NU Study Number: NU 16C01

A Phase 0 first-in-human study using NU-0129: a spherical nucleic acid (SNA) gold nanoparticle targeting BCL2L12 in recurrent glioblastoma multiforme or gliosarcoma patients

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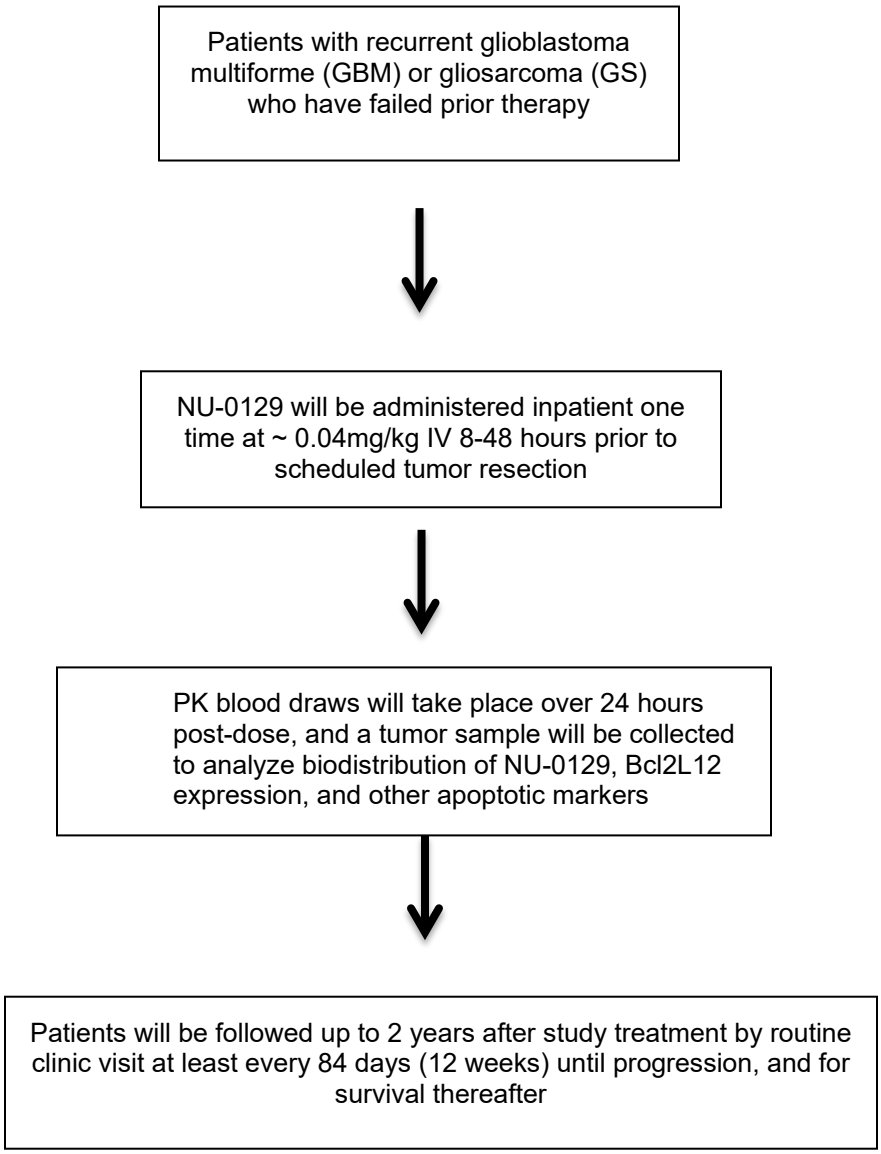
LIST OF ABBREVIATIONS

AE	Adverse Event
AED	Antiepileptic Drug
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
API	Active Pharmaceutical Ingredient
AST	Aspartate Aminotransferase
BBB	Blood Brain Barrier
BTB	Blood Tumor Barrier
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data and Safety Monitoring Board
GLP	Good Laboratory Practices
ECOG	Eastern Cooperative Oncology Group
EIAED	Enzyme-inducing Antiepileptic Drug
HED	Human equivalent dose
H&PE	History & Physical Exam
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IHC	Immunohistochemistry
IV (or iv)	Intravenously
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NOAEL	No-observed-adverse-effect level
ORR	Overall Response Rate or Objective Response Rate
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PFS	Progression Free Survival
PO (or p.o.)	Per os/by mouth/orally
PR	Partial Response
RANO	Response Assessment in Neuro-Oncology
REMBRANDT	Repository of Molecular Brain Neoplasia Data
RT-qPCR	Quantitative Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
SNA	Spherical Nucleic Acid
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling

ULN	Upper Limit of Normal
V_{SS}	Volume of distribution at steady state
WBC	White Blood Cells

STUDY SCHEMA



STUDY SUMMARY

Title	A Phase 0 first-in-human study using NU-0129: a spherical nucleic acid (SNA) gold nanoparticle targeting BCL2L12 in recurrent glioblastoma multiforme or GS patients
Version	December 19, 2018 (Amendment 5)
Study Design	This is a single-arm, open-label, phase 0 first-in-human trial to determine the safety of NU-0129.
Study Center(s)	Northwestern University
Objectives	Primary Objective: • To evaluate the safety of NU-0129 SNA gold nanoparticle infusion in patients with recurrent GBM or GS Secondary Objectives: • To evaluate the bio-distribution of NU-0129 in tumor tissue • To evaluate the pharmacokinetics of NU-0129 in blood samples collected at 1, 3, 5, 10, 30, 60 minutes and 4, 8, and 24 hours post-dose • To evaluate the feasibility of NU-0129 administration Exploratory Objectives • To evaluate the Bcl2L12 expression levels and apoptotic markers in the tumor tissue post-treatment • To evaluate preliminary response (Overall Survival and Progression Free Survival at 2 years, as well as Overall Response Rate)
Sample Size	6-8 patients will be enrolled
Diagnosis & Key Eligibility Criteria	• Patients should have a diagnosis of recurrent glioblastoma multiforme (GBM) or gliosarcoma (GS) after failing prior therapy. • Eligible patients must be surgical candidates where surgery is felt to be an appropriate treatment option. • Bone marrow and organ function must be adequate as defined below • WBC ≥ 3,000/μL • ANC ≥ 1,500/mm3 • Platelet count of ≥ 100,000/mm3 • Hemoglobin ≥8 g/dL • Bilirubin ≤ 2 x ULN • AST/ALT ≤ 2 x ULN • Creatinine ≤ 1.5 x ULN Patients must not have had prior cancer therapy (including biologic, cytotoxic, and experimental therapies and nitrosoureas) within 21 days of registration.
Treatment Plan	Once registered, eligible patients will receive intravenous NU-0129 nominal dose ~ 0.04mg/kg (based on dosing chart) one time. Patients will be monitored with telemetry, vital signs, and labs before, during and after the infusion as indicated in section 5.0. Treatment will be followed by a tumor resection 8-48 hours later. The tissue will be collected and analyzed for bio-distribution and exploratory studies.

Statistical Methodology	All patients properly included in the study who undergo surgery for tumor removed will be included in the analysis. Thus, an incorrect treatment schedule or drug administration would result in exclusion from the analysis of the tumor accumulation study. All conclusions will be based on all eligible patients. Sub-analyses may then be performed on the basis of a subset of patients, excluding those for who major protocol deviations have been identified. However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients form the analysis should be clearly reported.

1.0 INTRODUCTION – BACKGROUND & RATIONALE

1.1 Disease Background

Glioblastoma (GBM) is a neurologically debilitating disease, which culminates in death 16-18 months after diagnosis. An incomplete understanding of genetic aberrations leading to tumor growth, invasion and anti-apoptosis, mechanisms of resistance, combined with the lack of effective drugs have conspired to make GBM an incurable disease. In addition to issues noted, drug delivery has long been an issue in treating brain tumors that is not an issue for most other solid tumors.

Glioblastoma (GBM) is a highly malignant brain tumor of astrocytic origin which accounts for over 50% of all gliomas. GBM has an annual incidence rate of 3 to 4 cases per 100,000 people resulting in 240,000 newly diagnosed cases worldwide each year [1,2]; .

The current standard treatment for GBM is surgical resection followed by radiotherapy with concurrent and adjuvant temozolomide (TMZ). For newly diagnosed GBM, the addition of TMZ to surgery and radiation therapy prolongs median survival (from 12.1 to 14.6 months) and increases the five-year survival rate (from 2% to 10%; hazard ratio 0.63; 95% CI 0.53-0.75; p<0.0001) [3].

Bevacizumab, a monoclonal antibody against vascular endothelial growth factor (VEGF), was approved in the US for the treatment of recurrent GBM based on a clinically meaningful and durable objective tumor response rate (ORR 28%-38%, median duration of response 4.2 months). The 6-month-PFS rate in the multiple studies ranged from approximately 29% to 50% and represented a significant improvement over the historical 10-20% 6-month-PFS rate. Despite the improvement in response provided by bevacizumab, most patients with GBM experience relapse within months [4,5] and options for salvage remain extremely limited.

Genetically, GBM is characterized by complex chromosomal abnormalities and extensive intratumor cytogenetic and histological heterogeneity displaying the genetic alterations that govern the processes of cellular proliferation, survival, invasion and angiogenesis [6,7,8]. Common mutations found in GBM include: EGFR overexpression (70%) [9]; TP53, RB, INK4a, PDGF-R, VEGF-R, C- Met and HGF and PTEN [10].

Besides GBM, which features the highest grade of malignancy among glioma (grade IV), lower grade glioma which include grade II and grade III are a heterogeneous group of tumors in which specific molecular features are associated with divergent clinical outcome.

Currently approved therapy for a newly diagnosed GBM patient in the United States includes maximal surgical resection followed by radiation and temozolomide [11]. Upon recurrence there are few approved options and these include surgical implantation of chemotherapy bearing wafers[12] (polifeprosan 20 with carmustine implant, Gliadel® Wafer) and systemic administration of the anti-angiogenic agent bevacizumab, which has shown a partial response rate of 20% in one trial, and 26% in another [13, 14]. Each of these therapies has shown modest improvement in survival of recurrent GBM patients, with notable treatment related toxicities including wound breakdown after surgical resection [15].

1.2 Intervention Background & Overview

NU-0129 is an investigational spherical nucleic acid (SNA) designed to target the silencing of Bcl2L12 gene expression, an overexpressed protein that plays an important role in driving the pathogenesis of glioblastoma multiforme (GBM) and mediating therapeutic resistance [16]. SNAs are three dimensional conjugates consisting of densely functionalized and highly oriented nucleic acids covalently attached to the surface of nanoparticles [17]. NU-0129 is composed of 13nm gold nanoparticles surrounded by siRNAs tailored to recognize Bcl2l12 sequence. Gene silencing using siRNAs conjugated

to these SNAs represents a promising new approach for systemic RNA interference (RNAi)-based therapy of this aggressive malignant brain tumor in humans. This nanoconjugate is one of the first to report stable and robust RNAi delivery to intracranial tumors in rodent GBM models, as they have the capacity to cross both intact and tumor-compromised blood brain barrier (BBB), and has helped develop SNAs as a platform for biotherapeutic gene silencing. Non-human primate and non-primate toxicology studies were completed with NU-0129, with no significant clinical observations.

The impact of NU-0129 on tumor progression in xenogeneic grafts in mice show intravenous administration of NU-0129 at a total injection of 7 mg/kg of the active pharmaceutical ingredient (API) resulted in intraglioma protein down-regulation and impaired tumorigenicity as measured by decreased tumor weight and prolonged survival of xenogeneic mice. Increased intratumoral apoptosis was evidenced by enhanced levels of DNA strand breaks as assessed by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and active caspase-3 staining. Thus, blood-brain barrier/blood-tumor barrier (BBB/BTB) penetration and intra-glioma dissemination of NU-0129 upon systemic administration resulted in increased survival of glioma-bearing animals.

1.2.1 Pre-clinical Pharmacokinetic studies

Summary of finding from 126501 AD TK report Text

NU-0129, in the vehicle (phosphate-buffered saline, pH 7.4), was administered by IV bolus once to 3 groups of male and female Cynomolgus monkeys. A concurrent control group received the vehicle on the same regimen. Table T1 presents the study group assignment for the Toxicokinetic portion of the study.

Table T1. ! Study Group Assignment for the Toxicokinetic Portion of the Study

Group Number	Test Article	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Animals	
				Males	Females
1	Vehicle	0	5	5	5
2	NU-0129	1	5	5	5
3	NU-0129	4	5	5	5
4	NU-0129	8	5	5	5

*The dose levels correspond to the amount of siRNA, the actual test article component, in the neat powered material.

On the day of dosing, blood samples were collected from all animals prior to dosing and at approximately 3, 10, and 30 minutes, and 2, 8, and 24 hours post-dose. Plasma was analyzed for the concentration of NU-0129 using liquid chromatography with FLD. The results of these analyses were used for toxicokinetic analysis.

The toxicokinetic parameters for NU-0129 in plasma are summarized in Table T2.

Table T2. Toxicokinetic Parameters for NU-0129 in Male and Female Monkeys

	Males						Females					
Dosage (mg/kg)	1		4		8		1		4		8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC_{last} (µg*h/mL)	0.232†[1]	NA	2.37	1.04	9.82	2.82	0.321†[3]	0.104	3.62	0.717	14.2	1.90
C_0 (µg/mL)	1.67	0.879	34.0	19.8	131	51.4	3.90	2.31	68.9	18.1	174	36.3
$T_{1/2}$ (h)	NC	NA	0.06	0.00	0.08	0.04	0.05†[3]	0.00	0.05	0.00	0.07	0.01
Cl (mL/h/kg)	NC	NA	1980	863	863	224	3340†[3]	1070	1140	250	570	93.2
V_{ss} (mL/kg)	NC	NA	158	86.7	72.6	26.0	213†[3]	75.3	66.1	22.4	50.2	7.52

N = 5, except where designated with †[N]

Following a single IV bolus dose of NU-0129 to male and female monkeys, exposure increased with increasing dose. The increase in AUC_{last} was greater than dose proportional as NU-0129 dose increased from 1 mg/kg to 8 mg/kg. NU-0129 exposure, in terms of AUC_{last}, was slightly higher in females than in males, but differences were <2-fold.

Clearance, V_{ss}, and terminal half-life were not calculable at 1 mg/kg for males. Mean Clearance, ranging from approximately 600 to 3000 mL/h/kg, was moderate to high (approximately 20% to 120% of hepatic blood flow in the monkey as reported by Davies and Morris, 1993) and decreased as dose increased. Mean V_{ss} was small, ranging from approximately 50 to 200 mL/kg (approximately 10% to 30% of total body water in the monkey as reported by Davies and Morris, 1993) and also decreased with increasing dose. This resulted in a short terminal elimination halflife that ranged from approximately 2 to 9 minutes for individual animals. Clearance- and V_{ss} values were approximately 1.4- to 2-fold higher for males than females, but half-life values were similar.

1.2.2 Pre-clinical toxicity studies

The non-clinical toxicity studies for Bcl2l12-2-SNA in cynomolgus monkeys and rats showed no significant test article-related clinical observations. Purple and/or blue discoloration of various body surfaces (facial area, oral cavity, limbs and/or trunk) were noted in medium and high dose groups, along with macroscopic observations of blue discolorations in some tissues of high dose group. Consistently, microscopic findings indicated black pigment within macrophages and endothelium in corresponding tissues, i.e., adrenals, liver, lymph nodes, and spleen. These pigments are likely attributed to the gold-nanoparticles. Importantly, toxicity was not observed at the cellular or tissue level in the presence of the gold-nanoparticles.

Administration of Bcl2l12-2-SNA to male and female cynomolgus monkeys was well tolerated with all animals surviving to the scheduled primary (Day 2 post-dose) or recovery (Day 14 post-dose) necropsies. Significantly, there were no clinical observations in hematology, coagulation, serum chemistry, or urinalysis parameters, or organ weight. Blue discoloration was noted in many of the tissues of the monkeys, more so at the higher dose levels, and this was correlated to the

presence of pigment within macrophages and endothelium in the various tissues. The pigment appeared black by light microscopy and is likely attributed to the gold-nanoparticles. There did not appear to be any cellular or tissue level toxicity to the presence of the gold-nanoparticles. Bcl2l12-2-SNA appears to be a promising, clinically-viable drug product. There is no significant toxicity associated with the chemistry or manufacturing of the drug product. All pre-clinical studies were performed under conditions of Good Laboratory Practice (GLP).

1.2.3 More in-depth results and summary.

The dose levels tested were 1mg/kg (low), 4mg/kg (medium) and 8mg/kg (high) via intravenous (bolus) injection to male and female cynomolgus monkeys. The dose levels correspond to the amount of siRNA, the actual test article component, in the neat powered material. There was 0.025 grams of siRNA per 1.00 gram of the neat, powered material.

All toxicology and cardiovascular animals survived to the end of the study. There were no test article-related effects on body weights, hematology, coagulation, serum chemistry, urinalysis, or organ weights. In addition, administration of a single dose of NU-0129 at high dose (8 mg/kg) via intravenous (bolus) injection to male and female cynomolgus monkeys resulted in moderately decreased blood pressure (systolic, diastolic, and mean blood pressure); this effect was only detected in the test article formulation group and not the control group. NU-0129 at 8 mg/kg had no effect on heart rate, pulse pressure, body temperature, ECG intervals (PR, QRS, RR, QT, or QTcB), or ECG waveform morphology.

Test article-related clinical observations of purple and/or blue discoloration of various body surfaces (facial area, gums, forelimb[s], hindlimb[s], oral cavity, and/or trunk [dorsal and ventral]) were noted in the 4 and 8 mg/kg toxicology group males and females and in the 8 mg/kg cardiovascular group males and females throughout the study, and in a single 1 mg/kg toxicology group male on study days 4 and 5. In general, purple discoloration was noted only on the day of dose administration and blue discoloration was noted for the remainder of the recovery period.

Test article-related macroscopic observations of blue discoloration were noted in many tissues (adrenals, gastrointestinal tissues [esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum], epididymis, lymphoid tissues [spleen, lymph nodes, and thymus], skin, liver, tongue, urogenital tissues [seminal vesicles, testes, vagina, and urinary bladder]) of most males and females at 8 mg/kg on study days 2 and 14. Blue discoloration of the liver, spleen, lymph nodes, skin, tongue, and scattered other tissues was also noted in the 4 mg/kg/day group males and females on study days 2 and 14. In the 1 mg/kg/day group males and/or females, blue discoloration was noted only in the liver, adrenals, epididymis, testes, and spleen. In addition, blue discoloration of the bone was noted in the 4 and 8 mg/kg group males and females on study day 14. Procedurerelated- macroscopic findings of dark red area(s) at the injection site were noted in all animals, including the control group, on study days 2 and 14.

Test article-related microscopic findings of pigment in the adrenals, bone marrow (femur and sternum), Kupffer cells in the liver, lymph nodes (axillary, mandibular, and/or mesenteric), and spleen were noted in all test article-treated male and female groups on study day 2 and in all test articletreated male groups as well as 4 mg/kg and 8 mg/kg group females on study day 14. In addition, pigment was noted in the skin and tongue in the 4 and 8 mg/kg group males and females on

study days 2 and 14. Injection site findings of acute perivascular hemorrhage were noted in all groups, including the control group on study days 2 and 14. -Procedurerelated- injection site findings of perivascular acute inflammation (neutrophils, edema, and fibrin exudation) was noted at all dose levels on study day 2, and subacute inflammation was noted in the 4 and 8 mg/kg group males on study day 14.

Since the neat test article is comprised of spherical nucleic acids in the presence of gold nanoparticles and the dosing formulation was described as being an opaque dark red liquid, it is hypothesized that it is the gold nanoparticles that are responsible for the blue discoloration of the tissues.

Based on the results of this study, a single intravenous (bolus) injection of NU-0129 to cynomolgus monkeys resulted in a no-observed-adverse-effect level (NOAEL) of 8 mg/kg for both systemic and local assessments.

1.3 Rationale for the Current Study

NU-0129 has undergone extensive pre-clinical testing and appears to be a viable, safe, and potentially efficacious drug product in GBM. The NOAEL determined for non-human primates is 8mg/kg (nucleic acid dose concentration). One fiftieth of the human equivalent dose (HED) is around 0.04 mg/kg. Being that this is a Phase 0 first in human trial, micro-dosing will be utilized with a target dose of 0.04 mg/kg.

NU-0129 is an ideal potential agent for the treatment of GBM for its ability to cross the blood brain barrier giving it potential bioavailability within the tumor bed. Additionally, targeting the tumorigenic Bcl-2 could promote apoptosis and tumor kill for patients treated with NU-0129. Given the preclinical data as well as data from animal studies in rats, mice and non-human primates, it has been shown that NU-0129 successfully crosses the blood brain barrier, successfully induces apoptosis and in the mouse model leads to improved survival in animal models with GBM.

1.4 Exploratory Studies

An exploratory objective will be to assess the level of Bcl2L12 expression in the primary tumor. Expression of Bcl2L12 is upregulated in most GBM patients as higher expression results in resistance to apoptosis. A survival analysis in 343 GBM patients with different levels of Bcl2L12 demonstrated a decreased survival rate correlating with increased expression of Bcl2L12.

2.0 OBJECTIVES & ENDPOINTS

2.1 Primary Objective & Endpoint

- The primary objective is to assess the safety of intravenous NU-0129 in patients with recurrent GBM or GS.
- The total incidence of adverse events will be evaluated using CTCAE v4.03 from the time of informed consent to 21 days after study drug administration. Adverse events will be monitored during and immediately after the infusion as well as weekly for 21 days after treatment administration.

2.2 Secondary Objectives & Endpoints

2.2.1 Pharmacokinetics

- To analyze drug concentration in serum at specific time points after drug administration.
- PK blood samples will be collected post-infusion at 1, 3, 5, 10, 30, and 60 minutes, and 4, 8, and 24 hours post infusion.

2.2.2 Bio-distribution
- To demonstrate intratumoral penetration of NU-0129
- Tissue will be collected during the scheduled surgery and analyzed using ICP-MS

2.2.3 Feasibility
- To assess the feasibility of giving NU-0129 as a standard treatment for recurrent GBM or GS.
- Feasibility will be calculated as the rate of successful production, delivery, and administration of the investigational product and subsequent resection.

2.3 Exploratory Objectives & Endpoints

2.3.1 Bcl2L12 expression levels
To analyze tumor tissue for Bcl2L12 expression levels after NU-0129 administration.
Tissue collected during surgery will be analyzed using RNA analysis techniques. A comparison will be drawn with archival tissue collected at screening for any patients where it is available.

2.3.2 Preliminary response (PFS and OS at 6 months; ORR)
Preliminary response will be measured as an exploratory outcome. Progression Free Survival (PFS) and Overall Survival (OS) will be measured at 6 months after treatment.
Overall Response Rate will also be measured by brain MRI at least every 12 weeks after treatment using RANO criteria

3.0 PATIENT ELIGIBILITY

The target population for this study is patients with recurrent glioblastoma multiforme or gliosarcoma. This will be a single-center trial conducted at Northwestern Medicine.

A total of 6-8 subjects will be needed for this trial. Approximately 8 potentially eligible patients are seen per month, and it is anticipated that at least 1 per month will be accrued. Potential patients may be referred to the Principal Investigator (PI) at Northwestern University, Dr. Priya Kumthekar, at (312) 503-1818.

Eligibility will be evaluated by the study team according to the following criteria. Eligibility waivers are not permitted. Subjects must meet all of the inclusion and none of the exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered. Please refer to Section 11 for complete instructions regarding registration procedures.

3.1 Inclusion Criteria

3.1.1 Patients must have histologically proven glioblastoma multiforme (GBM) or Gliosarcoma (GS).

3.1.2 Patients must have measurable disease by RANO 2010 criteria at the time of registration (pre-operative).

3.1.3 Patients must have failed at least one regimen of chemo or radiation therapy.
NOTE: There is no limit to the number or types of prior therapy.

3.1.4 The patient must be a candidate for surgical debulking (either subtotal or gross total resection). Biopsy-only candidates will not be eligible.

3.1.5 All patients must be capable to voluntarily sign an informed consent indicating that they are aware of the investigational nature of this study prior to registration.

3.1.6 Patients must be ≥ 18 years old.

3.1.7 Patients must have a Karnofsky performance status of ≥ 70.

3.1.8 Patients must have adequate bone marrow, liver, coagulation and renal function within 7days prior to study registration, as defined below:

- WBC ≥ 3,000/µL
- ANC ≥ 1,500/mm^3
- Platelet count of ≥ 100,000/mm^3 (*Note: Transfusion or growth factor may be used for eligibility outside of 7 days)*
- Hemoglobin ≥ 8 g/dL (*Note: Transfusion may be used for eligibility outside of 7 days*)
- Bilirubin ≤2 x ULN
- AST/ALT ≤2 x ULN
- Creatinine ≤1.5 x ULN
- Urine protein ≤ 30 mg/dL
- Cholesterol ≤ 300 mg/dL
- INR ≤ 1.5 x ULN
- PT/PTT ≤ 1.5 x ULN

3.1.9 Any patient who has had a recent surgery should have recovered from all effects of the surgery and be cleared by their surgeon.

3.1.10 Females of child-bearing potential (FOCBP) and males must agree to use adequate contraception (e.g. hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 21 days following completion of therapy. Should a female patient, or a male patient's partner, become pregnant or suspect she is pregnant while participating in this study, the patient should inform her or his treating physician immediately.

NOTE: A FOCBP is any woman (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:
•Has not undergone a hysterectomy or bilateral oophorectomy
•Has had menses at any time in the preceding 12 consecutive months (and therefore has not been naturally postmenopausal for > 12 months)

3.1.11 FOCBP must have a negative pregnancy test (either urine or serum) within 14 days prior to registration.

3.2 Exclusion Criteria

3.2.1 Patients must not have any significant infections or medical illnesses that in the investigator's opinion cannot be adequately controlled with appropriate therapy or would compromise the patient's ability to tolerate NU-0129.

3.2.2 Patients must not have a history of any other cancer unless they are in complete remission and off of all therapy for that disease for a minimum of 3 years.

NOTE: Non-melanoma skin cancer or carcinoma in-situ of the cervix are exceptions and may be permitted after discussion with study QAM.

3.2.3 Patients must not have had radiation therapy ≤12 weeks prior to starting study treatment.

3.2.4 Patients must not have had prior cancer therapy (including biologic, cytotoxic, and experimental therapies, nitrosoureas, and Gliadel wafers or other surgically implantable antitumor treatment) within 21 days of registration. If questions arise, please contact the PI.

NOTE: Patients must not have had Novocure within 24 hours of registration

3.2.5 Hormonal tumor therapies should not be administered within 14 days of registration. Exceptions may be discussed with the PI.

3.2.6 Patients must not have symptomatic hypertension.

3.2.7 Patients with known human immunodeficiency virus (HIV) infection or chronic or acute Hepatitis B or C are not eligible.
Note: Patients do not need to have HIV, Hepatitis B, or Hepatitis C testing at screening.

3.2.8 Female patients who are pregnant or breast feeding are not eligible.

3.2.9 Patients are not eligible if they are unwilling or unable to comply with the protocol.

4.0 TREATMENT PLAN

4.1 Overview

The phase 0 trial will evaluate the safety, pharmacokinetics, and bio-distribution of a systemically (IV) administered SNA gold nanoparticle, NU-0129. Once registered, eligible patients will receive intravenous NU-0129 one time as an inpatient followed by tumor resection within 8-48 hours post-infusion. Patients will be monitored with telemetry, vital signs and labs before and after the infusion as indicated in section 5.0. Bio-distribution will be analyzed with PK blood draws over 24 hours and collection of tissue during the scheduled surgery.

4.2 NU-0129 Administration

Patients will be admitted for a one-time administration of IV NU-0129 at a nominal dose of 0.04mg/kg (approximately 0.03 to 0.05mg/kg), based on the patient's weight at screening. Patients will be dosed based on total body weight and the resulting dose will be rounded to one of the 5 dose groups as shown in dosing chart below.

Patient's weight in kg	Dose in mg (Oligonucleotide component)	Corresponding dose (mg/kg)
Up to 37.0 kg	0.987 mg	Up to 0.027
≥ 37.0 to < 61.7 kg	1.974 mg	0.053 to 0.032
≥ 61.7 to < 86.4 kg	2.96 mg	0.048 to 0.034
≥ 86.4 to < 111.0 kg	3.95 mg	0.046 to 0.036
≥ 111.0 to 135.7 kg	4.94 mg	0.045 to 0.036

The infusion rate will be 5.0-8.0 mL/minute over approximately 20 - 50 minutes. The flow rate will be monitored closely and the tissue surrounding the catheter examined frequently for any signs of extravasation. Patients will be monitored with telemetry from the beginning of the infusion for at least 8 hours post-infusion. Thereafter, vitals will be monitored at least every 4 hours for a total of 24 hours from the end of infusion.

Immediately after the infusion is completed, PK blood draws will take place at 1, 3, 5, 10, 30, and 60 minutes (± 1 minute) post-dose as well as 4, 8 and 24 hours (± 5 minutes)

post-dose. If necessary, blood draws may take place during the patient's resection surgery, however this should be avoided when possible.

Patients will remain inpatient for a tumor resection, occurring 8-48 hours after treatment with NU-0129. Vitals will be measured continuously throughout the surgery. Standard of care post-operative procedures will take place per institutional guidelines, including a brain MRI within 48 hours after the resection. Physician determined response will be determined using RANO (Response assessment in neurology-oncology) criteria. (NOTE: patient response is not expected based on a single sub-therapeutic dosing. In addition, the surgery may confound response assessment.)

4.3 Toxicity Management & Dose Delays/Modifications

Each patient will be assessed for the development of toxicity from the time of consent to 21 days post-dose. Toxicity will be assessed according to CTCAE v4.03. Any patient who receives at least one dose of study therapy will be evaluable for toxicity endpoints.

Patients should be closely observed for infusion-related reactions and any other form of toxicity. Treatment for infusion reactions, and all events, will be based on institutional guidelines. Grading and resolution of the event will be reported using CTCAE v4.03. For example, if a severe infusion reaction occurs, the infusion rate should be cut in half to 2.0-5.0mL/minute.

During the surgical resection, the resected tumor sample should be observed for potential discoloration. The locations and degree of discoloration must be carefully recorded and assessed for relationship to treatment with NU-0129.

There will be no dose delays or modifications as this is a single-dose therapy. However, each patient will be closely monitored for toxicities during the first 21 days after receiving study treatment. Any adverse event that is reported as a Grade 2 toxicity or higher, that is at least possibly related to NU-0129, will be referred to the external DSMB (Data Safety Monitoring Board) for review. The DSMB will review the event within 24 hours of notification to determine a plan for dosing future patients and immediate action if necessary. In addition, each patient's safety data will be reviewed at the end of the 21-day period post therapy and the DSMB will provide a recommendation for future dosing. No additional patients will be registered until the DSMB provides their analysis.

4.4 Concomitant Medications/Treatments

All concomitant therapies must be recorded in the appropriate eCRF and source documents throughout the study, beginning with the time of written informed consent up to 21 days after NU-0129 administration.

4.4.1 Permitted Concomitant Medications

- Standard supportive care therapies needed for the management of symptoms are permitted, as clinically indicated.
- Hormonal birth control is permitted.

4.4.2 Prohibited Medications

The following medications are prohibited during the study:

- Any chemotherapy
- Anticancer immunotherapy
- Experimental therapy
- Radiation therapy

4.4.3 Adjuvant Treatment Post-Surgery

Patients often receive adjuvant treatment post resection as standard of care for GBM or GS. Patients should not receive such adjuvant treatment until at least 21 days after receiving NU-0129. After 21 days, patients may receive “physician’s best choice” treatment.

4.5 Other Modalities or Procedures

Tumor resection surgery is a standard of care procedure that will follow Northwestern Medicine institutional guidelines. The tissue collected during surgery will undergo testing for research purposes, however any other procedures related to surgery are standard of care.

4.6 Duration of Therapy

Within 14 days of study registration, patients will receive intravenous NU-0129 one time at nominal dose 0.04mg/kg. Patients will be admitted the morning of the planned NU-0129 infusion, and a tumor resection will occur 8-48 hours after administration. Patients will remain inpatient for standard of care post-operative procedures, including an MRI of the brain. Discharge will take place when clinically appropriate.

4.7 Duration of Follow Up

Patients will have a routine follow-up visit 7, 14, and 21 days after NU-0129 administration and then at least every 84 days (± 10 days) until disease progression or patient withdrawal for up to 2 years from treatment administration. They will be monitored for toxicities by physical exam and laboratory results as outlined in Table 5.0. They will also undergo a brain MRI at least every 84 days (± 10 days).

4.8 Removal of Subjects from Study Treatment and/or Study as a Whole

Patients can be taken off the study treatment and/or study as a whole at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation must be clearly documented on the appropriate eCRF and may include:

- Patient voluntarily withdraws from follow-up or prior to treatment
- Patient withdraws consent (no follow-up permitted)
- Patient is unable to comply with protocol requirements

4.9 Patient Replacement

If a patient withdraws for any reason after consent or registration but before receiving investigational product, he or she may be replaced.

If a patient receives study drug and does not have a post-treatment surgical resection, or the surgery is delayed beyond 48 hours of treatment for any reason, the patient will be evaluable for toxicity, but will be inevaluable for all other study endpoints; another patient may be added to accrual.

5.0 STUDY PROCEDURES

Table 5.1 – On-Study Procedures (see table 5.2 for follow-up procedures)**

		Inpatient		
Time Period	**Screening[1]**	**Treatment Administration**	**Surgery**	**Post-op[12]**
Informed Consent[1]	X			
Medical history	X			
Physical exam[2]	X			X
Karnofsky PS	X			X
Vitals	X	X[3]	X	
Toxicity assessment	X	X		X[13]
Conmeds	X	X		X
MRI[4]	X			X
CBC with diff[5]	X	X[5]		X
Chemistry panel[5]	X			X
Phosphorus + Mg[5]	X	X		X
PT/PTT/INR[5]	X			X
Cholesterol panel[5]	X			X
Urinalysis	X			
Pregnancy test[6]	X			
ECG	X			X[7]
NU-0129 Infusion[8]		X		
Tumor Resection			X[11]	
PK blood samples		X[9]		
Tissue collection	X[10]			X[11]

[1] All screening procedures must be completed within 14 days of registration unless otherwise noted (Labs and pregnancy test must be within 14 days of registration). Informed consent can be within 28 days prior to registration.

[2] Includes complete neurologic exam and weight. Height will be recorded at screening.

[3] Vital signs should include pulse and blood pressure. On the day of treatment administration, vitals will be measured using continuous telemetry immediately prior to, during, and for at least 8 hours post infusion. Vital signs will then be measured at least every 4 hours for a total of 24 hours post-infusion. Vital signs will also be assessed continuously throughout the patient's surgical resection.

[4] A brain MRI with and without contrast will be performed at baseline (within 21 days of registration) and after surgery as part of standard of care post-op procedures, likely within 48 hours of surgical resection.

[5] Laboratory tests (including, CBC, Chemistry, PT/PTT/INR, Phosphorus, Mg, and Cholesterol panel) must be drawn within 7 days of registration and treatment. If treatment occurs more than 7 days after the indicated labs, they should be repeated. CBC w/ differential should include WBC, ANC, ALC, and Platelets. A complete chemistry panel will be drawn including Sodium, Potassium, Chloride, Bicarbonate, BUN/Creatinine, AST, ALT, Total Bilirubin, Calcium, and Albumin. CBC with diff will be repeated pre-dose on the day of study treatment.

[6] A pregnancy test will be performed for females of child-bearing potential at screening (serum or unine) within 14 days of registration.

[7] An ECG will be performed at screening and only as needed for standard of care toxicity management during post-operative observation.

[8] NU-0129 will be administered once at 0.04mg/kg IV. The infusion will take place as an inpatient 8-48 hours prior to a planned tumor resection.

[9] Blood samples will be collected for PK analysis at 1, 3, 5, 10, 30, and 60 minutes (± 1 minute), and 4, 8, and 24 hours (± 5 minutes) post infusion. See section 9.1 for further details.

[10] Archival tissue will be collected at baseline if the patient has tissue available. See section 9.0 for details.
[11] The patient will have a tumor resection surgery, following institutional procedures, 8-48 hours after NU-0129 infusion. Tumor tissue collected during this surgery will be processed for research analysis as outlined in section 9.0 and the lab manual (provided as a separate document).
[12] Standard-of-care post-operative procedures will take place inpatient, including a brain MRI, physical exam, and KPS after surgery, likely within 48 hours of surgery. All other procedures indicated in Table 5.1, including labs, must be completed post-operatively. Patient should be monitored closely for late onset toxicities and discharged only when clinically appropriate. See table 5.2 for study follow-up procedures.
[13] The tumor should be closely observed for potential discoloration. If discoloration is observed in the tissue, the location, color, and degree of color should be recorded.

Table 5.2 – Follow-Up Procedures

	Follow-Up			
Time from Study Treatment	**Day 7** (± 3 days)	**Day 14** (± 3 days)	**Day 21** (± 3 days)	**q 84 days[3]** (± 10 days)
Physical exam	X	X	X	X
Karnofsky PS	X	X	X	X
Vitals	X	X	X	X
Toxicity assessment	X	X	X	X[5]
Conmeds[1]	X	X	X	X
Tumor Measurements[2]			X[2]	X
CBC with diff	X	X	X	
Complete Chemistry panel	X	X	X	
Phosphorus + Mg	X	X	X	
PT/PTT/INR	X	X		
Cholesterol panel	X	X		
Survival status				X[4]

[1] Concomitant medications will be collected from consent up to 21 days after study drug administration. After 21 days, patients may begin adjuvant treatment for GBM/GS, and such therapies should also be recorded in the patient's study chart.
[2] Patients will have a brain MRI 21 days (± 10 days) after study drug administration (prior to adjuvant therapy, if applicable) and at least every 84 days (12 weeks) thereafter until disease progression for up to 2 years. If a response is suspected (CR or PR), it should be confirmed with a repeat scan four weeks later per RANO criteria 2011 (see section 6.1).
[3] Patients will be followed by routine clinic visit at least every 84 days (12 weeks) for up to 2 years until radiologic disease progression or withdrawal of consent. Post-treatment therapies for GBM/GS during this time should be collected for data purposes.
[4] After progression, patients will be followed for survival, including subsequent therapies for GBM/GS, for up to 2 years post-treatment. OS and PFS will be analyzed at 6 months post-treatment for all patients.
[5] If a patient has a continuing adverse event at 21-days post-treatment, the AE should be followed until resolution to ≤ Grade 2 or baseline

6.0 ENDPOINT ASSESSMENT

6.1 Definitions

6.1.1 RANO Criteria 2010 [18]

Table 6.1. Criteria for Response Assessment Incorporating MRI and Clinical Factors

Response	Criteria
Complete response	Requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions; patients must be off corticosteroids (or on physiologic replacement doses only); and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a complete response; the best response possible is stable disease.
Partial response	Requires all of the following: ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks; no progression of nonmeasurable disease; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan; and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a partial response; the best response possible is stable disease.
Stable disease	Requires all of the following: does not qualify for complete response, partial response, or progression; stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
Progression	Defined by any of the following: ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids* ; significant increase in T2/FLAIR nonenhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (eg, radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects); any new lesion; clear clinical deterioration not attributable to other causes apart from the tumor (eg, seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose; failure to return for evaluation as a result of death or deteriorating condition; or clear progression of nonmeasurable disease.

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

These RANO Criteria are also summarized in Table 6.2:

Table 6.2 Summary of the RANO Criteria

	CR	PR	SD	PD#
T1-Gd +	None	≥50% decrease	<50% decrease but ≤ 25% increase	≥25% increase*
T2/FLAIR	Stable or decrease	Stable or decrease	Stable or decrease	Increase*
New Lesion	None	None	None	Present*
Corticosteroids	None	Stable or decrease	Stable or decrease	NA**
Clinical Status	Stable or improve	Stable or improve	Stable or improve	Deterioration*
Requirement for Response	All	All	All	Any*

CR=complete response; PR=partial response; SD=stable disease; PD=progressive disease

*: Progression when this criterion is met **: Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration

6.2 Primary Endpoint

Safety will be measured by recording adverse events using CTCAE v4.03 from the time a patient signs consent to 21 days after study drug administration. Any patient who has received at least a partial dose of NU-0129 will be evaluable for this endpoint. If a patient has a continuing adverse event at 21 days which is related to treatment, it will be followed until resolution to baseline or Grade ≤ 2.

6.3 Secondary Endpoints

6.3.1 Pharmacokinetics

PK blood samples will be collected post-infusion at 1, 3, 5, 10, 30, and 60 minutes (+/- 1 minute), and 4, 8, and 24 hours (+/- 5 minutes).

6.3.2 Bio-distribution / Tumoral Penetration

A fresh tumor sample will be collected at the time of the patient's tumor resection surgery. It will be assayed with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine accumulated dose.

6.3.3 Feasibility

To assess feasibility, the rate of successful production, delivery, and administration of the investigational product and subsequent resection will be calculated.

6.4 Exploratory Endpoints

6.4.1 Bcl2L12 and apoptotic marker expression levels

An exploratory objective will assess for Bcl2L12 levels in tumor tissue collected post administration of NU-0129 using IHC/IF as well as RT-qPCR. Other apoptotic markers will be analyzed as well using IHC.

6.4.2 Preliminary Response will be measured by Overall Survival and Progression Free Survival at 6 months post treatment as well as Overall Response Rate. Brain MRI's will be performed at least every 12 weeks during follow-up.

7.0 ADVERSE EVENTS

This study will be conducted in compliance with the study-specific standard operating procedure (SOP) and per the DSMP guidelines of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University for in-house research. The level of risk attributed to this study requires High Risk Monitoring as outlined in the SOP. In addition, the study will abide by all safety reporting regulations, as set forth in the Code of Federal Regulations.

7.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (see Section 5 for time points). In addition, certain adverse events must be reported in an expedited manner to allow for optimal monitoring and patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be followed until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

7.2 Definitions & Descriptions

7.2.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

Recording of AEs should be done in a concise manner using standard, acceptable medical terms. In general, AEs are not procedures or measurements, but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Preexisting conditions that worsen in severity or frequency during the study should also be recorded (a preexisting condition that does not worsen is not an AE). Further, a procedure or surgery is not an AE; rather, the event leading to the procedure or surgery is considered an AE.

If a specific medical diagnosis has been made, that diagnosis or syndrome should be recorded as the AE whenever possible. However, a complete description of the signs, symptoms and investigations which led to the diagnosis should be provided. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, “hepatitis” and not “elevated liver function tests” should be recorded. If the cause is not known, the abnormal test or finding should be recorded as an AE, using appropriate medical terminology (e/g/ thrombocytopenia, peripheral edema, QT prolongation).

7.2.2 Severity of AEs

All adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The CTCAE v4.03 is available at http://ctep.cancer.gov/reporting/ctc.html

If no CTCAE grading is available, the severity of an AE is graded as follows:

- Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
- Moderate (grade 2): the event causes discomfort that affects normal daily activities.
- Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (grade 4): the patient was at risk of death at the time of the event.
- Fatal (grade 5): the event caused death.

7.2.3 Serious Adverse Events (SAEs)

All SAEs, regardless of attribution, occurring from time of signed informed consent, through 21 days after the administration of study drug, must be reported upon discovery or occurrence.

An SAE is defined in regulatory terminology as any untoward medical occurrence that:

- Results in *death*.
  If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.
- Is life-threatening.
  The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly/birth defect.
- Is an important medical event.
  Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of “Serious Adverse Event“.
  For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

7.2.4 Unanticipated Problems Involving Risks to Subject or Others

A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:

- Is *unanticipated* in terms of nature, severity, or frequency
- Places the research subject or others at a different or *greater risk of harm*
- Is deemed to be *at least possibly related* to participation in the study.

7.3 Adverse Event Reporting

7.3.1 Routine Reporting

All routine adverse events, such as those that are expected, or are unlikely or definitely not related to study participation, are to be reported on the appropriate eCRF. Routine AEs will be reviewed by the external Data Safety Monitoring Board (DSMB) according to the study’s phase and risk level, as outlined in the DSMP. NU’s Data Monitoring Committee (DMC) will also be informed.

7.3.2 Determining if Expedited Reporting is Required

This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

1) Identify the type of adverse event using the NCI CTCAE v 4.03.
2) Grade the adverse event using the NCI CTCAE v 4.03.
3) Determine whether the adverse event is related to the protocol therapy. Attribution categories are as follows:
   - Definite: AE is clearly related to the study treatment.
   - Probable: AE is likely related to the study treatment.
   - Possible: AE may be related to the study treatment.
   - Unlikely: AE not likely to be related to the study treatment.
   - Unrelated: AE is clearly NOT related to the study treatment.
4) Determine the prior experience of the adverse event.
   Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:
   - the current protocol
   - the drug package insert
   - the current Investigator's Brochure

7.3.3 Expedited Reporting of SAEs/Other Events

7.3.3.1 Reporting to the external DSMB

All SAEs must be reported to the DSMB within 24 hours of becoming aware of the event. The study coordinator must complete the Medwatch and NU SAE Forms, provided as a separate documents, and submit to the appropriate QAM at croqualityassurance@northwestern.edu. The QAM will distribute to the DSMB appropriately.

The completed form should assess whether or not the event qualifies as a UPIRSO. The report should also include:

- Protocol description and number(s)
- The patient's identification number
- A description of the event, severity, treatment, and outcome (if known)
- Supportive laboratory results and diagnostics
- The hospital discharge summary (if available/applicable)

All SAEs will be reported to, and reviewed by, the external DSMB within 24 hours, and will also be reviewed by the NU DMC at their next meeting.

7.3.3.2 Reporting to the Northwestern University IRB

The following information pertains to the responsibilities of the lead site (Northwestern University). Additional participating sites should follow their local IRB guidelines for reporting to their local IRBs.

- Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.
- Any death of an NU subject that is actively on study treatment (regardless of whether or not the event is possibly related to study treatment)

- Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to the NU IRB within 5 working days of notification.
- All other deaths of NU subjects not previously reported, other non-NU subject deaths that were unanticipated and unrelated, and any other SAEs that were not previously reported as UPIRSOs will be reported to the NU IRB at the time of annual continuing review.

7.3.3.3 Reporting to the FDA

The FDA will be notified within 7 calendar days of any SAE that is associated with study treatment, is unexpected, and is fatal or life-threatening.

The FDA will be notified within 15 calendar days of any SAE that is associated with the study treatment, unexpected, and serious but *not fatal or life-threatening*. This includes any previous SAEs that were not initially deemed reportable, but are later determined to meet the criteria for reporting (i.e. by the DMC).

All other SAEs will be reported on an annual basis as part of the annual FDA report.

8.0 DRUG INFORMATION

8.1 Agent NU-0129

8.1.1 Other names

n/a

8.1.2 Classification - type of agent

Bcl2L12-targeting Spherical Nucleic Acid (SNA)

8.1.3 Mode of action

NU-0129 is spherical nucleic acid (SNA) designed to target the silencing of Bcl2L12 gene expression, an overexpressed protein that plays an important role in driving the pathogenesis of glioblastoma multiforme (GBM) and mediating therapeutic resistance. SNAs are three dimensional conjugates consisting of densely functionalized and highly oriented nucleic acids covalently attached to the surface of nanoparticles. NU-0129 is composed of 13nm gold nanoparticles surrounded by siRNAs tailored to recognize Bcl2l12 sequence.

8.1.4 Storage and stability

NU-0129 in powder form should be at -20^{0}C (±5^{0}C) freezer. Once prepared in liquid/solution form, if not used immediately, NU-0129 could be stored up to 8 hours in room temperature (20-25 °C). Remaining or unused solution should be stored at 4ºC. Solution must NOT be frozen.

8.1.5 Protocol dose specifics

Each patient's dose will be based on his or her weight at screening. Patients will be dosed based on total body weight and the resulting dose will be rounded to one of the 5 dose groups as shown in dosing chart below. Nominal dose will be 0.04mg/kg (approximately 0.03 to 0.05 mg/kg).

Patient's weight in kg	Dose in mg (Oligonucleotide component)	Corresponding dose (mg/kg)
Up to 37.0 kg	0.987 mg	Up to 0.027
≥ 37.0 to < 61.7 kg	1.974 mg	0.053 to 0.032

≥ 61.7 to < 86.4 kg	2.96 mg	0.048 to 0.034
≥ 86.4 to < 111.0 kg	3.95 mg	0.046 to 0.036
≥ 111.0 to 135.7 kg	4.94 mg	0.045 to 0.036

8.1.6 Preparation. Refer to pharmacy manual for complete study drug preparation procedures.

- Prior to reconstitution, the vials should be equilibrated to room temperature to reach room temperature for 5-30 minutes. .
- Vials can be stored at room temperature (20-25 °C) for up to 3 hours. This includes thawing time and reconstitution time.
- Vials should not be refrozen after thawing.
- Each NU-0129 manufactured clinical vial should be reconstituted in 1mL SWFI for final concentration approximately 0.987 mg/ml.
- Mix the solution in the vial by gentle swirling or gentle shaking by benchtop sonicator, if available.
- Visually inspect for particulate matter and discoloration prior to administration. Reconstituted solution should red or maroon color, free of visible particles.
- Calculate the dose and the amount of vials required based on the dosing chart.
- Withdraw the full content of the NU-0129 vials and inject into an appropriate size bag of normal saline (0.9% sodium chloride).
- Reconstituted solution should be further diluted with normal saline (0.9% sodium chloride) to 1:50 dilution.
- Preparation should take place immediately prior to administration, but, if needed, reconstituted vials and/or IV bags may be stored at room temperature (20-25 ºC) for up to 8 hours (including administration time).
- Administer study drug using non-PVC/DEHP administration set with a sterile, non-pyrogenic, low-protein binding 0.22 µm filter.
- Visually inspect final product for particulate matter and discoloration prior to administration. Do not use and discard the drug product vial if extraneous particulate matter is observed.

8.1.7 Route of administration for this study
Intravenous

8.1.8 Incompatibilities
Given unknown compatibility data with PVC/DEHP materials, PVC/DEHP-free bags and administration set should be used for drug preparation. Refer to pharmacy manual for a full list of materials acceptable and required.

8.1.9 Availability & Supply
NU-0129 is an investigational product, which will be manufactured and supplied by University of Iowa Pharmaceuticals. The drug product is supplied as approximately 0.987mg per vial lyophilized powder to be reconstituted with SWFI and further diluted with 0.9% sodium chloride by pharmacy on the day of administration. .

8.1.10 Side effects
As this is a first-in-human trial, side effects in this population are not completely known. In the primate and non-primate treatment trials, a few noted side effects were observed. Gold nanoparticle accumulation in the liver has occurred in some pre-clinical studies as well as skin discoloration following the infusion have been seen with animal models displaying a purple hue to their baseline skin

tone. Hypercholesterolemia was also observed in the non-primate model (rat model).

Possible side effects:
Liver Damage
Skin Discoloration
Infusion Reaction
Hypercholesterolemia
Low blood pressure

There is no significant toxicity associated with the chemistry or manufacturing of the drug product.

8.1.11 Nursing implications

The dose (based on dosing chart) will be based on the patient's weight at baseline. The infusion rate will be 5.0-8.0 mL/minute. A sterile, non-pyrogenic, low-protein binding 0.22 µm in-line filter must be used during administration to remove any adventitious particles. If the infusion set does not contain in-line filter, 0.22 µm add-on filter should be used. The flow rate will be monitored closely and the tissue surrounding the catheter examined frequently for any signs of extravasation.
Drug Administration Log should be completed by administering nurse and returned to study coordinator, documenting date and time of infusion start/ stop, interruptions (if any). Any staining or discoloration of the syringe, IV bag, delivery device, and /or administration set should be documented in the Administration Log.

8.1.12 Return and Retention of Study Drug
Drug accountability records will be maintained for all clinical trial supplies. Remaining or unused solution should be stored at 4ºC. Refer to pharmacy manual for management of all unused clinical trial supplies. .

9.0 CORRELATIVES/SPECIAL STUDIES

All patients must provide blood samples for pharmacokinetic analysis as well as tumor tissue during their surgery to assess accumulated dose, expression of BcL2L12 levels, and other apoptotic markers. Archival tissue will be collected at baseline if available.

9.1 Blood Sample Collection Guidelines

Blood samples will be collected for PK analysis at 1, 3, 5, 10, 30, and 60 minutes (± 1 minute), and 4 hours, 8 hours, and 24 hours (±5 minutes) post NU-0129 infusion. Each sample will contain 1mL of blood collected in pre-chilled 4mL K2EDTA tubes and maintained on wet ice throughout collection and processing. See separate lab manual for full collection and processing details.

9.2 Fresh tumor samples

Patients will undergo a tumor resection 8-48 hours after treatment with NU-0129. The mass that is removed during surgery will be collected and processed for the study as detailed in section 9.4.2 below.

9.3 Archival tissue

All patients must provide an archival tissue sample if it is available. This tissue will undergo histology testing for Bcl2L12 and other apoptotic markers as indicated in section 9.4.2.1.

9.4 Sample Processing, Storage, and Shipment

9.4.1 Pharmacokinetic Blood Samples

PK blood samples should be drawn into pre-chilled 4mL K2EDTA tubes and must be immediately chilled on ice and transported to PCF-CTU (Pathcore Facility Clinical Trials Unit). Samples will be spun down within 15 minutes of collection in a temperature-controlled (4ºC) centrifuge. Samples should be immediately flash-frozen on dry ice and stored frozen (-65ºC to -85ºC). The plasma samples will be shipped on dry ice to Covance. See separate lab manual for shipping instructions.

Note: While the patient's tumor resection surgery should ideally be planned around the timing of PK blood draws, it is acceptable for blood draws to take place during surgery. Such cases should be planned closely with the surgical team.

9.4.2 Tissue

All patients will undergo a tissue resection 8-48 hours after NU-0129 administration. The full tissue sample will be sent for pathology processing and samples will be processed and shipped for the study as described below.

In addition, patients will provide an archival tissue sample at screening if it is available. 10 unstained slides will be processed for histological testing as in section 9.4.2.1.

9.4.2.1 Histology (apoptotic markers e.g. caspase)

For both archival and fresh tissue samples, 10 unstained slides will be requested for Bcl2L12 Immunohistochemistry (IHC), Immunofluorescence (IF), TUNEL (Terminal deoxynucleotidyl transferase), and active caspase-3 staining. See separate lab manual for details of processing and analysis.

9.4.2.2 Bcl2L12 expression in tissue (RNA)

A tissue sample (0.5cm x 05cm) will be collected and processed for RNA analysis as detailed in a separate lab manual. Half of the sample will be used for RNA extraction, and the other half will be stored in -80ºC for reserve use.

9.4.2.3 Tissue Bio-Distribution

Any remaining tissue (whether necrotic or not) will be processed by PCF-CTU and shipped frozen to QBIC for bio-distribution studies. Please see lab manual for shipping and processing details.

9.5 Assay Methodology

9.5.1 PK blood samples

See lab manual for details on PK assay methodology.

9.5.2 Tissue – IHC

Tissue samples will be processed for Bcl2L12 IHC, IF, TUNEL, and active caspase-3. See lab manual for details.

9.5.3 Tissue – RNA

Extracted RNA will be frozen and sent to NUSeq Core for RACE analysis.

9.5.4 Tissue – Biodistribution

Remaining tissue will be sent to QBIC for ICP-MS of biodistribution. See lab manual for details.

9.6 Specimen Banking

Any excess tissue should be stored in Northwestern University's biorepository for future unspecified use. Samples will be stored indefinitely until exhausted or the patient withdraws consent for storage.

10.0 STATISTICAL CONSIDERATIONS

10.1 Study Design/Study Endpoints

This is a single group Phase 0 study. A small number n=6-8 of patients will be given the treatment and observed for a fixed period of time.

10.2 Sample Size and Accrual

The sample size n=6, 7, or 8 was determined on the basis of feasibility of recruitment and of conducting the study. This is the first study in humans or this treatment, and it was felt that both the primary and secondary outcomes can be estimated with sufficient precision with such n, while few patients would be put to risk.

10.3 Data Analyses Plans

10.3.1 Primary Objective

To evaluate the safety of NU-0129 SNA gold nanoparticle infusion in patients with recurrent GBM or GS, we will count and record the number of adverse events.

10.3.2 Secondary Objectives

To evaluate the bio-distribution of NU-0129 in tumor tissue we will provide descriptive statistics regarding intensity of concentration of particles in various parts of tumor tissue.

To evaluate the pharmacokinetics of NU-0129 in blood samples collected at 1, 3, 5, 10, 30, 60 minutes and 4, 8, and 24 hours post-dose, we will provide appropriate pharmacokinetic curves and parameters such as Cmax, Tmax etc.

To evaluate the feasibility of NU-0129 administration, we will record a) successes/failures, b) number of attempts to administrate nanoparticles.

10.3.3 Exploratory Objectives

To evaluate the Bcl2L12 expression levels and apoptotic markers in the tumor tissue post-treatment, and to evaluate preliminary response (Overall Survival and Progression Free Survival at 2 years, as well as Overall Response Rate) we will describe expression levels and PFS and OS Kaplan-Meier curves corresponding to in study time up to 24 months.

In general, all patients properly included in the study who undergo surgery for tumor removed will be included in the analysis. Thus, an incorrect treatment schedule or drug administration would result in exclusion from the analysis of the tumor accumulation study. All conclusions will be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for who major protocol deviations have been identified. However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients form the analysis should be clearly reported.

11.0 STUDY MANAGEMENT

11.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

11.2 Amendments

The Principal Investigator will formally initiate all amendments to the protocol and/or informed consent. All amendments will be subject to the review and approval of the appropriate local, institutional, and governmental regulatory bodies. Amendments will be distributed by the lead institution (Northwestern) to all affiliate sites upon approval by the Northwestern University IRB.

11.3 Registration Procedures

For potential patients, study teams are asked to inform the QAM of the date and time that the patient will need to be registered (croqualityassurance@northwestern.edu).

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive an identification number:

- Patient's signed and dated informed consent form (upload to NOTIS and keep original hard copy in a secure location/study chart)
- Eligibility checklist (signed and dated by the treating physician – upload to NOTIS)
- Eligibility eCRF (complete in NOTIS)
- Copy of the pathology report (upload to NOTIS)

The QAM will review all source documentation required to confirm eligibility that is readily available in the patient's electronic medical record (EMR). Any information that is not available in the EMR must be de-identified and emailed to the QAM. Once the QAM confirms the patient is eligible, he or she will register the patient, assign a subject identification number, provide a cohort assignment, and send a confirmation of registration to involved personnel. Registration will then be complete and the patient may begin study treatment.

11.4 Data Submission

Once a subject is confirmed and registered to the study, eCRFs should be submitted according to the detailed data submission guidelines (provided in a separate document in NOTIS).

11.5 Data Management and Monitoring/Auditing

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University in-house research (please refer to NOTIS for additional information). The level of risk attributed to this study requires High Risk Monitoring, as outlined in the DSMP. The assigned QAM, with oversight from the external DSMB and NU Data Monitoring Committee, will monitor this study in accordance with the study phase and risk level. Please refer to the NOTIS for additional data submission instructions.

11.6 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.6.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within 5 business days of making the change, and the QAM must be notified within 24 hours of such change.

11.6.2 Other Protocol Deviations

All other deviations from the protocol must be reported to the assigned QAM using the appropriate form.

A protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs.
- Has no substantive effect on the risks to research participants.
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected.
- Did not result from willful or knowing misconduct on the part of the investigator(s).

A protocol deviation may be considered an instance of Promptly Reportable Non-Compliance (PRNC) if it:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

11.7 Investigator Obligations

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The PI is responsible for personally overseeing the treatment of all study patients. The PI must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected, entered onto the appropriate eCRFs, and submitted within the study-specific timeframes. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. The study may also be subject to routine audits by the Audit Committee, as outlined in the DSMP.

APPENDIX A

Karnofsky Performance Status and Neurological Function

Patient's performance status and Neurologic Functions will be graded according to the following scales:

Karnofsky Performance Status

KPS 100	Normal; no complaints; no evidence of disease
KPS 90	Able to carry on normal activity; minor signs or symptoms of disease
KPS 80	Normal activity with effort; some sign or symptoms of disease
KPS 70	Cares for self; unable to carry on normal activity or do active work
KPS 60	Requires occasional assistance, but is able to care for most personal needs
KPS 50	Requires considerable assistance and frequent medical care
KPS 40	Disabled; requires special care and assistance
KPS 30	Severely disabled; hospitalization is indicated, although death no imminent
KPS 20	Very sick; hospitalization necessary; active support treatment is necessary
KPS 10	Moribund; fatal processes progressing rapidly
KPS 0	Dead

APPENDIX B

Summary of Changes

Amendment 1 – February 2nd, 2017 *Approved by Scientific Review Committee: February 7th, 2017*			
Sections Affected	*Original Version*	*Amendment 1*	*Rationale*
Study Summary; 3.1.8 (Inclusion Criteria)	Listed hemoglobin requirement in mg/dL	Changes units to g/dL	Correction for accuracy
3.1.10 (Inclusion Criteria); 9.3 (Archival tissue)	Patient was required to have archival tissue available prior to study enrollment	Removes requirement for archival tissue	Clarification of discrepancy – archival tissue is only required if available at baseline
3.2.4 (Exclusion Criteria)	"NOTE: Patients must not have Novocure within 24 hours"	"NOTE: Patients must not have **had** Novocure within 24 hour **of registration**"	Clarification
4.9 (Patient Replacement)	If patient receives study drug but surgical resection is delayed or does not occur, patient is inevaluable for endpoints and will be replaced.	Removes "and will be replaced" and states "another patient may be added to accrual".	Since such patients are still evaluable for toxicity endpoints, it is not appropriate to fully replace the patient.
5.0 (Study Procedures, #3)	Vitals were to include respirations	Removes respirations as part of vitals requirement	Not clinically relevant or part of standard care
5.0 (Study Procedures, #10); 9.4.2 (Tissue)	Archival tissue collection is stated as being optional if the patient agrees	Removes optional component of archival tissue collection	Clarification - tissue is required if available, and patients do not need to sign consent
7.3.3.1 (Reporting to the external DSMB)	The Medwatch form was required for SAE reporting	Both Medwatch and NU SAE forms will be required for SAE reporting	Administrative – NU SAE form facilitates internal reports and monitoring
9.0 (Correlatives/Special Studies)	n/a	Adds sentence "Archival tissue will be collected at baseline if available."	Clarification for consistent listing of correlative requirements
11.2 (Amendments)	Protocol amendments were to be reviewed by Janssen Scientific Affairs	Removes reference to Janssen Scientific Affairs	Correction of typo, added inadvertently
11.5 (Data Management and Auditing)	Contained incorrect hyperlink for DSMP	Updates hyperlink for DSMP	Administrative
Amendment 2 – August 1st, 2017 *Approved by Scientific Review Committee: August 17th, 2017*			
Sections Affected	***Original Version***	***Amendment 2***	***Rationale***
Cover Page	Listed "Pre-IND" number	Changes to "IND Number"	Administrative; IND number now available
Study Summary	Contained short title of study protocol	Removes short title	Short title is no longer a template requirement; avoids discrepancies on clinicaltrials.gov
2.1 (Primary Objective & Endpoint); 3.1.10	Immediate follow-up procedures were to take place for up to 28	Immediate follow-up procedures will now take place up to 21 days after	A shorter follow-up window is felt to be clinically appropriate and

(Inclusion Criteria); 4.3 (Toxicity Management & Dose Delays / Modifications); 4.4 (Concomitant Medications / Treatments); 4.4.3 (Adjuvant Treatment Post-Surgery); 4.7 (Duration of Follow Up); 5.0 (Table 5.2); 6.2 (Primary Endpoint); 7.2.3 (SAE's)	days after dosing with NU-0129.	dosing with NU-0129. These include: • Safety (AE's, DLT's and SAE's) • birth control requirements • recording of concomitant therapies • weekly follow-up visits • initial follow-up brain MRI • permission to start adjuvant treatment post-surgery	safe based on prior pre-clinical and clinical data. Since patients with recurrent GBM are progressing at a rapid rate, it is important that the next line of therapy be started as soon as safely possible.
3.2.3 (Exclusion Criteria)	"Patients must not have had radiation therapy within 12 weeks prior to registration"	"Patients must not have had radiation therapy ≤28 days prior to starting study treatment"	Window for prior radiation therapy has been shortened to allow patients on study who are rapidly progressing and in need of more urgent resection. This will not affect the primary outcome of safety.
5.0 (Study Procedures Table 5.1, #2)	Informed consent can be within 28 days "of registration"	Informed consent can be within 28 days "**prior to** registration"	Clarification
5.0 (Study Procedures Table 5.1, #5)	CBC with diff was required at screening and post-operatively	Adds CBC with diff on the day of treatment administration, pre-dose	An additional CBC on the day of treatment allows the team to better determine whether any changes/ fluctuations are actually due to study drug
Amendment 3 – November 1st, 2017 *Approved by Scientific Review Committee:*			
Sections Affected	***Original Version***	***Amendment 3***	***Rationale***
5.0 (Study Procedures, Table 5.1 & 5.2)	• n/a • Magnesium was listed as part of the Chemistry panel • Footnote 12 indicated that post-operative procedures were to take place per standard of care. It was unclear whether labs were required.	• Adds a phosphorus requirement at Screening, Treatment Administration, and Day 7, 15, and 21 of follow-up • Moves magnesium to its own line with phosphorus • Adds language that MRI, physical exam, and KPS will be done per SOC and all other procedures are required	• DSMB recommendation to account for the possibility of toxicities related to phosphorus levels • Clarification; magnesium is a separate lab and was easily missed as previously listed • Clarification of ambiguous language
3.2.3 (Exclusion Criteria)	"Patients must not have had radiation	"Patients must not have had radiation therapy	Extends RT window back to original criteria. There

	therapy ≤28 days prior to starting study treatment"	≤12 weeks prior to starting study therapy"	was concern that shortening the window for RT might impact secondary efficacy endpoints from the original concept.
4.2.3 (Toxicity Management & Dose Delays/ Modifications)	Referred to DLT's occurring within the first 21 days after therapy.	Removes any reference to DLT's and instead refers to just adverse events that are Grade 2 or higher, which will be reported to the DSMB	There are no dose adjustments given that this study involves micro-dosing. Therefore, "DLT" is not felt to be an appropriate term for related toxicities. Toxicities will still be monitored with the same method and caution.
Amendment 4 – March 1st, 2018 *Approved by Scientific Review Committee:*			
Sections Affected	***Original Version***	***Amendment 4***	***Rationale***
3.1.8 (Inclusion Criteria)	Urine protein was required to be ≤ 3x ULN	Updates urine protein requirement to ≤ 30 mg/g	Previous requirement did not make sense since the ULN is 0 or negative. Updates to clinically appropriate upper limit in the opinion of the PI.
5.0 (Study Procedures, #5)	n/a	Adds Phosphorus and Magnesium to footnote 5	Clarification; Phosphorus and Mg should be within 7 days prior to registration
6.0 (Endpoint Assessment)	Contained inaccurate reference to Table 6.1	Updates to reference Table 6.2	Correction of discrepancy
Amendment 5 – December 19, 2018			
Sections Affected	***Original Version***	***Amendment 5***	***Rationale***
2.3.1 (Exploratory Objectives & Endpoints); 9.4.2.2 (Bcl2L12 expression in tissue (RNA)); 9.5.3 (Tissue–RNA)	Tissue collected was to be analyzed using qRT-PCR as part of RNA and Bcl2L12 analysis Section 2.3.1: "Tissue collected during surgery will be analyzed using **qRT-PCR techniques**." Section 9.4.2.2: "A tissue sample (0.5cm x 05cm) will be sent to **Pathcore's Molecular Laboratory**. Half of the sample will be used for RNA extraction, and the other half will be stored in -80ºC for	Removes qRT-PCR and refers more vaguely to "RNA analysis" or "RACE" techniques Section 2.3.1: "Tissue collected during surgery will be analyzed using **RNA analysis techniques."** Section 9.4.2.2: "A tissue sample (0.5cm x 05cm) will be collected **and processed for RNA analysis as detailed in a separate lab manual**. Half of the sample will be used for RNA extraction, and the other half will be	Samples will have RNA analyzed using techniques which will no longer include qRT-PCR

	reserve use. **Extracted RNA will be stored in -80ºC until PCF transfers it to NUSeq, who will then perform RT-qPCR on the RNA samples.**" Section 9.5.2: "Extracted RNA will be frozen and sent to NUSeq Core for **RT-qPCR** analysis"	stored in -80ºC for reserve use." Section 9.5.2: "Extracted RNA will be frozen and sent to NUSeq Core for **RACE** analysis"	
3.1.8 (Inclusion Criteria)	Urine protein cutoff was listed with units in mg/g	Updates urine protein cutoff to reflect units in mg/dL	Correction of error; md/dL is the standard unit for urine protein levels
5.0 (Study Procedures)	The window for baseline pregnancy test was inconsistent throughout the protocol	Updates study procedures to match the eligibility criteria for pregnancy test window; pregnancy test is required within 14 days of registration	Correction of discrepancy
9.1 (Blood Sample Collection Guidelines)	Specific details were included outlining tube types, processing instructions and locations "Blood samples will be collected for PK analysis at 1, 3, 5, 10, 30, and 60 minutes (± 1 minute), and 4 hours, 8 hours, and 24 hours (±5 minutes) post NU-0129 infusion. Each sample will contain 1mL of blood collected in pre-chilled 4mL K2EDTA tubes **(additional 5.4mg/mL of K2EDTA should be added)** and maintained on wet ice throughout collection and processing. **Samples are to be spun down within 15 minutes of blood collection in a temperature-controlled (4ºC) centrifuge"**	Removes details on processing and location of analysis for correlative samples "Blood samples will be collected for PK analysis at 1, 3, 5, 10, 30, and 60 minutes (± 1 minute), and 4 hours, 8 hours, and 24 hours (±5 minutes) post NU-0129 infusion. Each sample will contain 1mL of blood collected in pre-chilled 4mL K2EDTA tubes and maintained on wet ice throughout collection and processing. **See separate lab manual for full collection and processing details**."	By removing specific information from the protocol and limiting it to the lab manual, we aim to avoid unnecessary protocol amendments / deviations in the case of changes to analysis methods.

Section 9.4.2.1 (Histology (apoptotic markers e.g. caspase); Section 9.5.2 (Tissue - IHC)	Protocol references NU Pathcore's Histology IHC lab for where histology will be performed	Removed specific details on location of analysis and refer to separate lab manual for details.	By removing specific information from the protocol and limiting it to the lab manual, we aim to avoid unnecessary protocol amendments / deviations in the case of changes to analysis methods.

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