DUKE CANCER INSTITUTE

A National Cancer Institute-designated Comprehensive Cancer Center

A Phase 0 Study

PEskE: A phase 0/surgical window-of-opportunity study to evaluate the pharmacokinetics and pharmacodynamics of Evolocumab in patients with recurrent glioma or glioblastoma

Sponsor:	PI – Duke Cancer Institute
Funding Source:	Departmental Funds
Protocol Source:	PI - Duke Cancer Institute
Duke IRB#:	Pro000108375

Principal Investigator
Mustafa Khasraw, MBChB, MD, FRCP, FRACP
Room 047 Baker House
DUMC 3624
Ph: (919) 684-5301
Fax: (919) 684-6674
Mustafa.khasraw@duke.edu

Sub-Investigators
David Ashley, MBBS, FRACP, PhD
David.ashley@duke.edu

Annick Desjardins, MD, FRCPC
Annick.desjardins@duke.edu

Margaret Johnson, MD
Margaret.O.Johnson@duke.edu

Daniel Landi, MD
Daniel.landi@duke.edu

Katherine Peters, MD, PhD
Katherine.peters@duke.edu

Kirit Singh, MBBS
Kirit.singh@duke.edu

Statistician
James E. Herndon, II, PhD
James.herndon@duke.edu

Study Coordinator
Erin Severance
Erin.Severance@duke.edu

Regulatory Coordinator
Kate Hogan, MS
Katherine.hogan@duke.edu

Data Manager
Eric Lipp, MD
Eric.lipp@duke.edu

Original version 1.0:	20210528	
Amended version 1.1:	20210718	Revise order of secondary outcomes in Sections 3.1 and 6.
Amended version 2.0:	20210831	Revise Table 4 and Sections 10.1, 10.3, and 10.4 to separate the lipid panel from the CMP as lipid panel is only required at screening and to make language in Section 10.1 consistent with Table 4. All screening tests/procedures should be within 14 days of starting evolocumab with the exception of the pregnancy test (see also Section 9.1). MRI has been removed at screening from Table 4 and Section 10.1, as disease evaluation is not needed for the study endpoints. Physical and neurological exams and clinical labs removed from Table 4 and 10.4 as they are SOC tests/procedures for pre-op and no study-specified window is needed. Clarification regarding commercial agent in Section 8.4.
Amended version 2.1:	20210927	Updates to face page of the protocol
Amended version 3.0	20211027	Remove the term “high-grade” from study title and throughout protocol. While the study always included a

		comparator group, we are further defining this group as an unblinded, non-randomized, control arm that includes patients who consent to participate as a control OR specimens requested from the BTBR (see revisions throughout Sections 3, 6, 7.1, 10, 11, and 13). A new schema has been added in Section 4 for the control arm. Eligibility in Section 9 has been reformatted to indicate the criteria that apply to either both arms or treatment arms only. Units for eligible lab values have been modified.
Amended version 4.0	20220221	The inclusion criterion for platelets in the treatment arm has been revised to allow potential participants who received temozolomide in the past year to have lower platelets (Section 9.1). Evolocumab does not have myelotoxic side effects, so should not further lower platelets. Revise injection window prior to surgery from 7-14 days to 4-14 days (see revisions in Study Schema in Section 4, Table 4, and throughout Sections 7 and 10) to allow for subject scheduling flexibility. An exploratory objective has been added to assess the activity of T cells isolated from tumor specimens (Sections 6, 7.3, and 13.6).
Amended version 5.0	20220414	Biopsy is now allowed as a means to provide tissue in addition to surgical resection and debulking. Revised inclusion criterion #3 to include biopsy (Section 9.1). Revised language to be inclusive of biopsy material including reducing minimum amount of tissue collection needed to 20 mg from 50 mg. (See revisions throughout Sections 3, 6, 7, 9, 10, and 13; Study Schema Figure 1 and Figure 2, and Table 4)

1 TABLE OF CONTENTS

2 LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BBB	Blood Brain Barrier
BTBR	Brain Tumor Center Biorepository
CBC	Complete Blood Count
CFSE	Carboxyfluorescein Diacetate Succinimidyl Ester
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T-Lymphocyte
DLT	Dose Limiting Toxicity
DVT	Deep Vein Thrombosis
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
FACS	Fluorescence-activated cell sorting
GO	Gene ontology
H&P	History & Physical Exam
HRPP	Human Research Protections Program
ICI	Immune Checkpoint Inhibition
IDH	Isocitrate dehydrogenase
IV	Intravenously
KPS	Karnofsky Performance Status
LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
LDL	Low-density lipoproteins
MGMT	O-6-methylguanine-DNA methyltransferase
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
PCSK9i	Proprotein convertase subtilisin/kexin type 9 serine protease inhibitor
PK	Pharmacokinetics
PD	Pharmacodynamics
PD1	Programmed Death 1
PFS	Progression Free Survival
p.o.	per os/by mouth/orally
PR	Partial Response
PRTBTC	Preston Robert Tisch Brain Tumor Center
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SIL	Stable isotope-labeled
SPGT	Serum Glutamic Pyruvic Transaminase
STRING	Search tool from the retrieval of interactive genes/proteins
TIL	Tumor Infiltrating Lymphocyte

TME	Tumor Microenvironment
TMZ	Temozolomide
WBC	White Blood Cells
WHO	World Health Organization
XRT	Radiation therapy

3 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

3.1 Purpose

This Phase 0 surgical window of opportunity trial seeks to evaluate the pharmacokinetic (PK) and pharmacodynamic (PD) properties of an FDA-approved proprotein convertase/ kexin type 9 serine protease inhibitor (PCSK9i; Evolocumab) in patients with primary and recurrent glioma at the Preston Robert Tisch Brain Tumor Center (PRTBTC). Specifically, it will address the following objectives.

Primary Objectives:

Evaluate whether evolocumab crosses the blood brain barrier (BBB) and is measurable in the resected or biopsied tumor specimens of patients with primary and recurrent glioma.

Secondary Objectives:

1. Among patients treated with evolocumab, assess the association between serum and intratumoral levels of evolocumab in the patients treated with evolocumab.
2. Compare the PDs of evolocumab in treated patients relative to that observed in a control cohort of untreated patients. The characterization of the PD properties will include the following:
    a. Lipid metabolism of tumor cells taken from resected or biopsied samples
    b. Analysis of tumor cells expressing MHC-I will be performed by fluorescence-activated cell sorting (FACS)

Hypotheses:

We hypothesize that:

1. The PCSK9 inhibitor evolocumab can cross the BBB and accumulate intratumorally
2. Treatment with evolocumab induces measurable biologic effects when administered to patients with glioma.

3.2 Background and Significance

Glioma, specifically glioblastoma (GBM), accounts for 46.6% of primary brain tumors, affects approximately 10,996 people in the US annually [2], and results in a high mortality rate with a median survival ranging from 16.1 to 20.5 months for patients with newly diagnosed GBM [3-5]. Despite aggressive, image-guided tumor resection [6], high-dose external beam radiotherapy or brachytherapy, and recent advances in anti-angiogenic [7] treatments and chemotherapy, patients with GBM typically live less than 15 months from the time of diagnosis [8,9]. Immunotherapy has revolutionized treatment for several hard-to-treat cancers but has failed to make significant improvements in outcomes for patients with glioblastoma [10]. This reflects the multiple and unique mechanisms of immune evasion and escape in this highly heterogeneous tumor [11]. Glioblastoma engenders profound local and systemic immunosuppression and is remarkably effective at inducing T cell dysfunction, posing a challenge to any immunotherapy-based approach. Through the use of a genetic screen, proprotein convertase subtilisin/kexin type 9 serine protease (PCSK9) has been identified as important in tumor escape from immune mediated rejection. PCSK9 is a secreted protein that binds to the low-density lipoprotein (LDL) receptor redirecting it to the lysosome for degradation rather than recycling to the plasma membrane. In preclinical experiments, genetic depletion of PCSK9 in murine tumor cells significantly attenuates their abilities to form tumors in a T cell dependent manner. Furthermore, PCSK9 depletion overcomes resistance to anti-programmed death 1 (PD1) immune checkpoint therapy with a high fraction of mice free of tumors and resistant to later challenges with parental tumor cells. A significant increase has also been observed in both the total numbers and diversity of T cells in the PCSK9 deficient tumors. Importantly, PCSK9 down-regulates MHC-I expression, in a manner similar to its regulation of LDL receptor [1].

Evolocumab is a PCSK9i with a well-established safety profile, is FDA-approved as a lipid-lowering agent, and is widely used to treat patients who have elevated levels of LDL [12]. To explore the role of PCSK9i in glioma, we need first to confirm that Evolocumab crosses the blood brain barrier and achieves measurable concentration in human glioma. In this protocol, we will compare levels of Evolocumab in the tissue of treated patients to that observed in control subjects after we develop an assay that can measure the level of Evolocumab in resected or biopsied brain glioma tissue. This protocol uses a surgical window of opportunity approach, collecting tissue from patients undergoing procedures of their primary or recurrent glioma to characterize the pharmacokinetic and pharmacodynamics properties of PCSK9i in patients with glioma. We will initiate this trial as a first step to assess if it is worthwhile to repurpose Evolocumab as an immunotherapeutic agent for treatment of patients with glioma.

3.3 Design and Procedure

A maximum of 10 patients with pathologically documented glioma (either newly diagnosed or at first progression/ recurrence) will be treated with Evolocumab in this study after providing informed consent. Patients will receive 420 mg (the maximum single dose) of Evolocumab subcutaneously into thigh, abdomen or upper arm 4-14 days prior to a surgical procedure or biopsy of their tumor. After the procedure, leftover tissue not required for histological analysis will be collected, and the level of evolocumab will be quantified using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). At two time points, prior to injection of Evolocumab and at time of surgery or biopsy, peripheral blood will be drawn to analyze serum levels of the drug (for comparison to intratumoral). Twenty to one hundred milligrams of tumor per patient will be homogenized and normalized using a protein assay followed by reduction/alkylation and trypsin digestion. Stable isotope-labelled (SIL) peptides corresponding to evolocumab-specific peptides will be spiked into tryptic digests, and LC-MS/MS will be used for targeted quantification of the drug-derived and SIL forms of these Evolocumab-specific peptides. Calibration curves will be generated by spiking in of Evolocumab into blank matrix (tumor homogenates from the brain specimen repository) followed by targeted LC-MS/MS. Data analysis, including normalization to SIL standards and fitting of data to the calibration curve will be performed in Skyline as previously described [13]. If necessary, an immunoprecipitation step using Protein A/G agarose will be utilized to enrich for immunoglobulins (including evolocumab) prior to trypsin digestion and LC-MS/MS.

A contemporaneous control arm of 20 patients who undergo craniotomy (without receiving Evolocumab) will be consented to participate in this study as control subjects and prospectively observed. Although non-randomized, these specimens will provide valuable information for exploratory comparison of the evolocumab treated and evolocumab untreated tumor specimens and their immune microenvironment. If there are difficulties recruiting prospectively patients to serve as a control group, samples from the Brain Tumor Center Biorepository (BTBR) (Pro00007434) will be considered as possible control subjects.

3.4 Selection of Subjects

Please refer to Section 9.

3.5 Duration of Study

Patients' active participation in the treatment arm of the study will last approximately four weeks, including a two-week screening period, one day for their surgical procedure and two-week safety follow-up period.

Control patients' participation ends after their surgical procedure.

3.6 Data Analysis and Statistical Considerations

The primary and secondary objectives are comparisons of two cohorts with respect to PK and PD measures: treated and untreated brain tumor specimens. Specifically, tumor specimens will be assessed for lipid metabolism, and whether tumor cells express MHC-I. Depending upon the distribution of these

outcomes, either a Wilcoxon rank sum test or a two-sample t-test will compare cohorts. Spearman's correlation coefficient will describe the association between serum and intratumoral levels of Evolocumab.

In exploratory analyses, the hypothesis that Evolocumab treatment will lead to global changes in pro-tumorigenic pathways and the reduction in PSCK9 levels will be assessed. To test this hypothesis, non-targeted quantitative LC-MS/MS will be performed on resected or biopsied tumor from n=10 treated patients versus n=20 untreated patients. Furthermore, if PSCK9 is not among the proteins quantified by non-targeted LC-MS/MS, a more sensitive targeted LC-MS/MS analysis will be utilized as described for the PK analysis of Evolocumab as described above. Bioinformatics analyses of differentially expressed proteins will utilize protein set enrichment, gene ontology (GO) annotations, and search tool from the retrieval of interactive genes/proteins (STRING). Adjustments for multiple comparisons will be made.

4 STUDY SCHEMA

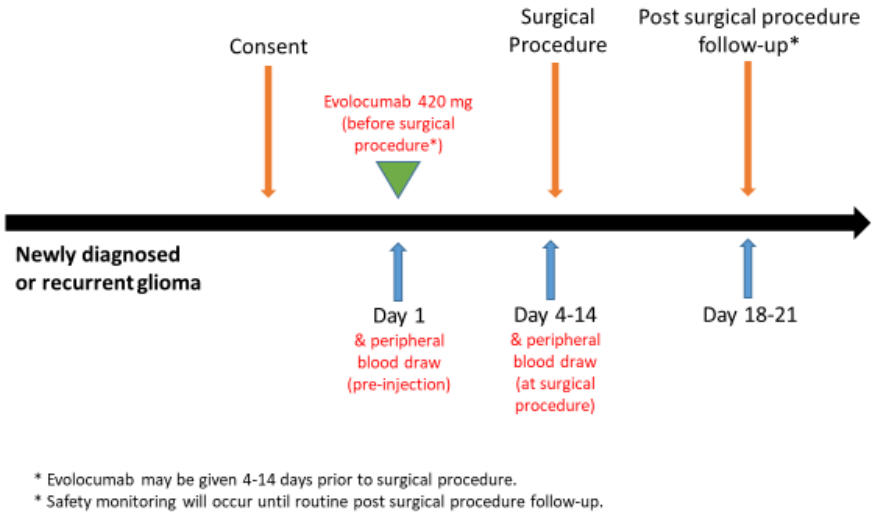


Figure 1. Study schema for newly diagnosed or recurrent glioma patients receiving Evolocumab

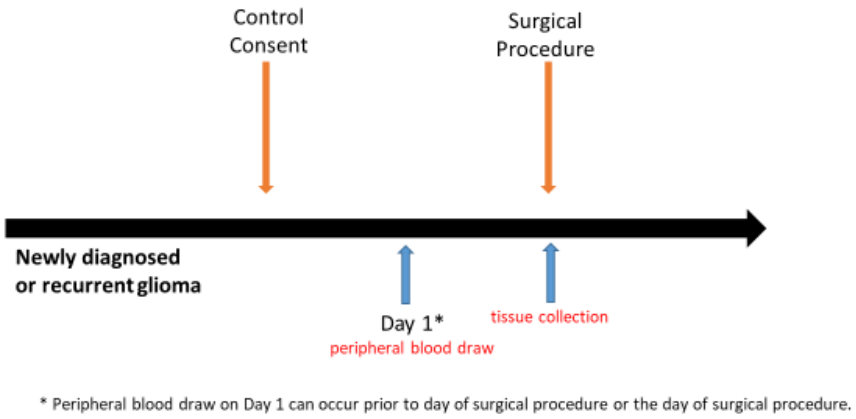


Figure 2. Study schema for control patients

5 BACKGROUND AND SIGNIFICANCE

5.1 Study Disease

Primary brain tumors are more common than Hodgkin's disease and account for more human deaths than melanoma or other cancers such as bladder or kidney. Glioblastoma (GBM) is the most common primary brain tumor, and despite aggressive, computer-guided tumor resection [6], high-dose external beam radiation therapy (XRT), and multi-mechanistic chemotherapy delivered at toxic doses, most patients with GBM live < 15 months from the time of diagnosis, and patients with recurrent tumors usually survive < 12 weeks [8,9,14-17]). The non-specific nature of standard of care therapy for brain tumors often results in incapacitating damage to surrounding normal brain and systemic tissues [18,19].

Immunotherapies have come to the forefront of treatment for many primary tumors, including gliomas. Unfortunately, gliomas are immunologically "cold" tumors that do not respond well to traditional immunotherapies targeted at checkpoint blockades. An important means by which some glioblastoma cells avoid immune-mediated rejection is through the reduced expression of MHC class I molecules, which are necessary for the binding of T cell receptors (TCRs) and therefore the tumor cells are ignored by T cells (He et al., 2019). However, recent work by Liu et al has demonstrated that antibody based inhibitors of proprotein convertase subtilisin/ kexin type 9 (PCSK9) can potentially enhance the efficacy of immune checkpoint inhibition by increasing MHC class I molecule expression [1].

5.2 Study Agent

Evolocumab is a fully human IgG2 monoclonal antibody PCSK9 inhibitor, currently approved by the FDA for use as a lipid lowering agent [20]. PCSK9 has important roles in LDL receptor trafficking and determination of cardiovascular risk. Evolocumab was studied in a randomized controlled phase III study of 27,564 patients, which demonstrated significant reduction in LDL cholesterol and the primary endpoint of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization, resulting in its FDA approval (11.3% vs 9.8% in placebo; HR 0.85, P<0.001) [12]. Evolocumab is typically administered as a 140-mg subcutaneous injection once every 2 weeks or 420 mg monthly and has been shown to have a favorable safety profile, with adverse events overlapping entirely with placebo except for injection site reactions [21,22].

5.2.1 Pre-clinical experience

5.2.1.1 PCSK9 Knockout Experiments

Liu et al performed several sets of experiments using immunocompetent syngeneic mice and multiple cancer cell lines (B16F10 – melanoma, 4T1 – mammary carcinoma, MC38 – colon adenocarcinoma and CT26 – undifferentiated colon carcinoma). By performing CRISPR-cas9 gene editing, tumors with PCSK9 knocked out exhibited significantly delayed growth when compared to wild-type (data shown in Figure 3).

Although PCSK9 inhibitors were developed to target the low-density lipid receptor (LDLR), and cause intracellular degradation, subsequent experiments lowering LDLR demonstrated no anti-tumor effects or effect on surface expression of major histocompatibility protein class I (MHC I) molecules. This indicates that the growth retardation induced by PCSK9 knockout was caused by a mechanism separate to that involving the LDLR [23].

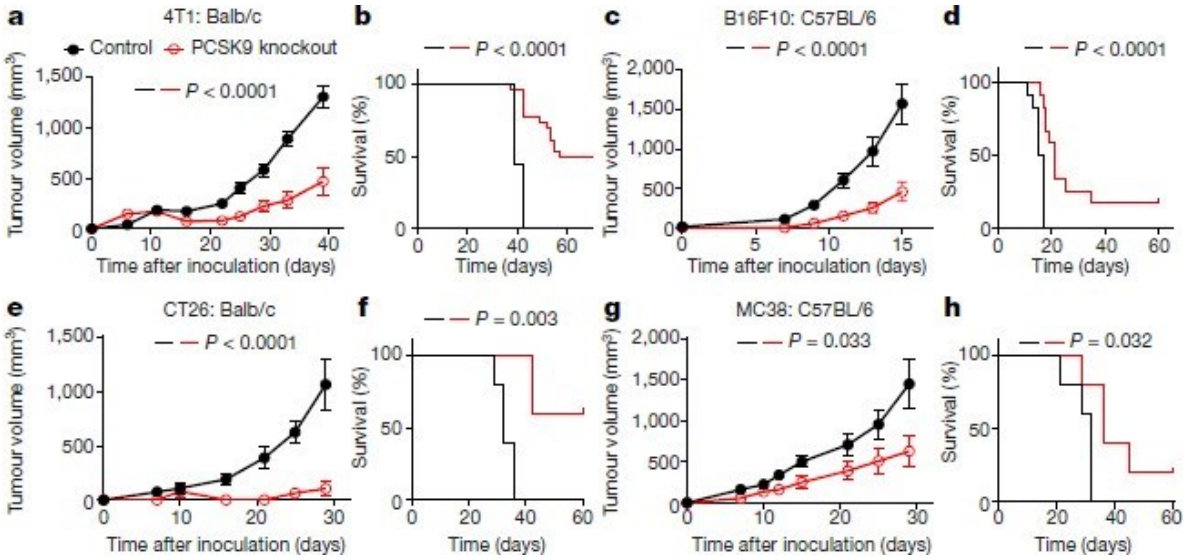
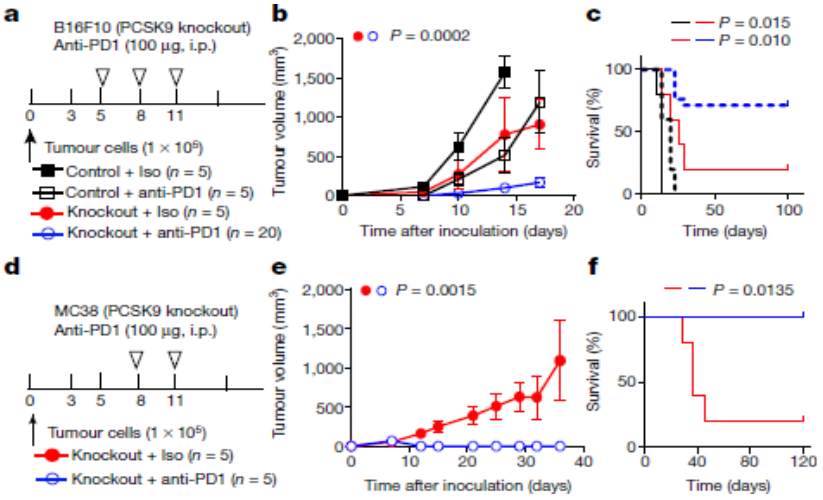


Figure 3. PCSK9 depletion attenuates tumor growth in syngeneic mice.

About 1 × 10^5 vector control and the same number of PCSK9-knockout mouse tumor cells of different typeswere inoculated subcutaneously into syngeneic mice and monitored for tumor formation. Both tumor size and overall survival were monitored. ***a****,* ***b****, 4T1 breast cancer line, grown in Balb/c mice. n = 9 and 20 micefor control and PCSK9-knockout tumor cells, respectively.* ***c****,* ***d****, B16F10 melanoma line, grown in C57BL/6mice. n = 12 mice for both groups.* ***e****,* ***f****, CT26 colon cancer line, grown in Balb/c mice. n = 5 mice for both groups.* ***g****,* ***h****, MC38 colon cancer line, grown in C57BL/6 mice. n = 5 mice for both groups. Data shown asmean =/- s.e.m. P values were calculated by two-way analysis of variance (ANOVA) in* ***a****,* ***c****,* ***e****,* ***g*** *and log- rank test in* ***b****,* ***d****,* ***f****,* ***h****. Figure taken from Liu et al in Nature 2020 [1].*

5.2.1.2 PCSK9 knockout combined with anti-PD1

Further work to evaluate if PCSK9 was a promising target for anti-cancer therapy involved treating syngeneic mice with PCSK9 knock out tumors with a mouse anti-PD1 antibody. Anti-PD1 blocks the binding of the immune checkpoint PD1 receptor on T cells with the PD-L1 ligand on tumor cells, and therefore increasing T cell activation and subsequentlytumor cell death. As described in Figure 4, knockout of PCSK9 combined with anti-PD1 resulted in significantly decreased tumor volume and greatly enhanced survival in both B16F10 melanoma models and MC38 colon cancer.



(a–c) *Treatment of vector control and PCSK9-knockout B16F10 melanoma with an anti-PD1 antibody in syngeneic mice. **(a)** Experimental protocol; **(b)** tumor growth curve; **(c)** overall survival. N = 5, 5, 5, and 20 mice in the four groups, respectively. i.p., intra-peritoneal. **(d–f)** Treatment of vector control and PCSK9-knockout MC38 colon cancer with an anti- PD1 antibody in syngeneic mice. **(d)** Experimental protocol; e, tumor growth curve; **(f)** overall survival. N = 5 mice per group. Data shown as mean ± s.e.m. Figure taken from Liu et al in Nature 2020 (Liu et al., 2020).*

Figure 4. PCSK9 knockout combined with anti-PD1 therapy enhances

5.2.1.3 PCSK9 inhibitors combined with anti-PD1

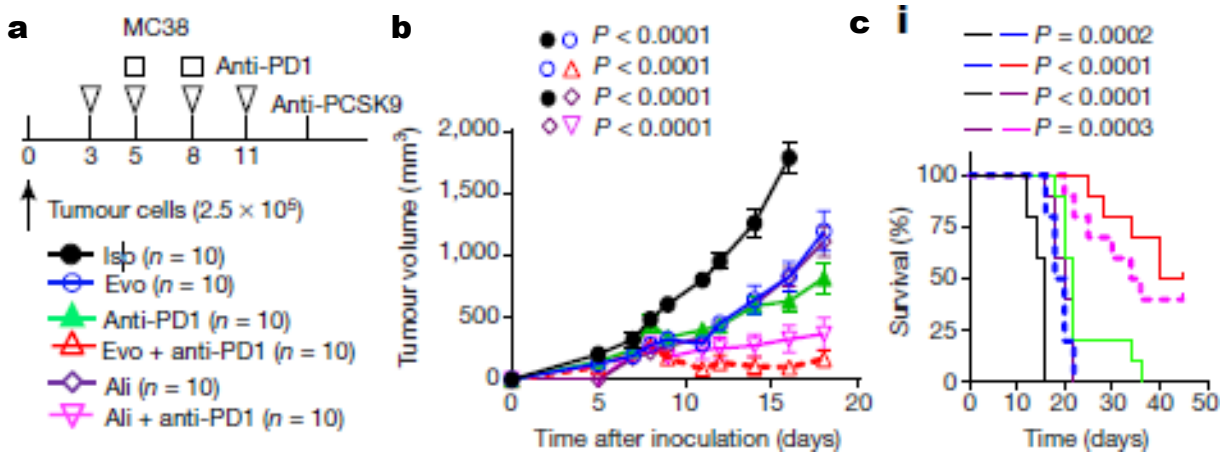


Figure 5. Inhibition of PCSK9 overcomes tumor resistance to anti-PD1 therapy

*Treatment of MC38 colon cancer with combined anti-PCSK9 antibodies (isotype antibody control, Iso; evolocumab, Evo; or alirocumab, Ali) and anti-PD1 antibodies in mice. **(a)** Experimental protocol; **(b)** tumour growth curve; **(c)** overall survival. n = 10 mice per group. Shown are combined results from two separate experiments. Data shown as mean ± s.e.m. Figure taken from Liu et al in Nature 2020 [1].*

When considering PCSK9 as an anti-cancer target, clinically approved agents are readily available. Evolocumab and Alirocumab are anti-PCSK9 blocking antibodies, approved by the FDA for treatment of hypercholesteremia or to reduce cardiovascular risk [20]. When evaluated in MC38 colon cancer tumors, combined with anti-PD1 antibody treatment, significant increases in long-term survival were seen. This is particularly of note given that anti-PD1 resistant tumors (MC38R) also responded to evolocumab, suggesting that PCSK9 plays an important role in the anti-PD1 resistance pathway (shown in Figure 5).

5.2.1.4 Mechanism of anti-tumor effect

These data appear to demonstrate that anti-PCSK9 antibodies when combined with anti- PD1 can enhance survival and overcome anti-PD1 resistance. As mentioned, the mechanism by which this occurs appears to be completely separate to the LDLR pathway for which it is currently in clinical use. To understand the mechanism by which evolocumab exerts this effect, flow cytometry was undertaken to profile immune effector cells present within PCSK9-knockout tumors (shown in Figure 6).

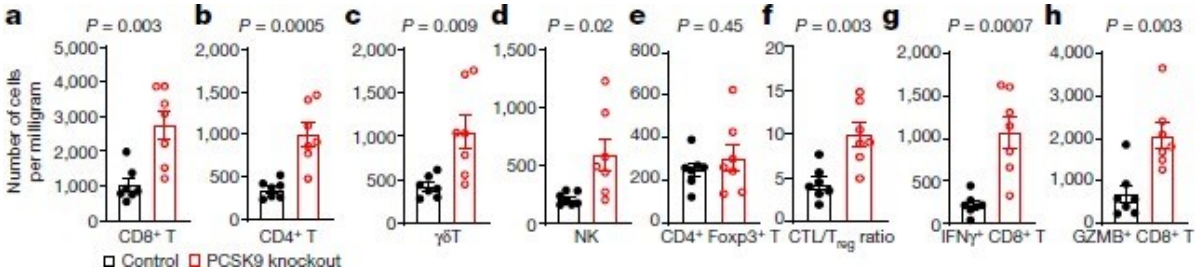
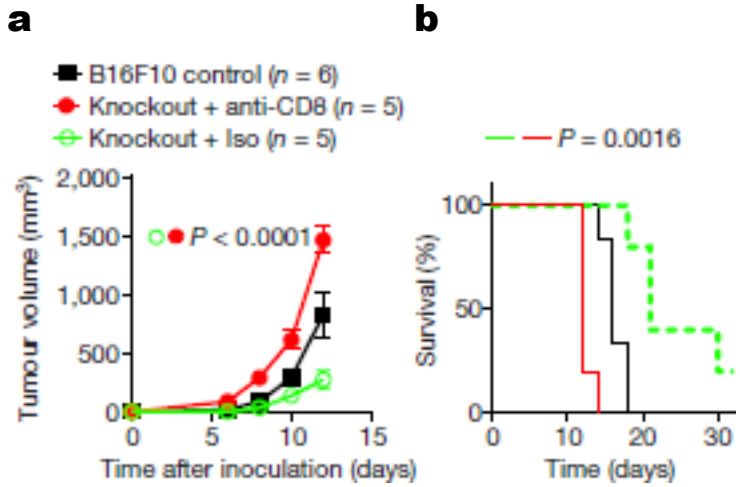


Figure 6. Quantitation of immune effector cells in PCSK9-knockout B16F10 tumors

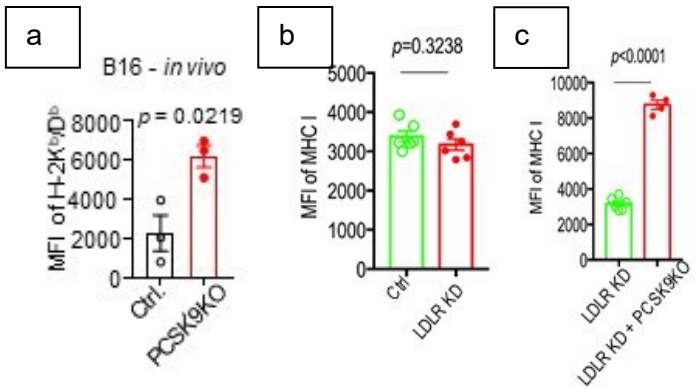
***(a–e)** Quantitative estimate of various immune effector cells per milligram of tumor tissue in vector controland PCSK9-knockout B16F10 tumors, as determined by flow cytometry. n = 7 tumors per group. NK, natural killer cell. **f**, Ratio of CD8+ cytotoxic T lymphocytes (CTLs) to CD4+ Foxp3+ Treg cells in controland PCSK9-knockout B16F10 tumors. n = 7 tumors per group. (**g, h**) Average numbers of tumor- infiltrating IFN-γ+ CD8+ T cells (**g**) and GZMB+ CD8+ T cells (**h**) per milligram of tumor tissue in controlor PCSK9-knockout tumors. n = 7 tumors per group. Figure taken from Liu et a in, Nature 2020* [1].

These knockout tumors displayed significantly increased number of tumor-infiltrating lymphocytes (TILs), with intratumoral IFN- γ+ cytotoxic T lymphocytes (CTLs) exhibiting grater response to evolocumab than on treatment with anti-PD1 antibody. Notably, the ability of PCSK9 knockout to inhibit tumor growth could be abrogated by depletion of CD8+ T cells, in contrast to CD4+ depletion which had minimal effect (shown in Figure 7) [1].



*Tumor growth (**a**) and host survival (**b**) from control and PCSK9-knockout B16F10 tumor cells in C57BL/6 mice depleted of CD8+ T cells. n = 6, 5, 5 tumors in the three groups. Figure takenfrom Liu et al in Nature 2020 (Liu et al., 2020).*

Figure 7. CD8+ T cell depletion in PCSK9-knockout in B16F10 melanoma models



MHC-I expression on the surface of (a) Control and PCSK9KO B16F10 cells grown in C57BL/6 mice; (b) control and LDLR-KO B16F10 cells grown in vitro; (c) LDLR KO with or without PCSK9 double knockout B16F10 cells grown in vitro. Figure taken from Liu et al in Nature 2020 [1]

Figure 8. MHC-1 Expression

The key effect of CD8+ cytotoxic T cells for PCSK9 knockout anti-tumor effect was shownto be correlated with significantly increased expression of MHC I molecules on the surfaceof tumors in which PCSK9 was absent (Figure 8). Notably this effect is absent in LDL receptor knockout mice, indicated a separate pathway. This increased expression was also shown to be accompanied by an increase in the diversity of T cell receptors, allowingfor greater binding potential for CTLs and subsequent tumor killing. MHC I expression could be induced by PCSK9 inhibitors, which could then be reversed when PCSK9 levelswere restored by means of an exogenous protein.

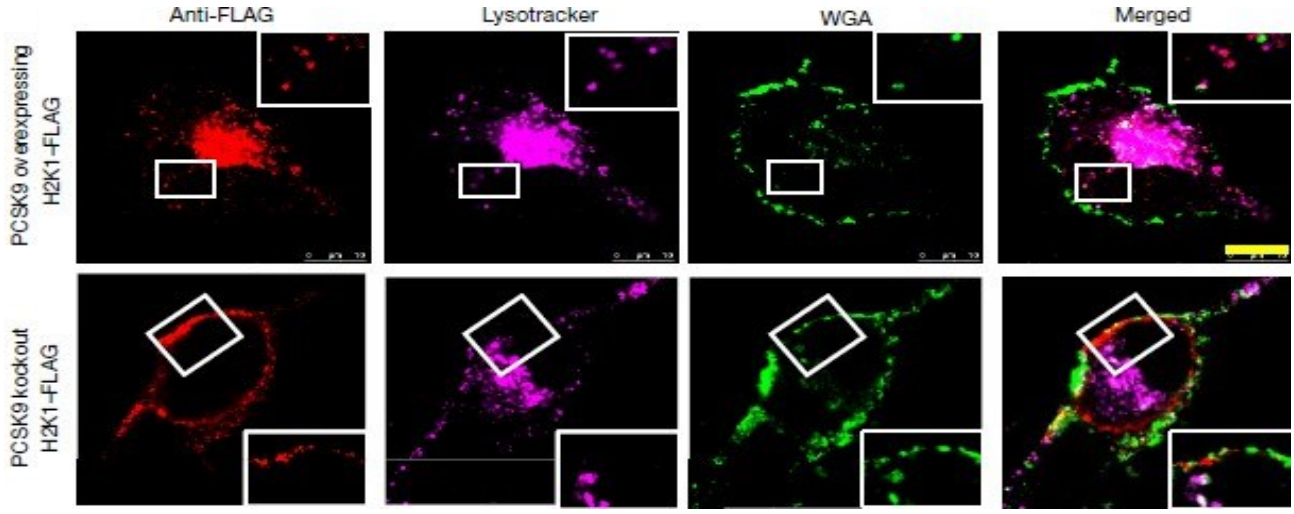


Figure 9. PCSK9 knockout effect on MHC I localization

*In PCSK9-overexpressing cells, more H2-K1 (mouse MHC class I antigen) was localized in the lysosome and less in the plasma membranes (**top panels**). On the other hand, in PCSK0-knockout cells, H2-K1 staining indicated greater localization to the plasma membrane (**lower panels**) Western blot analysis of fractionated cellular lysates confirmed the immunofluorescence staining results* [1].

By upregulating MHC I expression, greater numbers of intracellular peptides are displayed at the cell surface for recognition by CD8+ T cells. These MHC I-peptide complexes exist at the cell surface, awaiting recognition before being recycled into the cell membrane. This process begins with the formation of endocytic vesicles, which are then sorted by endosomes to be redirected to the cell surface or destroyed by lysosomes. Intriguingly, PCSK9 has been shown to interact

with MHC I, resulting in its increased trafficking to lysosomes and blocks its return to the cell membrane. This results in cells which express PCSK9 having reduced surface levels of MHC I which allows them to escape T cell recognition (confocal images shown in Figure 9 and mechanism described in Figure 10 [1]).

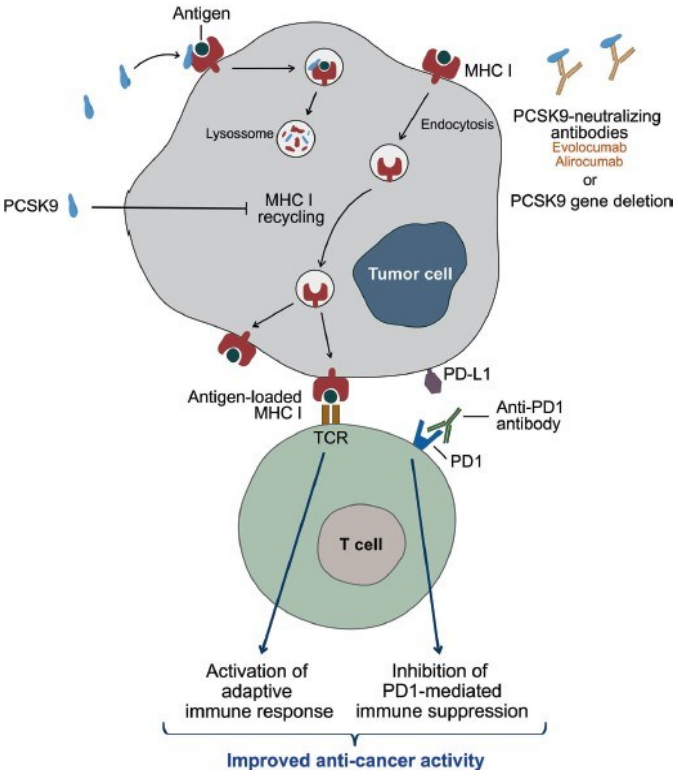


Figure 10. Proposed mechanism for PCSK9i on increasing cell surface MHC I expression

As PCSK9 has also been shown to induce degradation of other receptors such as LDLR, CD81 and CD36, it may be reasonable to assume that PCSK9 has a key regulatory role in endocytosis of membrane receptors and their diversion to lysosomal degradation [23]. Therefore, inhibition and its resultant upregulation of MHC I may result in greater recognition by CTLs and thus enhanced immune effector cell function.

5.2.2 Clinical Experience

Standard treatment for hypercholesterolemia relied on diet modification and use of statins until the introduction of antibodies, such as evolocumab, that inhibit proprotein convertase subtilisin-kexin type 9 (PCSK9). Evolocumab is an antibody that binds to PCSK9, where the antibody's therapeutic effect is to reduce LDL-cholesterol and where the therapeutic goal is to reduce risk for heart attack and stroke.

In phase II and III clinical trials (Open Label Study of Long Term Evaluation Against LDL- C [OSLER-1 and OSLER-2], respectively) evolocumab decreased LDL cholesterol by 52%, decreased total cholesterol by 36%, and raised high-density lipoprotein cholesterol by 7% when compared to standard treatment (Sabatine et al., 2015). A follow-up study (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Patients with Elevated Risk [FOURIER]) was performed to evaluate effects on reducing cardiovascular endpoints, such as myocardial infarction, stroke, unstable angina, or need for coronary revascularization. The combination of evolocumab plus standard statin therapy significantly reduced the risk of cardiovascular death, myocardial infarction, or stroke by 20%, which correlated with decreases in LDL levels [12]. Evolocumab is currently FDA-approved for treatment of hypercholesterolemia in combination with other lipid-lowering medications (e.g., statins, ezetimibe), and is only recommended as

single- agent therapy in patients diagnosed with primary hyperlipidemia, which is typically an inherited disease. It is given as a 140-mg subcutaneous injection once every 2 weeks or 420 mg monthly [24].

Peak plasma concentrations occur at 3–7 days [21] and evolocumab has a half-life of 11–17 days and like other monoclonal antibodies, is eliminated by degradation to short peptides and amino acids. When considering adverse reactions, this has been well characterized as part of the FDA approval process. In a 52- week, double-blind, randomized, placebo-controlled trial, 599 patients received 420 mg of evolocumab subcutaneously once monthly. The mean age was 56 years (range: 22 to 75 years), 23% were older than 65 years, 52% women, 80% White, 8% Black, 6% Asian; 6% identified as Hispanic ethnicity. Adverse reactions reported in at least 3% of evolocumab-treated patients, and more frequently than in placebo-treated patients are shown in Table 1.

Table 1. Adverse reactions occurring in ≥ 3% of Evolocumab-treated patients and more frequently than with placebo in a 52-week trial

	Placebo (N=302)%	Evolocumab (N=599)%
Nasopharyngitis	9.6	10.5
Upper respiratory tract infection	6.3	9.3
Influenza	6.3	7.5
Back Pain	5.6	6.2
Injection site reactions	5.0	5.7
Cough	3.6	4.5
Urinary tract infection	3.6	4.5
Sinusitis	3.0	4.2
Headache	3.6	4.0
Myalgia	3.0	4.0
Dizziness	2.6	3.7
Musculoskeletal pain	3.0	3.3
Hypertension	2.3	3.2
Diarrhea	2.6	3.0
Gastroenteritis	2.0	3.0

Adverse reactions led to discontinuation of treatment in 2.2% of evolocumab-treated patients and 1% of placebo-treated patients. The most common adverse reaction that led to evolocumab treatment discontinuation and occurred at a rate greater than placebo was myalgia (0.3% versus 0% for evolocumab and placebo, respectively) [24].

In seven pooled 12-week, double-blind, randomized, placebo-controlled trials, 993 patients received 140 mg of evolocumab subcutaneously every 2 weeks and 1059 patients received 420 mg of evolocumab subcutaneously monthly. The mean age was 57 years (range: 18 to 80 years), 29% were older than 65 years, 49% women, 85% White, 5% Black, 9% Asian; 5% identified as Hispanic ethnicity. Adverse reactions reported in at least 1% of evolocumab-treated patients, and more frequently than in placebo-treated patients, are shown in Table 2 [24].

Table 2. Adverse reactions occurring in ≥ 1% of Evolocumab-treated patients and more frequently than with placebo in pooled 12-week trials

	Placebo (N=1224) %	Evolocumab*(N=2052) %

Nasopharyngitis	3.9	4.0
Back Pain	2.2	2.3
Upper respiratory tract infection	2.0	2.1
Arthralgia	1.6	1.8
Nausea	1.2	1.8
Fatigue	1.0	1.6
Muscle spasms	1.2	1.3
Urinary tract infection	1.2	1.3
Cough	0.7	1.2
Influenza	1.1	1.2
Confusion	0.5	1.0

*140mg every 2 weeks and 420mg once monthly combined

5.3 Study Purpose/Rationale

Despite aggressive, computer-guided tumor resection [6], high-dose external beam radiation therapy (XRT), and multi-mechanistic chemotherapy delivered at toxic doses, most patients with glioblastoma (GBM) live < 15 months from the time of diagnosis, and patients with recurrent tumors usually survive < 12 weeks [8,9,14-17]. The non-specific nature of standard of care therapy for brain tumors often results in incapacitating damage to surrounding normal brain and systemic tissues [18,19]. Immunotherapies have come to the forefront of treatment for many primary tumors, including gliomas. Unfortunately, gliomas are immunologically "cold" tumors that do not respond well to traditional immunotherapies targeted at checkpoint blockades.

Recent work by Liu et al has been demonstrated that PCSK9 plays a role in regulating recycling of the major histocompatibility class I (MHC-I) and enhances tumor antigen presentation, independent of its role in LDL-receptor regulation [1]. PCSK9 deficient tumors show increased MHC-I expression and CD8+ T-cell infiltration. PCSK9 knockout or inhibition synergizes with anti-PD-1 therapy in murine models of melanoma, colorectal cancer, and breast cancer. We therefore intend to evaluate if a clinically licensed PCSK9i such as Evolocumab can be repurposed as a potential immunotherapeutic for glioma by testing its ability to access the intracranial space.

6 OBJECTIVES AND ENDPOINTS

	Objective	Endpoint	Analysis
Primary	Evaluate the pharmacokinetics (PK) of evolocumab and whether it can cross the blood brain barrier (BBB) to accumulate intratumorally in patients with primary and recurrent glioma or glioblastoma	Presence of evolocumab detected in surgical resection or biopsy samples by means of mass spectrometry	See Section 13.4
Secondary	Compare lipid metabolism of tumor cells taken from resected or biopsied samples after dose ofevolocumab to resected or biopsied samples obtained from s from untreated glioma patients.	Level of lipid metabolism within the resected or biopsied tumor as assessed via fluorescence-activated cell sorting (FACS)	See Section 13.5.1
Secondary	Compare MHC-I expression on tumor cells following administration of evolocumab to samples from untreated glioma patients with tissue	Extent of MHC-I expression within the resected or biopsied tumor, as assessed by fluorescence-activated cell sorting (FACS)	See Section 13.5.1
Secondary	Among patients treated with evolocumab, describe the strength of the association between serum and intratumoral levels of evolocumab. Peripheral blood samples to be taken before administration of evolocumab and at time of surgery or biopsy.	Correlation of serum and intratumoral levels of evolocumab	See Section 13.5.2.
Exploratory	Describe the immune characteristics of tumor cells taken from resected or biopsied samples	Sequencing characteristics as assessed by multi- omics analysis, immune infiltration, and T cell profiling	See Section 13.6
Exploratory	Assess the activity of T cells isolated from tumor specimens in patients treated with or without evolocumab	Characterization of tumor-infiltrating lymphocytes (TILs) based on flow cytometry immunostaining of TILs for expression of markers of exhaustion (e.g., PD-1, Lag3, TIM3) and activation (CD137)	See Section 13.6

7 INVESTIGATIONAL PLAN

7.1 Study Design

This phase 0/surgical window of opportunity study is being conducted to assess if Evolocumab, a clinically approved drug for hypercholesteremia, can cross the blood brain barrier and accumulate within the brain tumor of patients with glioma or glioblastoma. Study participants will include newly diagnosed or recurrent patients who are due to undergo resection or biopsy of their tumor. Up to 10 patients will be enrolled in the treatment arm of this study, while up to 20 patients will serve as controls.

Study treatment arm: Each eligible patient will receive 420 mg of Evolocumab subcutaneously (the maximum single dose) 4-14 days prior to surgical resection or biopsy of their tumor. Prior to injection of evolocumab (treatment arm only) and at time of surgery surgery, peripheral blood will be drawn to analyze serum levels of the drug (for comparison to intratumoral).

Following administration of Evolocumab and surgical procedure, patients in the treatment arm will be followed up for two weeks to observe for any evidence of side effects or toxicity. Although the safety profile of Evolocumab is well understood, the study will be paused and re-evaluated should any significant toxicities occur.

Patients can then proceed to standard of care treatment for their glioma following the trial, with no expected impediment caused by the use of Evolocumab.

Control arm: A control group of 20 patients who undergo craniotomy without Evolocumab treatment will be prospectively recruited to this study using similar eligibility criteria used to recruit patients to receive Evolocumab. If accrual of these controls is difficult, matched 2:1 (controls: cases) will be obtained from the BTBR (Pro00007434). The control arm will have peripheral blood drawn prior to their scheduled day of the surgical procedure or on the day of the surgical procedure. Patients who consent to participate as a control for this study will come off study after tissue is collected for research (if available) during their surgical procedure.

7.1.1 Dose Escalation and Expansion

The primary objective of this study is to determine whether Evolocumab can cross the blood brain barrier and accumulate intra-tumorally. This drug has been approved by the FDA, and the safety data are well understood [20]. Therefore, the maximum clinically indicated dose for hyperlipidemia (420 mg subcutaneously into thigh, abdomen or upper arm) will be used [24]. No escalation or expansion of the dosage regime is planned. If any evidence of dose-limiting toxicity among patients treated with Evolocumab as defined in Section 7.1.2 is observed, the trial will be paused and re-evaluated by the investigators.

7.1.2 Definition of Dose-Limiting Toxicity (DLT)

Among patients treated with Evolocumab, toxicities will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5 criteria. DLTs will be defined as any of the following events that are at least (possibly, probably, or definitely) attributable to study treatment (i.e., evolocumab):

- Any Grade 3 or any Grade 4 toxicity (at least possibly, probably, or definitely attributable to study treatment [i.e., evolocumab]) that is not reversible (i.e., reversible means the toxicity is trending towards improvement and has downgraded to at least Grade 2 or back to baseline) within 2 weeks, including cerebral edema or worsening neurologic symptoms

- Any life-threatening event (at least possibly, probably, or definitely attributable to study treatment [i.e., evolocumab]) that is not reversible (i.e., reversible means the toxicity is trending towards improvement and has downgraded at least to Grade 2 or back to baseline) within 2 weeks
- Treatment-related death at least possibly, probably, or definitely attributable to study treatment (i.e., evolocumab).
- Any grade 2 or higher serious autoimmune toxicities, particularly those affecting vital organs (e.g., cardiac, renal, CNS) occurring within 2 weeks of any protocol treatment (i.e., evolocumab).

Exceptions to the above DLT definition include:

- Seizures: Due to the nature of the disease under investigation in this protocol, patients may have pre-existing seizures or be susceptible to new seizures as a result of the underlying disease process. Although seizures may be defined as Grade 3 or 4 toxicities under NCI CTC, and will be reported as such in this protocol, seizures will not be considered an unacceptable adverse event if, in the opinion of the Principal Investigator they have not increased in frequency or can be attributed to another recognized cause of increasing seizure frequency such as sub- therapeutic anti-convulsant levels or biopsy proven tumor progression.
- New neurologic deficits: Due to the nature of the disease under investigation in this protocol, patients may develop new neurologic deficits as a result of tumor invasion. A new neurologic deficit, which resolves within 2 weeks after initiation of medical therapy, will not be considered an unacceptable adverse event. New neurological symptoms will not be an unacceptable adverse event if they can be ascribed to tumor progression (e.g. documented with histopathologic analyses of biopsy tissue; or; they respond to treatment (e.g., oral steroids, within 2 weeks)).
- Thromboembolism: Due to the high incidence of deep vein thrombosis (DVT) in this patient population, patients may have undiagnosed pre-existing DVTs or be susceptible to the development of DVTs due to the underlying disease process. Although DVT may be defined as Grade 3 or 4 toxicities under NCI CTC, and will be reported as such in this protocol, DVT will not be considered unacceptable adverse event in this protocol.
- Syndrome of Inappropriate Antidiuretic Hormone (SIADH): Due to the high incidence of SIADH in this patient population, patients may be susceptible to the development of SIADH due to the underlying disease process. Although SIADH may be defined as Grade 3 toxicity under NCI CTC, and will be reported as such in this protocol, SIADH will not be considered an unacceptable adverse event in this protocol unless it is refractory to medical management.
- Muscle Weakness and Weight Gain: Due to the high incidence of muscle weakness and weight gain in patients taking steroids, patients may be susceptible to the development of muscle weakness or weight gain, which is due to steroids alone. Although muscle weakness may be defined as Grade 3 or Grade 4 toxicity and weight gain ≥ 20% may be defined as Grade 3 toxicity under NCI CTC, and will be reported as such in this protocol. Muscle weakness or weight gain will not be considered an unacceptable adverse event in this protocol if the patient has required steroids greater than physiologic doses in the interval between the immunization and the development of the toxicity.
- Tumor Progression: Due to the nature of the disease under investigation in this protocol, patients may have an increase in pre-existing neurologic deficits or have an onset of new neurologic deficits due to tumor progression. Although such neurologic deficits may be defined as unacceptable adverse events under NCI CTC, and will be reported as such in this protocol, these clinical changes are not an unexpected phenomenon in this disease in the setting of tumor growth. As a result, neurologic deficits will not be considered an unacceptable adverse event if unequivocal tumor progression can be documented radiographically or histologically.
- Immune Related Exceptions: grade 2 Pneumonitis; grade 2 or 3 colitis or diarrhea; grade 2, 3, or 4 endocrinopathies (including but not limited to hypophysitis, adrenal insufficiency, hyperthyroidism, and type 1 diabetes mellitus). Any of the aforementioned immune-related AEs will not be considered unacceptable if the event resolves to Grade 1 after withholding therapy.

7.1.3 Dose Modification

7.1.3.1 Non-hematologic:
There will be no non-hematologic dose modifications in this study.

7.1.3.2 Hematologic:
There will be no hematologic dose modifications in this study.

7.1.3.3 Other considerations:
Not applicable

7.1.4 Safety Considerations

Evolocumab is not predicted to induce any specific immunologic adverse events given its well described safety profile. Although there is potential for immune-genicity, it has been evaluated using an electro-chemi-luminescent bridging screening immunoassay for the detection of binding anti-drug antibodies.

For patients whose sera tested positive in the screening immunoassay, an *in vitro* biological assay was performed to detect neutralizing antibodies. In a pool of placebo- and active-controlled clinical trials, 0.3% (48 out of 17,992) of patients treated with at least one dose of evolocumab tested positive for the development of binding antibodies. Patients whose sera tested positive for binding antibodies were further evaluated for neutralizing antibodies; none of the patients tested positive for neutralizing antibodies. There was no evidence that the presence of anti-drug binding antibodies impacted the pharmacokinetic profile, clinical response, or safety of Evolocumab [24]. Therefore, we do not anticipate any immunologic AEs that can affect the patients wellbeing or with standard of care treatment of radiation therapy and temozolomide (XRT/TMZ). If any reactions are observed, the study team will pause the study and re-evaluate.

7.1.5 Missed Doses

Not applicable.

7.1.6 Concomitant Medications

Concomitant medications will be managed by the treating physician and recorded at each study visit by the study team.

As we wish to monitor immune modulation in both treatment and control groups, corticosteroids should be used at the lowest dose possible [less than 0.1 mg/Kg/day (<4 mg of dexamethasone / day)], except for the 24 hours prior to surgery or biopsy, to control symptoms of edema and mass effect, and discontinued, if possible. Use of corticosteroids should be recorded in the electronic database.

7.1.7 Study Drug Blinding

Not applicable.

7.2 Rationale for Selection of Dose, Regimen, and Treatment Duration

The primary objective of this study is to determine whether evolocumab can cross the blood brain barrier and accumulate intratumorally. This drug has been approved by the FDA, and the safety data are well understood [20].

Therefore, the maximum clinically indicated dose for hyperlipidemia (420 mg subcutaneously into thigh, abdomen or upper arm) will be used [24]. Only a single dose is required 4-14 days prior to administration as this is the point at which peak plasma concentrations occur and maximal accumulation in the brain is likely to occur, but remaining well within the effective half-life of 11-17 days[21,25]. This will therefore allow for the

greatest chance for detected evolocumab in the brain, should it successfully cross the blood brain barrier. No dose escalation is planned.

7.3 Rationale for Correlative Studies

To compare the pharmacokinetics (PK) of evolocumab in treated patients, peripheral blood samples will be taken to compare serum concentrations of evolocumab to intratumoral concentrations. These are further detailed in Section 10.

Tumor-infiltrating lymphocytes (TILs) from tumor or liquid obtained from the surgical field will be dissociated into a single cell suspension or fragmented and TILs expanded as one batch following the TILs pre-rapid expansion protocol (preREP). The ability to successfully and reliably expand preREP TILs from lung cancer and glioblastoma samples has been previously demonstrated in Pro00106078. TILs, in general, display a profound degree of exhaustion. Therefore, cells will be stained for expression of surface and nuclear markers of exhaustion (PD-1, Lag3, TIM3) and activation (CD137) and differential levels assessed by flow cytometry. CD137 is upregulated on T cells during early activation immediately following interaction with tumor antigen in the Tumor Microenvironment (TME). We will explore the potential to select and expand the non-exhausted CD137+ subset and examine their anti-tumor efficacy in samples from patients treated with or without evolocumab. Autologous CD137+ TIL cell product will be co-cultured with disassociated CFSE labeled neurospheres for 24 and 48 hours. Cytotoxicity determined by flow cytometry of CFSE+ tumor cells will be compared to no TIL exposure. Patient-derived neurospheres will generated *in vitro* based on established protocols [26].

7.4 Definition of Evaluable Subjects, On Study, and End of Study

Once a patient has their baseline PK collection (both arms) and receives one dose of Evolocumab (treatment arm only), that patient will be considered “on study.” Different definitions for “on study,” “on treatment,” “off treatment,” and “off study” may be required in the electronic research record. Rationale for taking a patient off protocol treatment will be documented.

Any patient who receives one dose of Evolocumab will be included in safety analyses. Once all data has been collected, analyzed, and completely evaluated on all subjects, as defined in the statistical analysis plan, and subjects have completed follow up, expired, withdrawn, or been lost to follow up, the study will be ended.

7.5 Early Study Termination

This study can be terminated at any time for any reason by the PI or Sponsor- Investigator. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 10.7, which describes procedures and process for prematurely withdrawn patients.

8 STUDY DRUG

8.1 Names, Classification, and Mechanism of Action

Evolocumab (Repatha®) is a PCSK9 inhibitor and binds to a pro-protein involved in the regulation of LDL receptors on liver cells; receptor numbers are increased, which results in increased uptake of LDL-cholesterol from the blood. Evolocumab is currently indicated [24]:

- In adults with established cardiovascular disease to reduce the risk of myocardial infarction, stroke, and coronary revascularization
- As an adjunct to diet, alone or combined with other low-density lipoprotein cholesterol (LDL-C)-lowering therapies, in adults with primary hyperlipidemia, including heterozygous familial hypercholesterolemia (HeFH), reducing LDL-C

- As an adjunct to other LDL-C-lowering therapies in patients with homozygous familial hypercholesterolemia (HoFH), to reduce LDL-C

8.2 Packaging and Labeling

Please refer to the current evolocumab (REPATHA®) labeling on Drugs@FDA.
https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=125522

8.3 Supply, Receipt, and Storage

Evolocumab is available commercially from Amgen and is a clear to opalescent, colorless to pale yellow solution supplied as follows:

Table 3. Formulations of Evolocumab

140 mg/mL single-dose prefilled syringe	1 pack	NDC 72511-750-01 NDC 55513-750-01
140 mg/mL single-dose prefilled SureClick® autoinjector	1 pack	NDC 55513-760-01
140 mg/mL single-dose prefilled SureClick® autoinjector	2 pack	NDC 72511-760-02 NDC 55513-760-02
140 mg/mL single-dose prefilled SureClick® autoinjector	3 pack	NDC 55513-760-03
420 mg/3.5 mL single-dose Pushtronex® system (on-body infusor with prefilled cartridge) ***Formulation to be used in this study)***	1 pack	NDC 72511-770-01 NDC 55513-770-01

Per the package insert:
The needle cover of the glass single-dose prefilled syringe and the single-dose prefilled autoinjector contain dry natural rubber (a derivative of latex). The single-dose on-body infusor with prefilled cartridge is not made with natural rubber latex.

Store refrigerated at 2°C to 8°C (36°F to 46°F) in the original carton to protect from light. Do not freeze. Do not shake.

For convenience, evolocumab may be kept at room temperature at 68°F to 77°F (20°C to 25°C) in the original carton for 30 days. If not used within the 30 days, discard evolocumab.

8.4 Dispensing and Preparation

The commercial agent will be purchased from the pharmacy storeroom and managed by ICS. Evolocumab is a sterile, preservative-free, clear to opalescent, colorless to pale yellow solution for subcutaneous use. Each single-dose Pushtronex® system (on-body infusor with prefilled cartridge) delivers a 3.5 mL solution containing 420 mg Evolocumab, acetate (4.2 mg), polysorbate 80 (0.35 mg), proline (89 mg) in Water for Injection, USP. Sodium hydroxide may be used to adjust to a pH of 5.0 [24].

8.5 Compliance and Accountability

Evolocumab will be delivered from Duke Pharmacy directly to the clinic under the supervision of the research nurse, or his/her designee. Evolocumab will be administered according to the approved labeling. The patient's name, Study ID, DOB, and Duke history number will be double verified prior to subcutaneous injection as is standard Duke procedure.

8.6 Disposal and Destruction

All injection equipment will be disposed of according to standard Duke procedures. Unused medication will be returned to Duke pharmacy, if possible, or disposed of as per standard handling of clinical medicines.

9 SUBJECT ELIGIBILITY

9.1 Inclusion Criteria

Both arms:

1. Adult patients ≥ 18 years old
2. Newly diagnosed or recurrent glioma or glioblastoma (GBM) (if recurrent, prior pathology report indicating glioma)
3. A clinical indication for gross macroscopic resection, debulking of the glioma, or biopsy, with sufficient tumor size that can allow collection of specimens for the required analyses.

Treatment arm only:

4. Adequate hematologic function within 14 days prior to starting evolocumab defined as follows:
   a. Hemoglobin ≥ 10 g/dL *(Note: the use of transfusion or other intervention to achieve Hgb ≥ 10.0 g/dl is acceptable)*
   b. White Blood Cells ≥ 1.5 x 10^9/L
   c. Absolute Neutrophil Count (ANC) ≥ 1.0 x 10^9/L
   d. Platelets ≥ 100 x 10^9/L or ≥ 50,000 for patients who received TMZ within the past year
5. Adequate renal function within 14 days prior to starting evolocumab defined as calculated creatinine clearance (CrCL) of ≥ 30 mL/min/1.73m^2 by the Cockcroft-Gault formula
6. Adequate hepatic function within 14 days prior to starting evolocumab defined as follows:
   a. Total bilirubin ≥ 1.5 x institutional upper limit of normal (ULN) *(Note: Patients with known Gilbert disease without other clinically significant liver abnormalities are not excluded.)*
   b. AST(SGOT) and ALT(SGPT) ≥ 1.5 × ULN
7. Negative serum pregnancy test (in females of childbearing potential) within 48 hours of starting evolocumab.

9.2 Exclusion Criteria

Treatment arm only:

1. Any patient with a history of a serious hypersensitivity reaction to evolocumab or any of the excipients in evolocumab
2. Patients with severe hepatic impairment outside of the range defined in the inclusion criteria within 7 days of starting evolocumab.
3. History or evidence of central nervous system bleeding as defined by stroke or intraocular bleed (including embolic stroke) not associated with any antitumor surgery within 6 months before enrollment
4. Infection requiring intravenous antibiotics that was completed < 1 week of study enrollment (day 1) with the exemption of prophylactic antibiotics for long line insertion or biopsy
5. Females of reproductive potential and males who are unwilling to practice an acceptable method(s) of effective birth control while on study through 1 month (2 half-lives) after receiving the last dose of study drug.

10 SCREENING AND ON-STUDY TESTS AND PROCEDURES

Table 4. Schedule of Events for Treatment Arm

Description	Screening (within 14 days of starting evolocumab)	Treatment Period	Active Follow-up Period

	Pre-Evolocumab Screening	Evolocumab Injection	Surgical Procedure	Post-Operative Visit
Day(s)		1	4-14	Typically ~2 weeks following surgery
General Evaluations				
Evolocumab Informed Consent	X[1]			
Physical Exam	X			X
Neurologic Exam	X			X
Adverse Events		Continuous[2]		
Laboratory Evaluations				
CBC with differential	X			X
CMP	X			X
Lipid profile	X			
Serum PregnancyTest, if applicable	X[3]	X[3]		
Pharmacokinetic blood draws (5 mL)[4]		X	X*	
Tumor tissue analysis (estimated > 20mg available)[5]			X	
Treatment				
Evolocumab		X		

** This blood sample taken at time of surgery or biopsy so that drug level in the blood can be compared to intratumoral concentration.*

Table 4 shows the schedule of events for the treatment arm of this study, including all study-related general evaluations, laboratory evaluations, disease evaluations, and treatments. Of particular importance for this phase 1 study are the tumor sampling and pharmacokinetic blood draws to investigate evolocumab concentration profiles following agent administration. Patients who consent to participate as a control will only be asked to have the following:

- Informed consent on the control consent form
- PK blood draw prior to surgical procedure
- Tumor tissue analysis, if available

10.1 Screening Examination

The initial screening examination will take place at a Duke PRTBTC clinic visit. The patient will sign either the main Evolocumab treatment consent form or the control consent form for this study and then undergo peripheral blood draw (prior to administration of evolocumab for those in the treatment arm). All patients with resectable tumors or tumors amenable to biopsy will complete screening and confirm their eligibility per the inclusion/exclusion criteria in Section 9. Screening tests in the treatment arm will consist of a physical and neurological exam, CBC with differential, CMP, lipid panel, and Beta HCG quantitative[3] (if applicable).

[1] Consent can be obtained within 30 days before starting evolocumab.
[2] Patients in the treatment arm will be followed for ongoing serious adverse events and new onset serious adverse events for 30 days following evolocumab injection.

[3] Within 48 hours of the first evolocumab injection, if applicable. Will only be repeated on Day 1 if screening pregnancy test is outside of the 48-hour window.
[4] Tube type to be determined by the Proteomics Core.
[5] Estimated > 50-100 mg from surgical resection or de-bulking procedure; Estimated > 20 mg from a minimum of 2 cores from biopsy

Once the patient is confirmed eligible, patients in the treatment arm will then receive Evolocumab via subcutaneous injection 4-14 days prior to surgical procedure. If the first evolocumab injection does not start within two weeks of the screening visit, the CBC with differential, CMP, lipid panel, and clinic visit (physical/neuro exam) will need to be repeated.

Patients who consent to participate as a control will not undergo any screening tests or procedures.

10.2 Run-In Period

Not applicable.

10.3 Treatment Period

Day 1:

- Baseline pharmacokinetic (PK_ blood draw (applies to patients who consent to either arm of the study) (5 mL drawn). For patients in the treatment arm, PK blood draw should occur before Evolocumab injection. For patients in the control arm, this blood draw can occur prior to the scheduled day of the surgical procedure or on the day of the surgical procedure.[Error! Bookmark not defined.]

Treatment arm only:

- Beta HCG quantitative[3] (if applicable) only if screening pregnancy test is outside of the 48-hour window
- Evolocumab injection
    - Latex-sensitive patients will be cautioned that the needle cover of the glass single-dose prefilled syringe and the single-dose prefilled auto-injector contain dry natural rubber (a derivative of latex) that may cause allergic reactions in individuals' sensitive to latex
    - Evolocumab will be allowed to warm to room temperature for at least 30 minutes for the prefilled auto-injector or syringe and for at least 45 minutes for the single-dose Pushtronex® system (on-body infusor with prefilled cartridge) if evolocumab has been refrigerated.
    - Evolocumab will be administered subcutaneously into areas of the abdomen, thigh, or upper arm that are not tender, bruised, red, or indurated, avoiding injecting into areas with scars or stretch marks.
    - The 420 mg dose of Evolocumab can be administered either:
        - Over 5 minutes by using the single-dose Pushtronex® system (on-body infusor with prefilled cartridge)
        - By giving 3 injections consecutively within 30 minutes using the single-dose prefilled auto-injector or single-dose prefilled syringe.
- Adverse events monitoring

10.4 Active Follow-up Period

Days 4-14 – Surgical resection or biopsy

- Tumor tissue collection (applies to patients who consent to either arm of the study) – this tissue will be taken intraoperatively for pharmacokinetic LC-MS analysis to assess for the presence of evolocumab.
    - Sample will only be taken for analysis if greater than 20 mg of resected or biopsied tissue available (excess available beyond that required for histological analysis)
    - Sample to be aliquoted into 25 mg sections for resections and 10-20 mg (1-2 cores) for biopsies

Treatment arm only:

- Pharmacokinetic blood draw (5 mL, drawn at time of surgery or biopsy, i.e., PK draw may occur either immediately before or during surgery or biopsy)Error! Bookmark not defined.
- Adverse events monitoring[2]

About 2 weeks following surgery or biopsy (treatment arm only)

- Physical and neurological exam
- Laboratory evaluations (obtained as part of standard post-operative labs)
  - CBC with differential
  - CMP
- Adverse events monitoring[2]

10.5 End of Treatment

Once the baseline PK blood draw is collected (both arms) and the evolocumab injection (treatment arm only) is completed, the treatment phase of the study is over and the active follow-up period begins. During the active follow-up period (see Section 10.4), patients undergo their standard of care surgical resection or biopsy with tissue collection, if available. Patients in the treatment arm return for post-surgical wound care about 14 days post-surgical procedure as needed per standard neurosurgical practice.

10.6 Follow-up Period

Once active follow-up is complete, patients are followed per standard of care. The study team will review medical records of patients enrolled in the treatment arm to collect information for ongoing serious adverse events and new onset serious adverse events for up to 30 days following Evolocumab injection. If patients subsequently undergo additional biopsy or resection, a sample of leftover tissue may be requested for analysis.

10.7 End of Study

Once all data has been collected, analyzed, and completely evaluated on all subjects as defined in the statistical analysis plan and subjects have completed follow up, expired, withdrawn, or been lost to follow up, the study will be ended. The study may also be terminated early for any reason by the PI.

10.8 Early Withdrawal of Subject(s)

10.8.1 Criteria for Early Withdrawal

Subjects may voluntarily withdraw from the study at any time. The PI may also withdraw a subject from the study at any time based on his/her discretion. Reasons for PI-initiated withdrawal may include, but is not limited to the following:

- Adverse events
- Abnormal laboratory values
- Abnormal test procedure results
- Protocol deviation
- Administrative issues
- Disease progression
- Pregnancy

10.8.2 Follow-up Requirements for Early Withdrawal

Patients in the treatment arm will be assessed and followed for adverse event monitoring/safety analysis for 30 days following Evolocumab injection.

10.8.3 Replacement of Early Withdrawal(s)

Patients who are withdrawn by the PI prior to baseline PK collection (both arms) and Evolocumab injection (treatment arm) will be replaced. Patients who do not undergo surgery or biopsy or do not have sufficient tissue taken for the pharmacokinetic LC-MS analysis to assess for the presence of evolocumab (treatment arm) will be replaced. If there is no enough tissue available for analysis in either cohort, the patients should be replaced.

10.9 Study Assessments

10.9.1 Medical History

Medical history will be obtained from the Duke electronic system and from the subject and/or family at the screening visit and reviewed at each study visit. This data may include the following:

- All past medical and surgical history
- Current medications
- Changes in physical or neurologic symptoms
- Any adverse events

10.9.2 Physical Exam

Vital signs and physical and neurologic examinations will be assessed for patients in the treatment arm and recorded prior to enrollment and at each visit according to Table 4.

10.9.3 Correlative Assessments

The following will be tested from tumor and serum samples (both study arms):

1. Lipid metabolism and immune characteristics (multi-omics analysis, immune infiltration and T cell profiling) of tumor cells taken from resected or biopsied samples
2. Analysis of tumor cells expressing MHC-I will be performed by fluorescence-activated cell sorting (FACS)
3. Analysis of serum levels of evolocumab, for comparison to intratumoral concentrations
4. Differential levels of TILs based on flow cytometry after staining of cells for expression of surface and nuclear markers of exhaustion (e.g., PD-1, Lag3, TIM3) and activation (CD137)

11 SAFETY MONITORING AND REPORTING

The PI is responsible for the identification and documentation of adverse events and serious adverse events, as defined below. Among patients receiving evolocumab and at each study visit, the PI or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred.

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient receiving study drug in the treatment arm and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of Evolocumab, whether or not related to use of the Evolocumab. Abnormal laboratory findings without clinical significance (based on the PI's judgment) should not be recorded as AEs. But laboratory value changes that require therapy or adjustment in prior therapy are considered adverse events.

From the time a patient receives their Evolocumab injection to the End of Study visit (as defined in Section 10.7), all AEs must be recorded in the subject medical record and adverse events case report form.

AEs will be assessed according to the CTCAE version 5.0. If CTCAE grading does not exist for an AE, the severity of the AE will be graded as mild (1), moderate (2), severe (3), life-threatening (4), or fatal (5).

Attribution of AEs will be indicated as follows:

- Definite: The AE is clearly related to the study drug
- Probably: The AE is likely related to the study drug
- Possible: The AE may be related to the study drug
- Unlikely: The AE is doubtfully related to the study drug
- Unrelated: The AE is clearly NOT related to the study drug

11.1.1 AEs of Special Interest

Special Interest Adverse Events that may occur during the injection procedure include:

- Allergic reaction
- Anaphylaxis
- Pre-syncope
- Syncope
- Vasovagal reaction

Special Interest Adverse Events that may occur after the procedure, but may still be related to injection include:

- Injection site complications

11.1.2 Reporting of AEs

A summary of all adverse events (not just those considered related to the study drugs) will be kept. AEs will be categorized by organ system, relationship to which treatment, grade of severity, and resolution. Weekly review by the PI during weekly Adverse Event meeting of new AEs and the collective AEs will occur with the intention of identifying any trends or patterns in toxicity. If significant toxicity is observed in the initial 2 patients, or after the first month, the study will be paused, and protocol modification will be considered. If any DLT occurs, as defined in Section 7.1.2, the study will be paused for assessment/review to determine if any changes to the protocol are warranted.

11.2 Serious Adverse Events

An AE is considered “serious” if in the opinion of the investigator it is one of the following outcomes:

- Fatal
- Life-threatening
- Constitutes a congenital anomaly or birth defect
- A medically significant condition (defined as an event that compromises subject safety or may require medical or surgical intervention to prevent one of the three outcomes above).
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption to conduct normal life functions.

11.2.1 Reporting of SAEs

All SAEs should be reported immediately to the PI, Dr. Mustafa Khasraw (Pager: 919-206-0493) or his designee (919-684-8111).

All adverse events that are considered serious, unanticipated, and related or possibly related to the research (as defined by 21CRF312.32[a]) will be reported to the IRB using the appropriate SAE reporting process.

11.3 Emergency Unblinding of Investigational Treatment
Not applicable.

11.4 Other Reportable Information
Not applicable.

11.5 Special Warnings and Precautions
Not applicable.

11.6 Safety Oversight Committee (SOC)
The Duke Cancer Institute SOC is responsible for annual data and safety monitoring of DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent Data Safety Monitoring Board (DSMB). The primary focus of the SOC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The SOC in concert with the DCI Monitoring Team (see Section 12.1 for Monitoring Team description) oversees the conduct of DUHS cancer-related, sponsor-investigator greater-than-minimal-risk intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, standing operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements.

11.7 External Data and Safety Monitoring Board (DSMB)
There is no external DSMB for this study.

12 QUALITY CONTROL AND QUALITY ASSURANCE

12.1 Monitoring
This clinical research study will be monitored both internally by the PI, and institutionally by the Duke Cancer Institute (DCI). In terms of internal review, the PI will continuously monitor and tabulate adverse events. Appropriate reporting to the Duke University Medical Center IRB will be made. If an unexpected frequency of Grade III or IV events occur, depending on their nature, action appropriate to the nature and frequency of these adverse events will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Interim analyses occur as scheduled;
- Stopping rules for toxicity and/or response are met;
- Risk/benefit ratio is not altered to the detriment of the subjects;
- Appropriate internal monitoring of AEs and outcomes is done;
- Over-accrual does not occur;
- Under-accrual is addressed with appropriate amendments or actions;
- Data are being appropriately collected in a reasonably timely manner.

DCI Protocol Review and Monitoring systems (PRMS) review of this protocol begins with an initial review by the Cancer Protocol Committee (CPC). CPC new protocol review focuses on scientific relevance, study design, adequacy of biostatistical input, protocol prioritization, feasibility of completing the study within a reasonable time frame and risk assessment of the trial. The PI will abide by CPC assessment of the level of risk, which will determine the intensity of subsequent DCI monitoring. CPC also conducts annual scientific progress reviews on protocols that are open to enrollment and focus on protocol prioritization, accrual and scientific progress. These reviews are conducted at the time of IRB annual renewals and documentation of all CPC reviews will be maintained in eIRB/iRIS systems.

A determination for the degree of monitoring conducted by the DCI monitoring team is made at the time of initial CPC approval to commensurate with the type and level of intervention, phase, endpoints, degree of risk, size and complexity of the protocol. A formal, independent monitoring will be conducted by the DCI monitoring team according to the risk level and monitoring plan assigned by the CPC until the study is closed to enrollment or subjects are no longer receiving study drug or other interventions that are more than minimal risk. Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns. Monitoring visits may also be initiated upon request by DUHS and DCI Leadership, CPC, SOC, a sponsor, an investigator, or the IRB.

The DCI monitoring team reviews the adequacy of informed consent, enrollment of eligible patients, implementation of protocol-specified procedures and treatment, adequacy of data collection, and appropriateness of adverse event monitoring and reporting. The DCI monitoring team presents final monitoring reports to the DCI Safety Oversight Committee (SOC) highlighting safety concerns and unresolved issues. The SOC, at a convened meeting, assigns an overall rating of satisfactory, marginal, or unsatisfactory to reflect the overall quality of data, regulatory, consent, eligibility, study conduct and AE reporting. Corrective action plans (CAPs) are developed, implemented, and evaluated as indicated. The SOC will notify the sponsor-investigator and DUHS IRB when significant safety concerns are identified.

The SOC in concert with DCI monitoring team conducts data and safety monitoring for DUHS sponsor-investigator phase I and II, therapeutic interventional oncology studies that do not have an independent DSMB. These reviews occur at a minimum annually and more frequently for the high risk studies. The SOC safety reviews include review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The SOC, at a convened meeting, assigns a rating of satisfactory when adequate accrual with lack of excessive toxicity is present.

12.2 Audits

The Duke Office of Audit, Risk and Compliance (OARC) office may conduct confidential audits to evaluate compliance with the protocol and the principles of GCP. The PI agrees to allow the OARC auditor(s) direct access to all relevant documents and to allocate his/her time and the time of the study team to the CTQA auditor(s) in order to discuss findings and any relevant issues.

OARC audits are designed to protect the rights and well-being of human research subjects. OARC audits may be routine or directed (for cause). Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, Investigator initiated Investigational Drugs or Devices, federally-funded studies, high degree of risk (based upon adverse events, type of study, or vulnerable populations), Phase I studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized Institutional Official.

OARC audits examine research studies/clinical trials methodology, processes and systems to assess whether the research is conducted according to the protocol approved by the DUHS IRB. The primary purpose of the audit/review is to verify that the standards for safety of human subjects in clinical trials and the quality of data produced by the clinical trial research are met. The audit/review will serve as a quality assurance measure, internal to the institution. Additional goals of such audits are to detect both random and systemic errors occurring during the conduct of clinical research and to emphasize “best practices” in the research/clinical trials environment.

12.3 Data Management and Processing

12.3.1 Case Report Forms (CRFs)

The electronic CRF (eCRF) will be the primary data collection document for the study. The eCRFs will be updated in a timely manner following acquisition of new source data. Only approved study staff (e.g., the PI, the clinical research coordinator, the data management team, and the assistant research practice manager) are permitted to make entries, changes, or corrections in the CRF.

An audit trail will be maintained automatically by the 21 CFR Part 11 compliant electronic CRF management system. Designated personnel will complete user training, as required or appropriate per regulations.

12.3.2 Data Management Procedures and Data Verification

Designated personnel using the electronic CRF will have access based on their specific roles in the protocol as determined by the PRTBTC Data Manager.

Completeness of entered data will be checked automatically by the eCRF system, and users will be alerted to the presence of data inconsistencies. Additionally, the data management team and the statistical team will cross-reference the data to verify accuracy. Missing or implausible data will be highlighted for the PI requiring appropriate responses (i.e. confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

12.3.3 Study Closure

Following completion of the studies, the PI will be responsible for ensuring the following activities:

- Data clarification and/or resolution
- Accounting, reconciliation, and destruction/return of used and unused study drugs
- Review of site study records for completeness
- Shipment of all remaining laboratory samples to the designated laboratories

13 STATISTICAL METHODS AND DATA ANALYSIS

All statistical analysis will be performed under the direction of the statistician designated in key personnel. Any data analysis carried out independently by the investigator must be approved by the statistician before publication or presentation.

13.1 Analysis Sets

All patients who receive Evolocumab will be included in summaries of adverse events.

PK and PD analyses will include all patients in the treated and untreated groups who have baseline PK blood collected and underwent a resection.

Analyses of serum and intratumoral levels of Evolocumab will be conducted among treated and untreated patients who were resected or biopsied and had serum collected.

13.2 Patient Demographics and Other Baseline Characteristics

The clinical and sociodemographic characteristics of the patients who are enrolled and treated on this study will be summarized by arm. Frequencies and percentages will be provided for categorical variables;

means and standard deviations or medians and interquartile ranges will be provided for continuous variables.

13.3 Treatments

A CONSORT diagram will be generated to describe the flow of patients through the study, and will provide information about the number of patients who received Evolocumab treatment, the number of control patients (either consented on this study as a control or requested from the BTBR), and the number of patients who subsequently had a surgical procedure.

13.4 Primary Objective

The primary objective of this study is to assess whether Evolocumab crosses the blood brain barrier (BBB) and is measurable in the resected or biopsied tumor specimens of patients with primary and recurrent glioma.

13.4.1 Variable

By means of mass spectrometry, the level of Evolocumab within the resected or biopsied brain tumor of a patient treated with one dose of Evolocumab will be assessed.

13.4.2 Statistical Hypothesis, Model, and Method of Analysis

The level of Evolocumab within the resected or biopsied tumor will be determined for patients treated with Evolocumab, and within a cohort of resected or biopsied control patients who were not treated with Evolocumab. Either a Wilcoxon rank sum test or a two-sample t-test will compare cohorts.

13.4.3 Handling of missing values, censoring, and discontinuations

Not applicable.

13.5 Secondary Objectives

This study's three secondary objectives include a comparison of brain tumor tissues specimens with and without Evolocumab with respect to lipid metabolism, and tumor cells expressing MHC-I; and an assessment of the relationship between serum levels of evolocumab and intratumoral concentrations.

13.5.1 Key Secondary Objectives #1and #2: Lipid Metabolism and Tumor Cells Expressing MHC-I

The first two secondary objectives involve a comparison of the pharmacodynamics (PDs) of Evolocumab in treated patients relative to that observed in a control cohort of untreated patients. The characterization of the PD properties will include the following: (1) Lipid metabolism of tumor cells taken from resected or biopsied samples, and (2) Analysis of tumor cells expressing MHC-I will be performed by fluorescence-activated cell sorting (FACS). Analyses similar to those describe for the primary objective will be performed.

13.5.2 Secondary Objective #3: Serum and Intratumoral Levels of Evolocumab

Among patients treated with evolocumab, the third secondary objective is an assessment of the relationship between serum and intratumoral levels of the drug. These assessments will be obtained at the time of surgical procedure. A scatterplot will graphically show the relationship between these two measures. Spearman's rank correlation will assess their association.

13.6 Exploratory Objectives

This study has two exploratory objectives: (1) To describe the immune characteristics of tumor cells taken from resected or biopsied samples, and (2) To assess the activity of T cells isolated from tumor specimens in patients treated with or without evolocumab.

Exploratory Objective #1: We hypothesize that evolocumab treatment will lead to global changes in pro-tumorogenic pathways and the reduction in PSCK9 levels and therefore will seek to determine the immune characteristics (multi-omics analysis, immune infiltration and T cell profiling) of tumor cells taken from resected or biopsied samples. To test this hypothesis, non-targeted quantitative LC-MS/MS will be performed on resected or biopsied tumors from n=10 treatment patients versus n=20 untreated control specimens. Furthermore, if PSCK9 is not among the proteins quantified by non-targeted LC-MS/MS, a more sensitive targeted LC-MS/MS analysis will be utilized as described for the PK analysis of evolocumab above. Bioinformatic analyses of differentially expressed proteins will utilize protein set enrichment, gene ontology (GO) annotations, and search tool from the retrieval of interactive genes/proteins (STRING).[26]

Wilcoxon rank-sum test, or a two-sample t-test, will compare groups with respect to these endpoints. Given the number of comparisons that may be conducted in these exploratory analyses, adjustment for multiple comparisons will be made.

Exploratory Objective #2: Cells stained for expression of surface and nuclear markers of exhaustion (PD-1, Lag3, TIM3) and activation (CD137) will be characterized using flow cytometry. We will explore the potential to select and expand the non-exhausted CD137+ subset and examine their anti-tumor efficacy in samples from patients treated with or without evolocumab. Cytotoxicity determined by flow cytometry of stained tumor cells will be compared to no TIL exposure. Patient-derived neurospheres will generated *in vitro* based on established protocols [26]. Statistical methods described for exploratory objective #1 will be used.

13.7 Interim Analysis

Not applicable.

13.8 Sample Size Calculation

The sample size selected was chosen for practical considerations in the setting of a phase 0 study, to allow descriptive analysis of the changes of the biologic endpoints in the evolocumab treated (cases) and untreated patients (controls). This may provide important biologic insights into the secondary and exploratory endpoints.

14 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

14.1 Regulatory and Ethical Compliance

This protocol was designed and will be conducted and reported in accordance with the International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable federal, state, and local regulations.

14.2 DUHS Institutional Review Board and DCI Protocol Review and Monitoring Committee

The protocol, informed consent form, advertising material, and additional protocol-related documents must be submitted to the DUHS Institutional Review Board (IRB) and DCI Protocol Review and Monitoring Committee (PRMC) for review. The study may be initiated only after the Principal Investigator has received written and dated approval from the PRMC and IRB.

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form. The PRMC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, statistical analysis, etc.).

The Principal Investigator must obtain protocol re-approval from the IRB within 1 year of the most recent IRB approval. The Principal Investigator must also obtain protocol re-approval from the PRMC within 1 year of the most recent IRB approval, for as long as the protocol remains open to subject enrollment.

14.3 Informed Consent

The informed consent form must be written in a manner that is understandable to the subject population. Prior to its use, the informed consent form must be approved by the IRB.

The Principal Investigator or authorized key personnel will discuss with the potential subject the purpose of the research, methods, potential risks and benefits, subject concerns, and other study-related matters. This discussion will occur in a location that ensures subject privacy and in a manner that minimizes the possibility of coercion. Appropriate accommodations will be made available for potential subjects who cannot read or understand English or are visually impaired. Potential subjects will have the opportunity to contact the Principal investigator or authorized key personnel with questions, and will be given as much time as needed to make an informed decision about participation in the study.

Before conducting any study-specific procedures, the Principal Investigator must obtain written informed consent from the subject or a legally acceptable representative. The original informed consent form will be stored with the subject's study records, and a copy of the informed consent form will be provided to the subject. The Principal Investigator is responsible for asking the subject whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Principal Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

14.4 Study Documentation

Study documentation includes, but is not limited to, source documents, case report forms (CRFs), monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the DCI-mandated "Regulatory Binder," which includes, but is not limited to, protocol and amendments, approved informed consent forms, and laboratory certifications.

Source documents are original records that contain source data, which is all information in original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

An electronic case report form (CRF) will be the primary data collection document for the study. The CRFs will be updated within two weeks of acquisition of new source data. Only approved study staff (e.g., the PI, the clinical research coordinator, the data management team, and the assistant research practice manager) are permitted to make entries, changes, or corrections in the CRF. The Principal Investigator or

authorized key personnel will maintain a record of the changes and corrections. For eCRFs, an audit trail will be maintained by the 21CFR Part 11 compliant electronic CRF management system.

14.5 Privacy, Confidentiality, and Data Storage

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained. Research Data Security Plans (RDSPs) will be approved by the appropriate institutional Site Based Research group.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his family, if desired. For all future visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private exam room. All research related interactions with the participant will be conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using a 21 CFR Part 11 compliant dedicated database, which is housed in an encrypted and password-protected network server. Access to electronic databases will be limited to those authorized by the PRTBTC Data Manager. The security and viability of the IT infrastructure will be managed by DCI and/or Duke Medicine.

Upon completion of the study, research records will be archived and handled per DUHS HRPP policy.

Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

14.6 Data and Safety Monitoring

Data and Safety Monitoring will be performed in accordance with the DCI Data and Safety Monitoring Plan. For a more detailed description of the DSMP for this protocol, refer to Section 11 (Sections 11.6 and 11.7 in particular) and Section 12.

14.7 Protocol Amendments

All protocol amendments must be initiated by the Principal Investigator and approved by the IRB prior to implementation. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an immediate hazard. However, the Principal Investigator must inform the IRB and all other applicable regulatory agencies of such action immediately.

Though not yet required, the PRMC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e., amendments affecting subject population, inclusion/exclusion criteria, agent administration, etc.).

14.8 Records Retention

The Principal Investigator will maintain study-related records for the longer of a period of at least six years after study completion.

15 REFERENCES

1. Liu XJ, Bao XH, Hu MJ, et al: Inhibition of PCSK9 potentiates immune checkpoint therapy for cancer. Nature 588:693-+, 2020
2. Ostrom QT, Gittleman H, Fulop J, et al: CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. Neuro Oncol 17 Suppl 4:iv1-iv62, 2015
3. Chinot OL, Wick W, Mason W, et al: Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med 370:709-722, 2014
4. Gilbert MR, Dignam JJ, Armstrong TS, et al: A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med 370:699-708, 2014
5. Stupp R, Taillibert S, Kanner AA, et al: Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. JAMA 314:2535-43, 2015
6. Kelly PJ: Stereotactic resection and its limitations in glial neoplasms. Stereotact Funct Neurosurg 59:84-91, 1992
7. Vredenburgh JJ, Desjardins A, Herndon JE, 2nd, et al: Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol 25:4722-9, 2007
8. Stupp R, Mason WP, van den Bent MJ, et al: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987-96, 2005
9. Walker MD, Green SB, Byar DP, et al: Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med 303:1323-9, 1980
10. Tan AC, Ashley DM, López GY, et al: Management of glioblastoma: State of the art and future directions. CA Cancer J Clin 70:299-312, 2020
11. Khasraw M, Reardon DA, Weller M, et al: PD-1 Inhibitors: Do they have a Future in the Treatment of Glioblastoma? Clin Cancer Res 26:5287-5296, 2020
12. Sabatine MS, Giugliano RP, Keech AC, et al: Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. New England Journal of Medicine 376:1713-1722, 2017
13. Schaller TH, Foster MW, Thompson JW, et al: Pharmacokinetic Analysis of a Novel Human EGFRvIII:CD3 Bispecific Antibody in Plasma and Whole Blood Using a High-Resolution Targeted Mass Spectrometry Approach. Journal of Proteome Research 18:3032-3041, 2019
14. Walker MD, Hunt WE, Mahaley MS, et al: Evaluation of Bcnu and-or Radiotherapy in Treatment of Anaplastic Gliomas - Cooperative Clinical-Trial. Journal of Neurosurgery 49:333-343, 1978
15. Shapiro WR: Therapy of Adult Malignant Brain-Tumors - What Have the Clinical-Trials Taught Us. Seminars in Oncology 13:38-45, 1986
16. Salford LG, Brun A, Nirfalk S: 10-Year Survival among Patients with Supratentorial Astrocytomas Grade-Iii and Grade-Iv. Journal of Neurosurgery 69:506-509, 1988
17. Dinapoli RP, Brown LD, Arusell RM, et al: Phase-Iii Comparative-Evaluation of Pcnu and Carmustine Combined with Radiation-Therapy for High-Grade Glioma. Journal of Clinical Oncology 11:1316-1321, 1993
18. Imperato JP, Paleologos NA, Vick NA: Effects of Treatment on Long-Term Survivors with Malignant Astrocytomas. Annals of Neurology 28:818-822, 1990
19. Hall WA, Fodstad O: Immunotoxins and Central-Nervous-System Neoplasia. Journal of Neurosurgery 76:1-12, 1992
20. Traynor K: Evolocumab approved for reducing LDL cholesterol. Am J Health Syst Pharm 72:1599-600, 2015
21. Khatib RW, F.: Pharmacology of Medications Used in the Treatment of Atherosclerotic Cardiovascular Disease, in Vasan RSS, D. B. (ed): Encyclopedia of Cardiovascular Research and Medicine. Oxford, Elsevier, 2018
22. Sabatine MS: PCSK9 inhibitors: clinical evidence and implementation. Nat Rev Cardiol 16:155-165, 2019

23. Liu X, Suo R, Chan CZY, et al: The immune functions of PCSK9: Local and systemic perspectives. J Cell Physiol 234:19180-19188, 2019

24. Amgen : Repatha (evolocumab) injection, for subcutaneous use: US prescribing information, 2021

25. Kasichayanula S, Grover A, Emery MG, et al: Clinical Pharmacokinetics and Pharmacodynamics of Evolocumab, a PCSK9 Inhibitor. Clin Pharmacokinet 57:769-779, 2018

26. Hasselbach LA, Irtenkauf SM, Lemke NW, et al: Optimization of high grade glioma cell culture from surgical specimens for use in clinically relevant animal models and 3D immunochemistry. J Vis Exp:e51088, 2014