PROTOCOL TITLE:	Prospective, randomized, blinded, and placebo-controlled study of multi-stage bioelectric therapy for cancer tumor treatment in a murine glioma xenograft model
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Background

Glioblastomas are estimated to account for over 80% of primary malignant tumors in the Central Nervous System (CNS), result in death within five years of diagnosis in approximately 10% of patients, and subject patients to tremendous discomfort due to the toxic side effects of chemotherapy. Current innovative therapies using alternating electric fields have shown promise in the eradication of tumors without side effects and provide important evidence of a transition from invasive therapies with devastating side effects to objective, patient-specific, and precisely targeted therapies without side effects.

Bioelectric stimulation is a non-invasive, safe, and painless procedure that has shown promise in the inhibition of tumor growth across a number of in vitro and in vivo models of cancer. By adjusting frequency and other key tuning parameters of the stimulation, this provides an opportunity to objectively assess the tumor before treatment, deliver communication-jamming and blood-starving bioelectric signals to the tumor to inhibit growth, and ultimately eradicate the effected region to allow transition to brain regeneration.

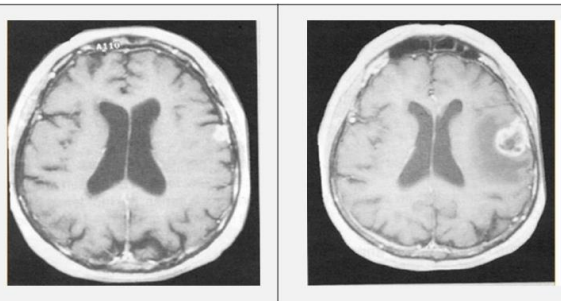
In this study, we will measure the effects of a multi-stage bioelectric therapy on a murine xenograft model of cancer to determine the optimal parameters using this approach. Bioluminescence imaging (BLI) and caliper measurements will be used to track tumor growth from two weeks post-inoculation of cancerous cells to four weeks post-inoculation. We expect a significant decrease in tumor size in the treatment condition when compared to the sham condition. Results showing a decrease in tumor size in the treatment arm will provide important evidence moving forward to determine optimal frequency and tuning parameters of multi-stage bioelectric therapy.

Glioblastoma

Glioblastoma (GBM) is the most common malignant tumor of the CNS. GBM accounts for 46% of primary malignant brain tumors, and its aggressive behavior presents unique treatment challenges.[1] After maximal safe surgical resection, the conventional standard of care is radiotherapy with concomitant temozolomide followed by maintenance temozolomide.[2] Despite aggressive multimodal therapy, the prognosis of patients with GBM is poor; historically, the 2-year survival rate is 27%, and only 10% of patients live longer than 5 years.[3] Therapeutic options are limited after progression following initial treatment, and clinical trials of investigational systemic therapies in the past decade have failed to improve outcomes of patients with GBM.4-7

GBM tumors are derived from glial cells, the most common cell type in the CNS. Glial cells and neuronal cells, the other category of CNS cells, work in concert to produce a functional nervous system. Neuronal cells work to transmit signals to and from the brain, while glial cells act as the "glue" of the CNS. Glial cells help to maintain homeostasis, generate myelin, and support and protect neuronal cells.8 Different types of glial cells include astrocytes, oligodendrocytes, and Schwann cells. When a glial cell becomes cancerous, it develops into a glioma, or a glial-derived tumor. GBM is derived specifically from astrocytes, and is therefore a type of astrocytoma.9

Brain imaging studies are performed in order to show the presence, size, and location of the GBM. The most common brain imaging study used in the diagnosis of GBM is gadolinium-enhanced magnetic resonance imaging (MRI). GBM is most



Initial Assessment | 68 days later

Figure 1. MRI of patient with glioblastoma showing rapid progression two months after initial assessment.

visible in T1-weighted MRIs and differences between white and gray matters are visible because of changes in contrast.10

Brain cancer therapies

Current standard treatments for GBM include surgery, radiotherapy, and chemotherapy. Alternative techniques including Tumor-treating fields (TTFields) and Nano-pulse stimulation (NPS) have emerged showing promise in eradicating tumors without side effects or harm to

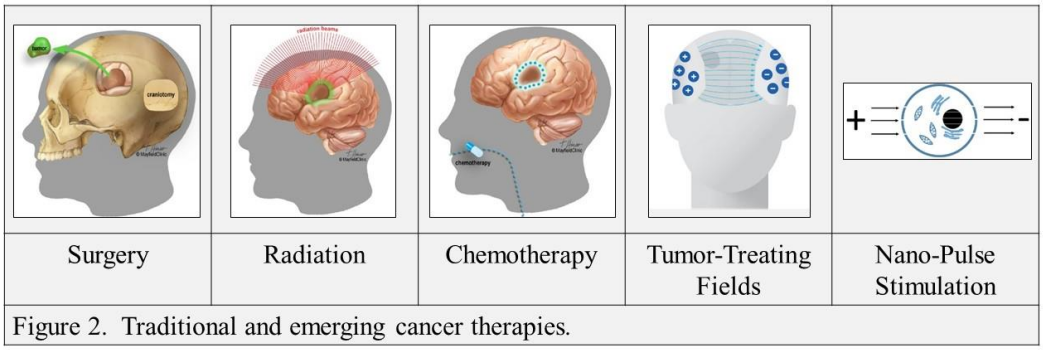


Figure 2. Traditional and emerging cancer therapies.

existing healthy cells.

Although some advances have been made in recent years, treatment remains palliative for most patients as a cure remains elusive. Particular challenges to treating GBM are its distinct tumor heterogeneity, the inability of treatments to reach all tumor cells, and the high likelihood of relapse, which is often rapid and aggressive.

Traditional cancer therapies

Surgery. Surgical resection is performed with the intent for a complete removal of the GBM tumor. If a complete resection is impossible due to the location of the tumor, a partial resection may be performed; however, partial resection is associated with significantly lower survival rates.

Radiation. Radiation therapy uses controlled high-energy rays to damage the DNA inside cells making them unable to divide and reproduce.[10] There are two ways to deliver radiation, external and internal beams. External beam radiation is delivered from outside the body by a machine that aims high-energy rays at the tumor. Stereotactic radiosurgery (SRS) delivers a high dose of radiation during a single session.[11] Fractionated stereotactic radiotherapy (FSR) delivers lower doses of radiation over many visits.[12] Whole brain radiotherapy (WBRT) delivers the radiation dose to the entire brain.[13] Internal radiation (brachytherapy) is delivered from inside the body by surgically placing radioactive material (sealed in catheters, seeds, or balloons) directly into the tumor.[14] The radiation dose is delivered to the first few millimeters of tissue that surrounded the tumor cavity where malignant cells may still remain.

Chemotherapy. Chemotherapy drugs work by disrupting cell division. Over time, chemotherapy causes the abnormal cells to die and the tumor may shrink. Treatment is delivered in cycles with rest periods in between to allow the body to rebuild healthy cells.[15] Chemotherapy drugs can be taken orally as a pill, intravenously (IV), or as a wafer placed surgically into the tumor. Some chemotherapy drugs are applied locally to the tumor bed after the tumor has been removed. By applying it directly to the diseased area of the brain, side effects are limited and the drug has a more beneficial effect. The current standard chemotherapy used is Temozolomide or Temodar. Temozolomide is an oral alkylating agent, and inhibits DNA repair mechanisms in tumor cells.[16]

Alternative brain cancer therapies

Tumor-Treating Fields. Tumor-treating fields (TTFields) is an approved treatment for patients with GBM that inhibits tumor cell growth by blocking cell division and replication. TTFields delivers low-intensity, intermediate-frequency (200 kHz) alternating electric fields via transducer arrays applied to the shaved scalp [17-24]. In the randomized Phase III trial (EF-14; NCT00916409) of TTFields in newly diagnosed patients with GBM after initial treatment with standard chemoradiation, the combination of TTFields plus maintenance temozolomide significantly improved progression-free survival (PFS) and overall survival (OS) compared with temozolomide alone in a prespecified interim analysis. Based on the observed survival benefits, the Independent Data Safety Monitoring Board recommended termination of the study.[22] TTFields is the first intervention in a decade to improve survival in patients with newly diagnosed GBM and was recently approved by the US FDA for use in combination with temozolomide

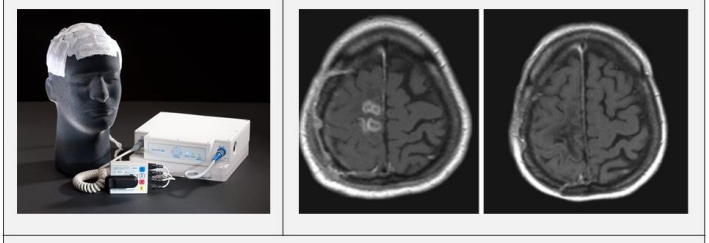


Figure 3. (Left) Novocure Optune device. (Right) MRI of before and after 6-month treatment.

Nano-pulse stimulation. Nano-pulse stimulation (NPS) pulses are applied directly to tissue, creating a transient opening of small pores in cell and organelle membranes. Result have shown that by controlling the disruption of the cellular organelles, the cellular response can be directed.[25-30] For the treatment of cancer, it is believed that this treatment signals a cascade within the tumor cells that ends in immunogenic apoptosis. Immunogenic apoptosis is a process in which cells are induced to die in a natural way, initiating their own programmed cell death,

engaging the immune system to clear damaged, diseased, or aged cells and enrolling cytotoxic T cells to recognize and eliminate cells of the same tumor type.

Next-generation brain cancer therapies

Multi-stage bioelectric therapy. CancerCell is a patented platform for reading cancer tumors in real-time and custom delivery of individualized bioelectric therapy.

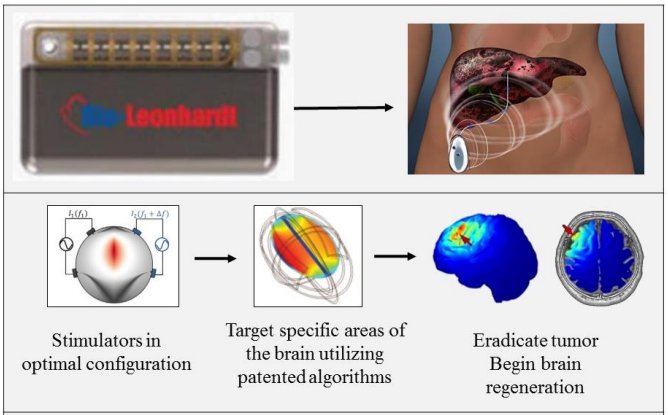


Figure 4. Multi-stage bioelectric therapy. (Top) Implantable lead with simulated therapy for liver cancer. (Bottom) Next-generation targeting of glioblastoma using advanced algorithms.

Multi-Stage Bioelectric Therapy
1. Bioelectric and cytokine reading of cancer tumor
2. Customized jamming of communications
3. Starving blood supply
4. Changing surface proteins and electrical charge to enable the person's own body to attack tumor (immunotherapy)
5. Stopping cell division
6. Inflammation management and modulation
7. Stem cell and protein expression based organ regeneration

Figure 5. Description of 7-step process for multi-stage bioelectric therapy.

Specific aim and hypotheses

Specific aim. Using bioluminescence imaging and caliper measurements, validate the efficacy of a multi-stage bioelectric therapy on tumor growth inhibition in a murine glioma xenograft model.

Hypothesis 1. For caliper measurements, it is expected that the treatment group condition will yield significantly lower measurements when compared to the sham group condition.

Hypothesis 2. For bioluminescence imaging, it is expected that the treatment group condition will yield significantly lower luminoscores when compared to the sham group condition.

Materials

Mouse strain. NSG NOD scid gamma (00557) will be acquired from Jackson Laboratory, shipped to UCLA Crump Institute, and then allowed to acclimate for three to seven days in the animal facility prior onset of experimental procedures.

Cancer cell line. Bioware Brite Cell Line - GL261 Red-FLuc. Codon-optimized luciferase from Luciola Italica (RedLuc) with a red-shifted emission peak wavelength of 617 nm (as compared to 550 nm [Luc] and 590 nm [Luc2]) and approximately 100-fold higher signal intensity compared to the other firefly luciferases.[32]

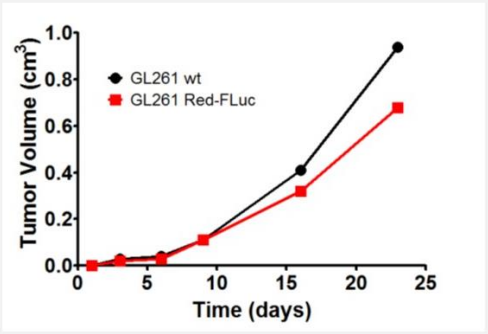


Figure 6. Tumor growth curve of GL261 Red-Fluc cancer cell line used in this study.

Tumor Model. An Ectopic tumor xenograft model will be used with subcutaneous injection of cells into the flank of the rodent.[31] Subjects will be inoculated with cancer cells shortly after arrival at UCLA Crump Institute.

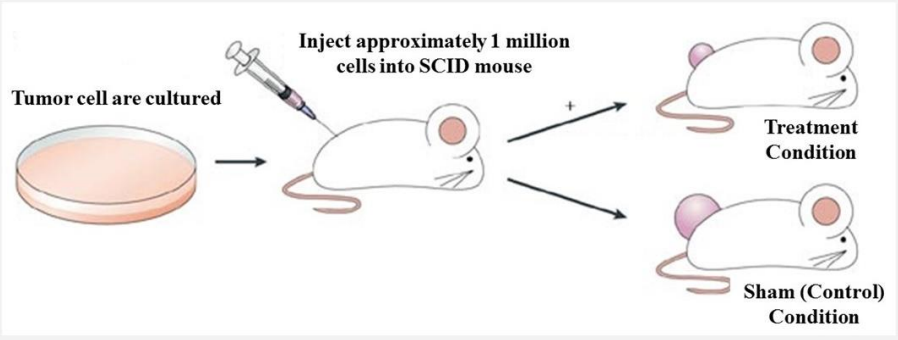


Figure 7. Xenograft model of murine cancer. Subcutaneous inoculation of tumor cells into the flank of the mouse followed by treatment or sham condition.

Bioelectric stimulation. The proposed treatment will be applied by means of (XX)-mm long pairs of wires/electrodes with leads (XX dimensions). They will be placed intradermally on the back of a mouse. Tumors will be innoculated intradermally in between the pair of implanted wires/electrodes with a separation of X mm between the pairs. A signal generator coupled with a voltage amplifier is set to apply electrical stimulation (stimulation parameters here; XX Hz, XX Volts, XX ns or ms, monophasic/biphasic) via the wire/electrode pair to tumors. Mice in the sham (control group) will have the wire/electrode setup, but will not receive therapy. Mettler, Rigol, or other suitable stimulator will be used for the study.

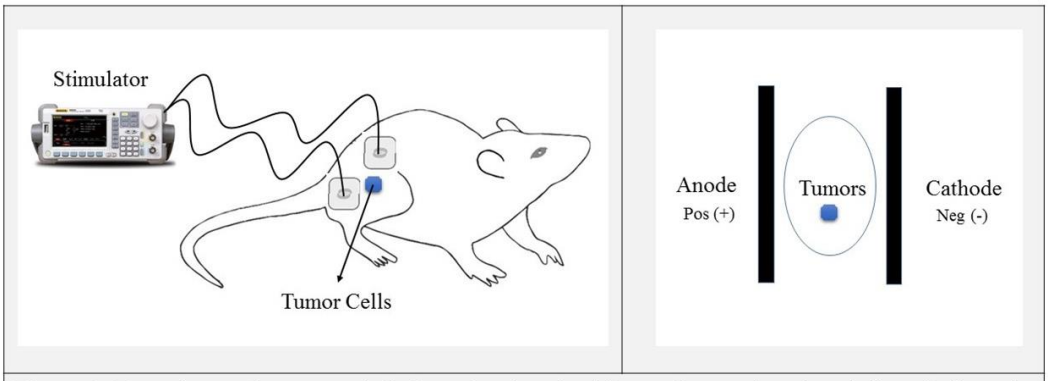


Figure 8. Experimental setup and design. Anode wire/electrode may be placed directly into the tumor or adjacent to the tumor (adjacent is Novocure animal methods).

Procedure

Schedule. The study will begin approximately 14 days post-inoculation of cancerous cells. Expected tumor size will be approximately 400 mm^3 based on growth rate curves from validation studies.

Day 0: Mice arrive from Jackson Labs
Day 3: Mice engrafted with tumor cells at UCLA
Day 17: Bioluminescence imaging; caliper measurement
Day 17: Treatment/sham 40 minutes
Day 20: Bioluminescence imaging; caliper measurement
Day 20: Treatment/sham 40 minutes
Day 24: Bioluminescence imaging; caliper measurement
Day 24: Treatment/sham 40 minutes

Day 27: Bioluminescence imaging; caliper measurement
Day 27: Treatment/sham 40 minutes
Day 31: Bioluminescence imaging; caliper measurement

All possible efforts will be made to minimize animals' suffering and the number of animals used. All experimental procedures will be conducted under the guidelines set forth by the UCLA and IACUC.

Outcome Measures

Bioluminescence. Over the past decade, in vivo bioluminescent imaging has emerged as a non-invasive and sensitive tool for studying ongoing biological processes within living organisms.[33-34] Based on the detection and quantitation of the photons produced by the oxidation of luciferin by luciferase enzymes, this technique has proved to be particularly useful in analyzing cancerous cells and monitoring tumor growth, providing a cost-effective insight into how the disease progresses in vivo, without the need of serial sacrifice of animals. Bioluminescence will be utilized in this study providing (1) the biodistribution of antibodies, (2) the distribution of immune cells in tumor-bearing animals, (3) quantification of cancer-related biomarkers, and (4) size and location of tumors. The luminescence, which is the consequence of the photon flux emitted by the luciferase-expressing cells, directly correlates to the size of the tumor and can be measured at the site of injection using a region of interest (ROI) tool.[35-41]

Caliper measurement. To determine tumor volume by external caliper, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were determined. Tumor volume based on caliper measurements were calculated by the modified ellipsoidal formula.[42-43]

Projected Results

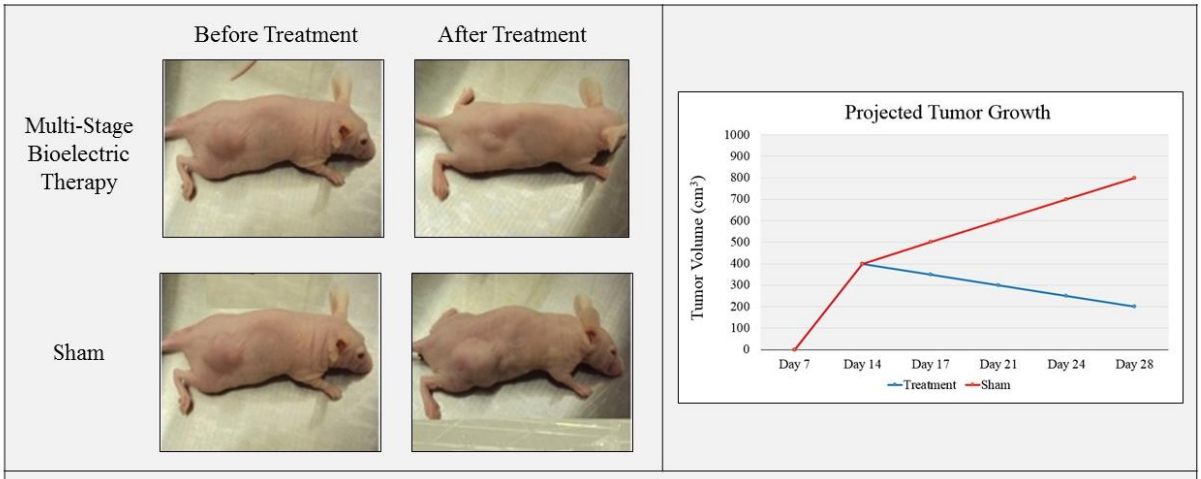


Figure 9. (Left) Projected visual observation results showing significant tumor reduction in treatment group and significant tumor growth in sham group. (Right) Projected tumor growth curves for treatment group and sham group, as determined by caliper measurements.

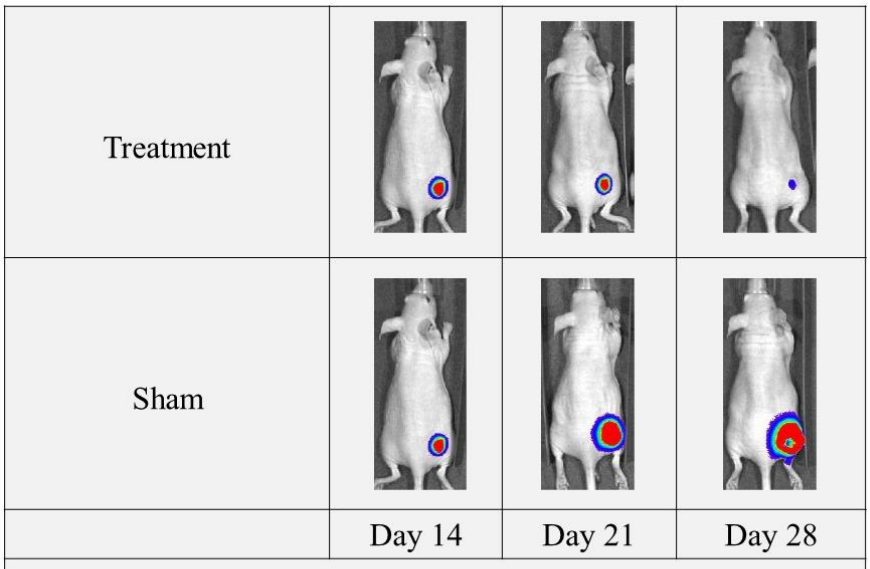


Figure 10. Projected results from bioluminescence imaging showing significant tumor signal intensity reduction in treatment group versus signal intensity in sham group.

Power analysis

Sample Size Calculations for Means					
	Anticipated Values				
	Mean	Stan. Dev			
Group 1	200	50			
Group 2	800	200			
		Sample Size Needed in Each Group			
alpha level			Power		
("p" value)		95%	90%	80%	50%
0.10		1	1	1	0
0.05		2	1	1	0
0.02		2	2	1	1
0.01		2	2	1	1

Figure 11. Optimistic power estimate. 6 rodents per group considered minimum; 8+ rodents per group closer to standard.

Costs

CancerCell Project Budget
Partnership with UCLA Crump Institute

		Number/Hours	Amount	Total
A.	**Mouse Strain**			
	Jackson NSG scid gamma (00557)	16	200	$3,200
B.	**Cell Line**			
	Bioware Brite Cell Line - GL261 Red-FLuc	1	3712	$3,712
	D-Luciferin	1	244	$244
C.	**UCLA Services**			
	Bioluminescent imaging scanner use	10	220	$2,200
	Animal handling, caliper measurement, etc.	25	150	$3,750
	Faculty support (design, amendments, training, analysis)	10	230	$2,300
D.	**Total Costs**			**$15,406**

Figure 12. Projected budget.