive and memory populations, we expect that the rhIL-7-hyFc induced increase in circulating T cells will preferentially expand naive cells, and therefore lead to an overall increase in TCR diversity. To evaluate the diversity of the T cell receptor repertoire, we will collect PBMC to characterize using TCR deep sequencing and analyze with Immunoseq Analyzer software (Adaptive Biotechnologies, Seattle, WA). Blood samples for T-cell repertoire analysis will be obtained at study week 1 (prior to the 1st injection of rhIL-7-hyFc; at study week 4; and then at study week 13 (prior to rIL-7-hyFc injection).

9.3 Cytokine analysis

We will measure serum cytokine levels such as IL-7, IL-6, IL-15, INF-γ, TNF-α and TGF-β using ELISA or Luminex, with modification to previously described methods (Levy 2012; Francois 2018) during the study. Plasma samples will be collected and stored for all subjects.

9.4 Immunogenicity

Anti-drug antibodies have been reported in patients after one dose of rhIL-7-hyFc. The immunogenicity frequency and the occurrence of anti-drug antibodies (ADA) and neutralizing anti-drug antibodies (NADA) could be higher with exposure to multiple doses of rhIL-7-hyFc. However, presence of ADA and NADA was not associated with an increase in the frequency or severity of AEs. We will collaborate with NIT to monitor immunogenicity in patients treated with rhIL-7-hyFc by monitoring the formation of ADA and NADA to rhIL-7-hyFc in serum. (Detail see below)

9.5 Tumor tissue analysis (optional)

Whenever it is available, pre- and post-treatment formalin-fixed, paraffin-embedded (FFPE) specimens will be examined to estimate CD8 and CD4+ TILs using immunohistochemistry analysis. As a complimentary method, using a new tissue-profiling method by Multiplex Immunofluorescence (GE Cell Dive^{TM} system) (Sood 2016) that Drs. John DiPersio and James Hsieh recently installed in the department, we will assess the spatial distribution of immune cells at the tumor site. When possible, fresh tissue can be collected and saved to TPC for future analysis.

9.6 Lymphocyte functional analysis (optional)

The ELISPOT assay will be used to determine the number of T-cells secreting IFN-γ in specific response to a viral antigen. Commercially available kits for virus-specific ELISPOT assays will be used and the number of spots will be read on an ELISPOT reader.

9.7 Specimen Processing for PBMC

A total of 32-40 mL of whole blood will be collected in 4-5 EDTA tubes for isolation of PBMCs at each of the following time points (NOTE-: +/- 7 days are allowed):

- Week 1 (prior to the 1st dose of rhIL-7-hyFc) – 40 mL (32 mL for FACS, 8 mL for TCR sequencing).
- Week 2 (one week after rhIL-7-hyFc) – 32 mL
- Week 3 (two weeks after rhIL-7-hyFc) – 32 mL
- Week 4 (three weeks after rhIL-7-hyFc) – 40 mL (32 mL for FACS, 8 mL for TCR sequencing).
- Week 13 (prior to the 2nd dose of rhIL-7-hyFc) – 40 mL (32 mL for FACS, 8 mL for TCR sequencing).
- Week 14 (one week after rhIL-7-hyFc) – 32 mL
- At the time of tumor progression – 32 mL for FACS

In addition, 2 mL of plasma will be saved during the isolation of PBMC. Saved plasma will be used for cytokine analysis.

9.7.1 Handling of Specimens

A total of 32-40 mL of whole blood will be collected in EDTA tubes and delivered to the DiPersio lab at room temperature within 2-hour of collection. PBMCs will be collected by density gradient centrifugation (Ficoll) per standard protocol. PBMC will be saved in -80°C for immunologic studies. Plasma will be saved in -80°C for cytokine analysis.

9.8 Specimen Processing for ELISPOT (optional)

A total of 5 mL of whole blood will be collected in one green top heparin tube at each of the following time points (NOTE-: +/- 7 days are allowed):

- Week 1 (prior to the 1st dose of rhIL-7-hyFc) (as baseline) – 5 mL
- Week 4 (three weeks after rhIL-7-hyFc) – 5 mL
- Week 13 (prior to the 2nd dose of rhIL-7-hyFc) – 5 mL
- Week 16 (three weeks after rhIL-7-hyFc) – 5 mL
- At the time of tumor progression – 5 mL

9.8.1 Handling of Specimens

A total of 5 mL of whole blood will be collected in heparin tube and delivered to the Hotchkiss lab or Dr. Dinesh Thotala's lab at room temperature within 2-hour of collection. ELISPOT will be performed per lab standard procedure.

9.9 Anti-drug antibodies and neutralizing anti-drug antibodies (immunogenicity)

The formation of anti-drug antibodies (ADA) and neutralizing anti-drug antibodies (NADA) to rhIL-7-hyFc will be evaluated: BioAgilytix will perform both Elisa Binding (non-neutralizing) and neutralizing antibody assays according to their Standard Operating Procedure. Serum from the above time points will be collected for these assays.

NeoImmuneTech transferred the validated assay method to BioAgilytix who will perform the assays and the determination of the main ADA/NADA parameters.

Collection of Specimen(s): Blood will be collected in serum separator tubes (SST) as follows:

- Week 1 (prior to injection of 1st dose of rhIL-7-hyFc) – 8 mL
- Week 13 (prior to the 2nd dose of rhIL-7-hyFc) – 8 mL
- Week 45 (8 weeks after the last dose of rhIL-7-hyFc) – 8 mL
- If ADA or NADA is observed in week 45, additional blood collections will be required every 2 months in order to monitor ADA/NADA levels until it decreases to the basal level.

To date substantial amount of ADA data was obtained from the ongoing trials in Korea and the USA in solid tumors and GBM. The current data obtained indicates that the presence of ADA does not seem to affect drug exposure or pharmacovigilance data and safety profile of the drug.

Handling of Specimens(s): For all samples, draw blood into SST tubes. Mix the content of the tube by slowly inverting several times (5 to 6 times) immediately after the blood draw, then sit at ambient temperature for 30-60 minutes until a clot has formed. Within 15 minutes of blood collection, centrifuge the sample at room temperature at 2000 rpm for 5 minutes. The supernatant (serum) will be collected carefully for immediate storage and aliquot each serum sample into 4 cryovials, each of them containing at least 600 µL. Prepare appropriate labels and write the patient ID and time of preparation using a cryopen. Affix the labels to the cryovials. Freeze the samples in an upright position and store at -20ºC until shipment.

Shipping of Specimen(s): Specimen should be packed on dry ice and shipped frozen in batches to the BioAgilytix (Kathryn Lindley, Director, Bioanalytical Operations, BioAgilytix, 2300 Englert Dr., Durham, NC, 27713 USA. Email: kathryn.lindley@bioagilytix.com; Telephone: 919-381-6097, Monday-Thursday 9:00AM-4:30PM EST; shipments should be made Monday through Wednesday, or at least 2 days before any official holiday.)

Site Performing Correlative Study: The ADA assay will be conducted by BioAgilytix, Durham, NC

10 STUDY CALENDARS

10.1 Phase I and Randomized Phase II

Screening / baseline evaluations are to be conducted within 2 weeks prior to the start of protocol therapy. Screening labs are used for screening purposes only and Baseline lab values will be considered those obtained on Week 1 Day 1, prior to first study dose injection. Unless otherwise noted study visits will be completed within a ± 1 week window.

	Screening	**W1[4]**	**W2**	**W3**	**W4**	**W8**	**W12**	**W13**	**W14**	**W16**	**W20**	**W24**	**W25**	**W26**	**W37**	**W41**	**W45**	**FU[9]**
Confirm eligibility	X[13]																	
Informed consent	X[5]																	
KPS	X	X					X	X					X		X			
Physical exam	X	X					X	X					X		X			
CBC w/diff incl. ALC[8]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	
CD4	X	X			X	X	X			X	X	X						
LFTs	X	X[11]			X		X[11]			X		X[11]			X[11]		X	
EKG[17]	X	X						X					X		X			
Cardiac Enzymes (TnI or TNT and CK)[17]	X	X[20]						X[20]					X[20]		X[20]			
Pregnancy test[1]	X																	
Brain MRI[18]		SOC MRIs (Q2-3Mo)																
Randomization[2]	X																	
NANO	X						X								X			
rhIL-7-hyFc / placebo[4]		X						X					X		X			
SOC adjuvant TMZ[3, 14,15]					X	X	X			X	X	X						
TMZ drug diary					X	X	X			X	X	X						
Ki67			X						X									
Research blood for PBMCs and plasma[16]		X[6]	X	X	X			X[6]	X									
Research blood for ADA and NADA		X[6]						X[6]									X[6]	X[10]
OPTIONAL research blood for ELISPOT[16]		X			X			X		X								
OPTIONAL research tissue[7]	X														X[12]			
Survival																		X
AE assessment[19]		X ---------- X																

1. Women of childbearing potential only
2. Phase II only
3. Given on Days 1-5 of a 28-day cycle for a total of 6 cycles.

4. Within 14 days after the end of SOC RT/TMZ
5. Does not have to occur within 2 weeks prior to the start of protocol therapy; participants may be consented as early as the start of standard of care treatment.
6. Before injection
7. If available
8. Weekly CBC will be performed as per SOC and results will be collected for this study. Weekly CBC will not be performed specifically for this study. It may be drawn at a local lab.
9. Assess survival at 2 years and 5 years following the end of treatment by chart review, phone call, or clinic visit.
10. If ADA or NADA is observed, additional blood collections will be required Q2Mo to monitor ADA/NADA levels until it decreases to the basal level
11. LFTs must be assessed no more than 7 days prior to each dose of rhIL-7-hyFc/placebo.
12. At end of treatment.
13. Eligibility to receive first study dose injection during Week 1 Day 1 will be determined by lab values obtained at Screening, and not reliant on lab values obtained on Day 1
14. In the event of a delay, SOC labs (CBC, LFTs, and/or CD4) coinciding with visits of SOC TMZ dispensing will only be re-drawn if deemed medically necessary per treating physician discretion.
15. Any delays in TMZ cycle dosing are permitted per SOC guidelines and per treating physician discretion and may not be indicative of exact weeks noted in study calendar.
16. At the time of tumor progression
17. If a patient has an EKG that is deemed clinically significant (e.g., sinus tachycardia would not meet this definition, and non-specific changes may or may not meet this definition), then an echocardiogram should be performed. If an echocardiogram is performed and is deemed not clinically significant, the patient may be enrolled/continue in the study, with cardiac enzymes drawn as noted in footnote 20.
18. MRI window is ±30 days
19. AE's will be followed through 30 days after the end of study treatment
20. Only for patients with a clinically significant EKG

10.2 Phase II Expansion Cohort

Screening / baseline evaluations are to be conducted within 2 weeks prior to the start of protocol therapy. Screening labs are used for screening purposes only and Baseline lab values will be considered those obtained on Week 1 Day 1, prior to first study dose injection. Unless otherwise noted study visits will be completed within a ± 1 week window.

	Screening	**W1[4]**	**W2**	**W3**	**W4**	**W8**	**W12**	**W13**	**W14**	**W16**	**W20**	**W24**	**W25**	**W26**	**W37**	**W41**	**W45**	**FU[6]**
Confirm eligibility	X[8]																	
Informed consent	X[4]																	
KPS	X	X					X	X					X		X			
Physical exam	X	X					X	X					X		X			
CBC w/diff incl. ALC[5]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	
CD4	X	X			X	X	X			X	X	X						
LFTs	X	X[7]			X		X[7]			X		X[7]			X[7]		X	
EKG[11]	X	X						X					X		X			
Cardiac Enzymes (TnI or TNT and CK)[11]	X	X[14]						X[14]					X[14]		X[14]			
Pregnancy test[1]	X																	
Brain MRI[12]		SOC MRIs (Q2-3Mo)																
NANO	X						X								X			
rhIL-7-hyFc [3]		X						X					X		X			
SOC adjuvant TMZ[2, 9,10]					X	X	X			X	X	X						
TMZ drug diary					X	X	X			X	X	X						

Survival																	X
AE assessment[13]		X ---------- X															

1. Women of childbearing potential only
2. Given on Days 1-5 of a 28-day cycle for a total of 6 cycles.
3. Within 14 days after the end of SOC RT/TMZ
4. Does not have to occur within 2 weeks prior to the start of protocol therapy; participants may be consented as early as the start of standard of care treatment.
5. Weekly CBC will be performed as per SOC and results will be collected for this study. Weekly CBC will not be performed specifically for this study. They may be drawn at a local lab.
6. Assess survival at 2 years and 5 years following the end of treatment by chart review, phone call, or clinic visit.
7. LFTs must be assessed no more than 7 days prior to each dose of rhIL-7-hyFc/placebo.
8. Eligibility to receive first study dose injection during Week 1 Day 1 will be determined by lab values obtained at Screening, and not reliant on lab values obtained on Day 1
9. In the event of a delay, SOC labs (CBC, LFTs, and/or CD4) coinciding with visits of SOC TMZ dispensing will only be re-drawn if deemed medically necessary per treating physician discretion.
10. Any delays in TMZ cycle dosing are permitted per SOC guidelines and per treating physician discretion and may not be indicative of exact weeks noted in study calendar.
11. If a patient has an EKG that is deemed clinically significant (e.g., sinus tachycardia would not meet this definition, and non-specific changes may or may not meet this definition), then an echocardiogram should be performed. If an echocardiogram is performed and is deemed not clinically significant, the patient may be enrolled/continue on study with cardiac enzymes drawn as noted in footnote 14.
12. MRI window is ±30 days
13. AE's will be followed through 30 days after the end of study treatment
14. Only for patients with a clinically significant EKG

11 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On-Study Form Treatment Assignment Form Medical History Form	Prior to starting treatment
rhIL-7-hyFc Treatment Form	Every treatment cycle (at W1, W12(Cycle 3), Week 24 (Cycle 6) and Week 37
CBC Form	As per SOC (see study calendar)
NANO Form Response Form	To coincide with SOC MRIs (Q2-3 months)
DLT Form	After Cycle 1 for phase I patients only
SOC Treatment Summary Form	At the end of SOC chemoradiation
Research Blood Form Research Tissue Form	Per Section 9.7 (phase I and randomized phase II patients only)
Toxicity Form Steroid Form	Continuous
Treatment Summary Form	Completion of treatment
Follow Up Form	Eat Year 2 and Year 5 Follow UP
Progression Form	Time of disease progression (not completed for expansion cohort)
Death Form	Time of death
MedWatch Form	See Section 7.0 for reporting requirements

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

11.1 Adverse Event Collection in Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 7) must be captured in the Toxicity Form. Baseline AEs should be captured on the Medical History Form.

Participant death due to disease progression should be reported on the Toxicity Form as grade 5 disease progression. If death is due to an AE (e.g. cardiac disorders; cardiac arrest), report as a grade 5 event under that AE. Participant death must also be recorded on the Death Form.

12 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, an independent Data and Safety Monitoring Board (DSMB) will be specifically convened for this trial to review toxicity data. A DSMB will consist of no fewer than 3 members including 2 clinical

investigators and a biostatistician. DSMB members must be employed by Washington University, Barnes-Jewish Hospital, or St. Louis Children's Hospital. Like investigators, DSMB members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMB will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMB must also be disclosed.

Until such a time as the first secondary site enrolls its first patient, a semi-annual DSM report to be prepared by the study team will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after study activation at Washington University (if at least one patient has been enrolled) or one year after study activation (if no patients have been enrolled at the six-month mark).

The DSM report for the DSMB will be prepared by the study team with assistance from the study statistician, will be reviewed by the DSMB, and will be submitted to the QASM Committee. The DSMB must meet at least every six months beginning six months after enrollment of the first patient at a secondary site no more than one month prior to the due date of the DSM report to QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date, accrual by site, and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites and separated by cohorts with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Further DSMB responsibilities are described in the DSMB charter.

The study principal investigator and coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines (please refer to Section 7.0).

Refer to the Washington University Quality Assurance and Data Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMB. This is located on the QASMC website at https://siteman.wustl.edu/research/clinical-research-resources/protocol-office-prmcqasmc/.

13 MEASUREMENT OF EFFECT

13.1 rhIL-7-hyFc effect

The biological effect of rhIL-7-hyFc will be determined by its effect on raising ALC. ALC will be measured per the study calendar. The levels of ALC and the changes of ALC over time prior to and post rhIL-7-hyFc treatment will be monitored and analyzed (see statistical analysis section).

13.2 Antitumor Effect – RANO

Brain MRI will be performed about every 2 months (±30 days) as per SOC to evaluate response.

Criteria for response: Response will be evaluated in this study using the updated response assessment criteria for high-grade gliomas: Response Assessment in Neuro-Oncology (RANO) working group guideline.

Criteria for Response Assessment Incorporating MRI and Clinical Factors

Response	Criteria
Complete response	• Requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks. • No new lesions; stable or improved nonenhancing (T2/FLAIR) lesions. • Patients must be off corticosteroids (or on physiologic replacement doses only) and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a complete response; the best response possible is stable disease.
Partial response	Requires all of the following: • ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. • No progression of nonmeasurable disease. • Stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan. • Stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a partial response; the best response possible is stable disease.
Stable disease	Requires all of the following: • Does not qualify for complete response, partial response, or progression. • Stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without

Response	Criteria
	confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
Progression	Defined by any of the following: • ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids*. The absolute increase in any dimension must be at least 5mm when calculating the products. • Significant increase in T2/FLAIR nonenhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). • Any new measureable lesion. • Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose. • Failure to return for evaluation as a result of death or deteriorating condition; or clear progression of nonmeasurable disease.

- NOTE. All measurable and nonmeasurable lesions must be assessed using the same techniques as at baseline.
- Abbreviations: MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery.
- Stable doses of corticosteroids include patients not on corticosteroids.

Criteria for progression: Pseudoprogression is a phenomenon where radiographic features are consistent with tumor recurrence/progression but is instead related to treatment effect. This is a common occurrence in glioblastoma following radiation that is generally seen in the first 3-6 months after completing therapy.

If follow-up imaging confirms progression, the date of actual progression should be back-dated to the date of initial radiographic evidence of progression.

Alternatively, progressive disease can be defined as radiographic evidence of progression PLUS significant clinical decline that is felt to be unrelated to a co-morbid event or concurrent medication.

13.3 Disease Parameters

Measurable disease: Bi-dimensionally measurable lesions with clearly defined margins by MRI scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable or evaluable disease: Uni-dimensionally measurable lesions or lesions with margins not clearly defined such as areas of T2/FLAIR signal abnormality or poorly defined enhancing abnormality.

Note: For cystic lesions, the only measurable part is any enhancement area around the

cyst that is clearly defined and bi-dimensionally measurable. The cyst itself should not be considered measurable or non-measureable disease.

Target lesions: All measurable lesions that are residual of the lesion treated with MLA should be identified as target lesions and recorded and measured. Target lesions should be selected on the basis of their size (lesions with the longest diameter), but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion, which can be measured reproducibly should be selected. When there are too many measurable lesions, choose the largest 3 lesions as target lesions to follow. The other measurable lesions should be considered evaluable for the purpose of objective status determination.

Non-target lesions: All non-measurable lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

13.4 Methods for Evaluation of Measurable Disease

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

Clinical lesions: Clinical lesions will only be considered measurable on brain MRI when they are ≥ 5 mm diameter as assessed using a ruler.

Histology: This technique can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases when biopsy or surgical resection of a measureable lesion is clinically indicated.

Perfusion/CBV: This advanced brain MRI technique can be used as an adjunct test to determine treatment response or disease status. However, it should not be used as the primary or sole method to determine response or disease status.

13.4.1 Evaluation of Target Lesions

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

Partial Response (PR): ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all target lesions sustained for at least 4 weeks.

Progressive Disease (PD): At least a 25% increase in the sum of products of perpendicular diameters of at least 1 target lesion, taking as reference the smallest sum of products of perpendicular diameters on study (this includes the baseline sum if that is the smallest on study). The absolute increase in any dimension must be at least 5mm when calculating the products of perpendicular diameters.

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

13.4.2 Evaluation of Non-Target Lesions

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

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

13.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Summary of the RANO Response Criteria (Adapted from [47])

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.
* Progression occurs when this criterion is present.
† Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

13.4.4 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.

13.4.5 Neurological Exam and Performance Status

Patients will be graded using the Karnofsky Performance Status scale and their neurological function evaluated as improved, stable or deteriorated in addition to objective measurement of tumor size. These parameters will be used to determine the overall response assessment.

13.4.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

14 AUDITING

As coordinating center of this trial, Washington University (via the Quality Assurance and Safety Monitoring Committee (QASMC) will monitor each participating site to ensure that all protocol requirements are being met; that applicable federal regulations are being followed; and that best practices for patient safety and data collection are being followed per protocol. Participating sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.
Items to be evaluated include:

- Subject screening and enrollment
- Reporting of adverse events
- Maintenance of HIPAA compliance
- Completeness of regulatory documentation
- Completeness of participant documentation
- Acquisition of informed consent
- IRB documentation
- Issues of protocol adherence

Additional details regarding the auditing policies and procedures can be found at https://siteman.wustl.edu/research/clinical-research-resources/protocol-office-prmcqasmc/.

15 STATISTICAL CONSIDERATIONS

15.1 Study Design

This is a single-center study consisting of a phase I study to identify MTD (/RP2D and a phase II placebo-controlled randomized study on target patients. In the phase I study, MTD will be determined and a recommended phase 2 dose (RP2D) will be decided. The maximum tolerated dose (MTD) is defined as the dose level immediately below the non-tolerated dose. A total of at least 6 patients must be treated at a dose level for it to be considered the MTD. RP2D is the dose recommended for phase 2 study based on the MTD from dose escalation and ALC data which can never exceed MTD.

During the randomized phase II portion of the study, eligible patients will be enrolled and randomized at a 1:1 ratio to either rhIL-7-hyFc at the RP2D dose level or placebo arm (see study Schema).

Phase I enrollment was completed with N=19 patients. The MTD was identified as 720 mcg/kg. Current collected data suggest an improvement in progression-free survival in patients with IDH1 wildtype as defined by immunohistochemistry negative staining using an IDH mutant-specific antibody and MGMT promoter unmethylated GBM; therefore, we have added an expansion cohort to the rhIL-7-hyFc arm with N=31 as justified in Section 14.4.

15.2 The phase I study

15.2.1 Study design

The phase I dose finding study will begin with an Accelerated Phase with 1 patient per cohort at the first 2 doses (60 mcg/kg, 120 mcg/kg) followed by a conventional 3+3 dose escalation phase on the remaining 4 doses (240 mcg/kg~960mcg/kg). A cohort of six patients need to be tested at the MTD/RP2D level.

The Accelerated Phase
The Safety data from current phase I studies on rhIL-7-hyFc in solid tumors and GBM show that patients tolerate either or both of the first 2 doses (60 mcg/kg, 120 mcg/kg) well with no DLTs observed at the 2 doses, see rationale section on ABTC-1403 (IND 137067) on healthy volunteers, GX-I7-CA-003 in solid tumors, GX-I7-CA-004 in GBM. The Accelerated Phase will be conducted for dose levels 1 to 2, i.e., dosage 60 mcg/kg and 120 mcg/kg, with 1 patient per dose level and DLT and adverse events will be monitored during the 1st cycle of the treatment. If no DLT is observed at dosage 60 mcg/kg, a new patient will be enrolled to dose level 2 (120 mcg/kg). If any DLTs are observed in the 1 patient at either of the two dose levels, the Accelerated Phase will convert to a conventional 3+3 design at the dose level where DLTs are observed and remain so for all the dose levels above. If excess DLTs (>=2 out of a total of 6 patients experiencing DLTs) are observed at dose level 1 (60 mcg/kg), the study will halt. If no DLTs, the phase I

study will proceed to the next conventional 3+3 phase for dose levels 3 to 6 (see dosage Table in Section 5.2).

The conventional 3+3 Phase
Assuming no conversion at the first 2 doses, the conventional 3+3 design with 3 independent patients per cohort will start at dose level 3 (240 mcg/kg) and will follow the dose escalation decision rule of 3+3 design to escalate along the dosages from dose level 3 (240 mcg/kg) to 6 (960 mcg/kg) sequentially from the lowest to the highest dose level, see dosage Table in Section 5.2. The dose escalation rules are summarized in the dose escalation criteria in Section 5.3.3. If excess DLT (≥2 out of a cohort of 3 patients or ≥2 out of a total of 6 patients from 2 cohorts) is observed at starting dose level 240 mcg/kg, a cohort of 5 patients (six, plus the one patient tested previously) will be tested at dose level 2 (120 mcg/kg) and if <=1 DLTs in 6 are observed, dose level 2 will be the recommended dose for the Phase II. If >=2 out of 6 patients are observed with DLTs at dose level 2, the trial will similarly test dose level 1 to have dose level 1 either as the MTD or the study halt in presence of excess toxicities. If no excess dose (0 DLT out of a cohort of 3 patients or 1 DLT out of a total of 6 patients) is observed at dose level 3, the Phase I study will follow the dose escalation decision rule to escalate along the dosages thereafter to dose level 6 until the MTD if no DLT at highest does level) is identified at which no more than one in six patients experiences DLT. A total of 6 patients will need to be treated at the MTD level. Integrating the MTD data and ALC data, a RP2D (which can be the MTD or not but will not exceed the MTD), will be determined.

If we do not find MTD at the proposed dose levels, we plan to amend the protocol to test new or higher dose levels per the recommendations from NIT. In addition, another Phase I trial is on-going. Dose escalation can be stopped if a MTD is determined through the on-going Phase I trial announced by the sponsor.

15.2.2 Endpoint

The primary endpoint of the phase I is adverse events (AEs), especially dose limiting toxicity (DLT). DLT will be defined as ≥ grade 3 AEs that occur within 30 days from the date when patients receive the 1st dose of rhIL-7-hyFc administration and are concluded to be possibly, likely or definitely related to the drug regimen that occurs during cycle 1, with severity graded according to the Common Terminology Criteria for Adverse Events (CTCAE) 5.0. Grade 3 dermatologic AEs, including injection site reaction, that resolves to Grade ≤ 2 in ≤ 3 days with supportive care; transient Grade 3 influenza-like symptoms or pyrexia controlled with medical management; Grade 3 fatigue that resolves to Grade ≤.2 in ≤ 3days; Grade 3 neurologic toxicity responding within ≤ 7 days to steroids, anticonvulsants, or electrolyte correction; or Grade 3 laboratory abnormalities that are asymptomatic and considered by the investigator not to be clinically significant will be excluded from the DLT definition.

The secondary endpoint is absolute lymphocyte count (ALC).

15.2.3 Sample size calculation

During the Phase I, total number of patients enrolled will depend on the number of dose levels and observed DLTs, ranging from a minimal of 6 patients to a maximum of 26 patients.

15.2.4 Statistical Analysis

Data from the Phase I study will be presented in a descriptive manner AEs and SAEs will be summarized by counts and percentages by patient, dose type and grade.

15.3 The randomized phase II study

15.3.1 Study design

For the phase II trial, eligible patients, stratified by concomitant use of steroids (Yes/No), will be randomized at a 1:1 ratio to receive either placebo or the study drug rhIL-7-hyFc. Patients on the study drug arm will receive multiple doses of the study drug rhIL-7-hyFc (see study schema). Lymphocyte count will be measured before and after rhIL-7-hyFc administration (details see treatment schema and study calendar).

15.3.2 Endpoint

The primary endpoint of Phase II is the % increase of ALC in evaluable patients prior to adjuvant TMZ (at ~ Week 4) that measures the “pure” drug effect before influences from TMZ in reference to the baseline prior to rhIL-7-hyFc injection. A patient is considered evaluable for the primary endpoint if (s)he completes CBC at baseline and at ~4 weeks after the first dose of NT-17 or placebo and has valid ALC measurements prior to and at ~week 4 before adjuvant TMZ of treatment with NT-17 or placebo. Enrolled patients who are not evaluable for the primary endpoint will be replaced so as to meet the study power requirements (see 15.3.3).

The secondary endpoints include lymphocyte counts over time in the setting of steroid use and chemotherapy, progression free survival (PFS) and overall survival (OS).

15.3.3 Sample size calculation

We summarized the ALC of all comers seen in our center at 1 month after completion of RT + TMZ and estimated a mean of ALC at 850 and a standard deviation (SD) of 480. This also agrees with data in published literatures (Mendez 2016). We assume that patients on the placebo arm (who usually have received radiation and TMZ post-operative) have the same mean of 850 and SD of 480. Based on 1-sided two sample t-test with equal variance at a 5% level, 8 patients per arm provides 80% power to test a mean ALC of 1486 in the treated patients in comparison to a mean of 850 in the placebo arm. Assuming ~10% dropout rate, we will enroll a total of 20 patients, 10 per arm.

For the whole study spanning through the Phase I and the Phase II study, approximately a minimum of 26 to a maximum of 46 evaluable patients will be enrolled.

15.3.4 Accrual

We see approximately 150 new patients with HGG (with ~70% are GBM) each year at Washington University with ~30% participating in clinical trials. We expect to be able to enroll approximately 1-2 patient per month on average, and thus it will take approximately 18 months to enroll ~29 patients and 30 months to enroll the expected maximum total of 46 evaluable patients.

15.3.5 Statistical Analysis

All data will be analyzed as observed and no imputation for missing values will be used. Patient characteristics will be summarized by descriptive statistics. Descriptive statistics will be used to summarize the trial results, i.e., statistics for continuous variables such as lymphocyte counts may include means, medians, ranges and appropriate measures of variability. Qualitative variables will be summarized by counts and percentages. The uncertainty of estimates will be assessed by confidence intervals (CIs). Markers for lymphocyte recovery in each group will be summarized as means and standard deviations (SD) at each time point. The primary endpoint, ALC at ~ week 4 and the change from the time point of radiation will be compared between the two arms by two sample t-test or Wilcoxon rank sum test as appropriate and by a linear model where other important factors such as concomitant use of steroid besides the treatment arm will be modeled. Within each arm, the primary endpoint will be compared to the ALC at the completion of radiation by paired sample t-test or Wilcoxon signed rank test as proper. Total lymphocyte counts at later cycles will be similarly analyzed but may be compared to a different reference time point. We will analyze ALC over time using linear models for repeated measurement data, followed by post-hoc multiple comparisons for between-group differences at other time points of interest. PFS and overall survival will be analyzed using the Kaplan–Meier method and survival difference will be compared between arms by log rank test. The univariate Cox proportional hazard model will be applied to evaluate ALC at each time point, in continuous scale and dichotomized scale by the cutoff of 1 (determined based on literature), for their prognostic effect on PFS and OS while multivariate Cox model will be applied to account for other covariates. The time varying Cox proportional hazard model will be applied to model the prognostic effect of the longitudinal ALC as a whole, without and with additional covariates.

15.4 The expansion cohort

15.4.1 Study design

Based on observed survival improvement in currently enrolled patients, we added (in August 2021) an expansion cohort to the experimental arm. For the expansion cohort, a total of N=31 IDH1 wildtype confirmed by immunohistochemistry and MGMT promoter unmethylated eligible patients will be enrolled to receive the study drug rhIL-7-hyFc.

15.4.2 Endpoint

The primary endpoint for the expansion cohort is progression free survival (PFS) defined from date of surgery to date of progression or death due to disease or date of last clinical follow up. The secondary endpoints include AE and OS.

15.4.3 Sample size calculation

The sample size calculation was based on testing the estimated median PFS of 11.5 months in the currently enrolled patients versus a null median PFS of 6.3 months (NEJM, 2014, 8:370), assuming PFS follows an exponential distribution.

A two-sided, one-sample log-rank test calculated from N=31 subjects achieves 81.05% power at a 5% significance level to detect a median survival time of 11.5 months (see Section 1.8.2) when the median survival time of the historic control group is 6.3 months, corresponding to a hazard ratio of 0.548. Subjects are accrued for a period of 18 months. Follow-up continues for a period of 12 months after the last subject is added. The probability that a subject experiences an event during the study is 0.7039. The expected number of events during the study with N=31 eligible patients is 22.

15.4.4 Accrual

We see approximately 150 new patients with HGG (with ~70% are GBM) each year at Washington University with ~30% participating in clinical trials. We expect to be able to enroll approximately 1-2patient per month on average, and thus we can enroll 31 patients within approximately 18-month accrual period.

15.4.5 Statistical Analysis

PFS will be analyzed by the KM method and median PFS will be estimated with 95% CI and tested against the null median PFS of 6.3 months by 2-sided one-sample log rank test. OS will be analyzed similarly. Other endpoints will be analyzed similarly as in Section 15.3.5 but without between arm comparison.

15.5 Analysis set

The safety analysis set include all patients who have received any dose of the rhIL-7-hyFc.

The efficacy analysis set for the randomized phase II portion of the study includes all patients who have received a dose of rhIL-7-hyFc, completes CBC at baseline and at ~4 weeks prior to adjuvant TMZ, and have evaluable ALC measurements from the CBC labs. The primary analyses will focus on the intent to treat (ITT) patients regardless what treatment they actually receive.

The PFS evaluable patients include patients who received at least one dose of rhIL-7-hyFc.

15.6 Early stopping rules

The study will stop if the following stopping rules are met:

In phase I part of the study, if ≥2 patients are observed with DLTs out of 6 patients at dose level 1 (60 mcg/kg), the trial will halt.

There is no efficacy stopping rule for the randomized phase II cohort or expansion cohort. The toxicity data for the expansion cohort will be reviewed regularly by the study team and the DSMB. If serious unexpected toxicities occur, the expansion cohort can be stopped early due to safety concern as recommended by the DSMB.

15.7 Ad hoc proof-of-concept check

In the randomized phase II portion of the study, when the number of patients enrolled into each arm is 5 (half of N per arm), we will perform one-time ad-hoc proof-of-concept check on the primary endpoint, the primary % ALC change at ~wk4 prior to adjuvant TMZ relative to baseline . If the ad-hoc check finds that the mean % ALC increase evaluated in the rhIL-7-hyFc treated patients is lower than that of placebo treated patients, the study will halt to be reviewed by the PI and DSMB.

16 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Site activation is defined as when the secondary site has received official written documentation from the coordinating center that the site has been approved to begin enrollment. At a minimum, each participating institution must have the following documents on file at Washington University prior to study activation:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMB (if applicable), the Washington University study team, and NeoImmuneTech.
- Documentation of FWA, signed FDA Form 1572 (if applicable), and the CVs of all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The coordinating center Principal Investigator (or designee) is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 4 weeks of obtaining Washington University IRB approval. Activated secondary sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted. Upon the secondary sites obtaining local IRB approval,

documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

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APPENDIX A: NANO Scale

8.5 Neurologic Function in Neuro-Oncology (NANO) Scale

Scoring assessment is based on direct observation and testing performed during clinical evaluation and is not based on historical information or reported symptoms.

Domain Levels of Function
Please check 1 answer for each domain

Domains	Key Considerations
Gait 0 ☐ Normal 1 ☐ Abnormal but walks without assistance 2 ☐ Abnormal and requires assistance (companion, cane, walker, etc.) 3 ☐ Unable to walk ☐ Not assessed ☐ Not evaluable	• Walking is ideally assessed by at least 10 steps
Strength 0 ☐ Normal 1 ☐ Movement present but decreased against resistance 2 ☐ Movement present but none against resistance 3 ☐ No movement ☐ Not assessed ☐ Not evaluable	• Test each limb separately • Recommend assess proximal (above knee or elbow) and distal (below knee or elbow) major muscle groups • Score should reflect worst performing area • Patients with baseline level 3 function in one major muscle group/limb can be scored based on assessment of other major muscle groups/limb
Ataxia (upper extremity) 0 ☐ Able to finger to nose touch without difficulty 1 ☐ Able to finger to nose touch but difficult 2 ☐ Unable to finger to nose touch ☐ Not assessed ☐ Not evaluable	• Non-evaluable if strength is compromised • Trunk/lower extremities assessed by gait domain • Particularly important for patients with brainstem and cerebellar tumors • Score based on best response of at least 3
Sensation 0 ☐ Normal 1 ☐ Decreased but aware of sensory modality 2 ☐ Unaware of sensory modality ☐ Not assessed ☐ Not evaluable	• Recommend evaluating major body areas separately (face, limbs and trunk) • Score should reflect worst performing area • Sensory modality includes but not limited to light touch, pinprick, temperature and proprioception • Patients with baseline level 2 function in one major body area can be scored based on assessment of other major body areas

Visual Fields

- 0 ☐ Normal
- 1 ☐ Inconsistent or equivocal partial hemianopsia (≥quadrantopsia)
- 2 ☐ Consistent or unequivocal partial hemianopsia (≥quadrantopsia)
- 3 ☐ Complete hemianopsia
- ☐ Not assessed
- ☐ Not evaluable

- Patients who require corrective lenses should be evaluated while wearing corrective lenses
- Each eye should be evaluated and score should reflect the worst performing eye

Facial Strength

- 0 ☐ Normal
- 1 ☐ Mild/moderate weakness
- 2 ☐ Severe facial weakness
- ☐ Not assessed
- ☐ Not evaluable

- Particularly important for brainstem tumors
- Weakness includes nasolabial fold flattening, asymmetric smile and difficulty elevating

Language

- 0 ☐ Normal
- 1 ☐ Abnormal but easily conveys meaning to examiner
- 2 ☐ Abnormal and difficulty conveying meaning to examiner
- 3 ☐ Abnormal. If verbal, unable to convey meaning to examiner OR non-verbal (mute/global aphasia)
- ☐ Not assessed
- ☐ Not evaluable

- Assess based on spoken speech. Non-verbal cues or writing should not be included.
- **Level 1**: Includes word finding difficulty; few paraphasic errors/neologisms/word substitutions; but able to form sentences (full/broken)
- **Level 2**: Includes inability to form sentences (<4 words per phrase/sentence); limited word output; fluent but "empty" speech.

Level of Consciousness

- 0 ☐ Normal
- 1 ☐ Drowsy (easily arousable)
- 2 ☐ Somnolent (difficult to arouse)
- 3 ☐ Unarousable/coma
- ☐ Not assessed
- ☐ Not evaluable

- None

Behavior

- 0 ☐ Normal
- 1 ☐ Mild/moderate alteration
- 2 ☐ Severe alteration
- ☐ Not assessed
- ☐ Not evaluable

- Particularly important for frontal lobe tumors
- Alteration includes but is not limited to apathy, disinhibition and confusion
- Consider subclinical seizures for significant

APPENDIX B: Safety Data

In Study GX-I7-CA-003 of single-agent rhIL-7-hyFc given in multiple doses in advanced solid tumor patients, there were no DLTs observed up to 1200ug/kg. Each of the cohorts (Cohort 1: 60 ug/kg; Cohort 2: 120ug/kg; Cohort 3: 240ug/kg; Cohort 4: 480ug/kg; Cohort 5: 720ug/kg; Cohort 6: 960ug/kg; Cohort 7: 1200ug/kg) consisted of 3 patients each that followed a 3+3 dose escalation design.

Following are the patient baseline characteristics:

Cohort 1 (60 µg/kg; n=3) Cohort 2 (120 µg/kg; n=3) Cohort 3(240 µg/kg; n=3) Cohort 4 (480 µg/kg; n=3) Cohort 5 (720 µg/kg; n=3) Cohort 6 (960 µg/kg; n=3) Cohort 7(1200 µg/kg; n=3)	Number of Patients (%) (n = 21)
Gender:	
Male	12 (57.14)
Female	9 (42.86)
Primary Sites:	
Synovial Sarcoma	1 (4.76)
Breast Cancer	2 (9.52)
Rectal Cancer	5 (23.81)
Colon Cancer	10 (47.62)
Anal Cancer	1 (4.76)
Ovary Cancer	1 (4.76)
Cervical Cancer	1 (4.76)

The most common AEs seen in the study were injection site reactions (Grades 1 or 2 only). Following is a list of AEs seen in each of the cohorts.

Status of the occurrence of entire adverse drug reactions (based on the data entered on July 2, 2019)

n(%),[case]		GX-I7 (hIL-7-hyFc)							Total (n=21)
		60 μg/kg (n=3)	120 μg/kg (n=3)	240 μg/kg (n=3)	480 μg/kg (n=3)	720 μg/kg (n=3)	960 μg/kg (n=3)	1200 μg/kg (n=3)	
ADR		3(100.0),[6]	2(66.7),[9]	3(100.0),[6]	2(66.7),[6]	1(33.3),[5]	2(66.7),[2]	3(100.0),[10]	16(76.2),[44]
	Gr1	2(66.7),[4]	2(66.7),[7]	3(100.0),[4]	2(66.7),[4]	1(33.3),[2]	1(33.3),[1]	2(66.7),[7]	13(61.9),[29]
	Gr2	1(33.3),[2]	2(66.7),[2]	2(66.7),[2]	2(66.7),[2]	1(33.3),[3]	1(33.3),[1]	3(100.0),[3]	12(57.1),[15]
Severity	Gr3	-	-	-	-	-	-	-	-
	Gr4	-	-	-	-	-	-	-	-
	Gr5	-	-	-	-	-	-	-	-
ADR by Preferred term									
Injection site reaction		3(100.0),[6]	2(66.7),[5]	3(100.0),[3]	2(66.7),[4]	1(33.3),[3]	-	3(100.0),[4]	14(66.7),[25]
Pyrexia			1(33.3),[1]	1(33.3),[1]	1(33.3),[2]	-	1(33.3),[1]	2(66.7),[3]	6(28.6),[8]
Rash / Rash papular			1(33.3),[1]	-	-	1(33.3),[1]	1(33.3),[1]	1(33.3),[1]	4(19.0),[4]
Decreased appetite			1(33.3),[1]	-	-	-	-	1(33.3),[1]	2(9.5),[2]
Asthenia		-	-	1(33.3),[1]	-	-	-		1(4.8),[1]
Back pain			1(33.3),[1]	-	-	-	-		1(4.8),[1]
Constipation		-	-	-	-	1(33.3),[1]	-		1(4.8),[1]
Influenza like illness		-	-	-	-	-	-	1(33.3),[1]	1(4.8),[1]
Myalgia		-	-	1(33.3),[1]	-	-	-		1(4.8),[1]

[Note] ADR; Adverse drug reaction

APPENDIX C: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term “research” encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

APPENDIX D: Reporting Timelines

Expedited Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Drug/Device Manufacturer**
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	All events being submitted to the FDA should be submitted concurrently to NeoImmune Tech
Unexpected fatal or life-threatening suspected adverse reaction			Report no later than 7 calendar days after initial receipt of the information	All events being submitted to the FDA should be submitted concurrently to NeoImmune Tech
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Report via email after IRB acknowledgment		All events being submitted to the QASMC should be submitted concurrently to NeoImmuneTech
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.			All events being submitted to the HRPO should be submitted concurrently to NeoImmuneTech
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.			
Protocol exception	Approval must be obtained prior to implementing the change			
Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	All events being submitted to the FDA should be submitted concurrently to NeoImmuneTech
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days.			

Expedited Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Drug/Device Manufacturer**
	If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.			
Breach of confidentiality	Within 10 working days.			
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			

Routine Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Drug/Device Manufacturer**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.	All events being submitted to the FDA should be submitted concurrently to NeoImmuneTech
Minor deviation	Report summary information at the time of continuing review.			
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.			

Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			

Expedited Reporting Timelines for Secondary Sites				
Event	**WU (Coordinating Center)**	**Local IRB**	**FDA**	**Drug/Device Manufacturer**
Serious AND unexpected suspected adverse reaction	Report no later than 11 calendar days after it is determined that the information qualifies for reporting.	Report all applicable events to local IRB according to local institutional guidelines.	The research team at Washington University is responsible for reporting all applicable events to the FDA as needed.	The research team at Washington University is responsible for reporting all applicable events to NeoImmuneTech as needed.
Unexpected fatal or life-threatening suspected adverse reaction	Report no later than 4 calendar days after initial receipt of the information.			
Unanticipated problem involving risk to participants or others	Report no later than 4 calendar days after initial receipt of the information.			
Adverse event or SAE that does not require expedited reporting	As per routine data entry expectations			
Protocol exception	Approval must be obtained prior to implementing the change.			

APPENDIX E: Washington University SAE Reporting Cover Sheet

SAE COVER SHEET- Secondary Site Assessment

Washington University HRPO#:	Sponsor-Investigator:
Subject Initials:	Subject ID:
Treating MD:	Treating Site:
EVENT TERM:	Event Start Date:
EVENT GRADE:	Date of site's first notification:

Treating MD Event Assessment:

Is this event **possibly, probably, or definitely** related study treatment?

☐ yes ☐ no

If yes, please list which drug (if more than one)_____

Explain

_____

_____

_____ _____ _____

Physician's Name **Physician's Signature** **Date**