Postoperative Infection May Influence Survival in Patients With Glioblastoma: Simply a Myth?

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BACKGROUND: It is a prevalent myth that a postoperative infection may actually confer a survival advantage in patients with malignant glioma. This contention is based largely on anecdotal reports. Recently, a single-center study showed there was no survival advantage in those patients who had glioblastoma with postoperative infection.

OBJECTIVE: To examine the impact of postoperative infections on outcome in patients with glioblastoma treated at our center.

METHODS: This study included 197 patients with newly diagnosed primary glioblastoma treated from January 2001 to January 2008. Of the 197 patients, 10 (5.08%) had postoperative bacterial infection. The Kaplan-Meier method, log-rank test, and Breslow test were used in the univariate approach; Cox regression was used in the multivariable approach.

RESULTS: The median survival was 16 months (95% confidence interval [CI], 14-18 mo). The infection group had a significant advantage in the median survival: 30 months (95% CI, 21-39) vs 15 months (95% CI, 13-17) for patients without postoperative infection. This advantage was also confirmed by Cox regression; in fact, patients not developing a postoperative infection showed an adjusted hazard ratio for death of 2.3 (95% CI, 1-5.3).

CONCLUSION: The association between infection and prolonged survival is not definitive; we acknowledge the considerable difficulties in undertaking this type of study in a retrospective manner. Our results can instead stimulate further multicentric studies (to increase the number of patients) or experimental studies using genetically modified bacteria for treatment of glioblastoma.

KEY WORDS: Glioblastoma, Infection, Outcome, Surgical infection, Survival

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Glioblastoma (GBM) is the most devastating and commonest of all primary brain tumors. It carries a poor prognosis with a median survival of approximately 14 months even with aggressive multimodality therapy.[1] Aggressive tumor removal, multimodal therapy, younger age, high Karnofsky Performance Score (KPS), and MGMT methylation are factors associated with longer survival.[2,3] It has long been stated, in various reports throughout the years, that having an infection within or near the resection cavity after removal of a brain tumor can actually stimulate the immune function of the patient and promote a longer survival. This contention has been based largely on anecdotal reports and has never been substantiated. Recently, Bohman et al[4] attempted to provide substantiation via a single center study examining the role of postoperative infection on patient outcome. They showed that there was no survival advantage in those patients who had GBM with postoperative infection.

The aim of our study was to examine the impact of postoperative infections on the outcome of patients with GBM treated at our center.

ABBREVIATIONS: CI, confidence interval; **GBM,** glioblastoma; **KPS,** Karnofsky Performance Score

PATIENTS AND METHODS

Patients >18 years of age with histologically confirmed newly diagnosed primary GBM (World Health Organization grade IV astrocytoma) from January 2001 to January 2008 at the Catholic University,

Rome, Italy, were evaluated for eligibility. All patients gave written consent for the processing of personal data for research purposes. Patient characteristics included sex, age, preoperative KPS (assessed the day before surgery), operation(s) performed, extent of surgical resection (ie, gross total [defined as >98%], subtotal/partial), hospital course, and follow-up records (including complications and adjuvant treatment received). Surgical resection extent was determined by comparison of postoperative images obtained up to 72 hours (contrast-enhanced magnetic resonance imaging [MRI] or computed tomography [CT] scans) after surgery with the latest preoperative images. In some cases (especially from 2001-2004), postoperative CT/MRI was performed only before radiation therapy was started (ie, between 3 and 4 weeks after surgery). Follow-up data were obtained by the outpatient section of the Radiation Therapy Department, where patients were followed up initially once a week until they completed adjuvant therapy and then every 3 months. All patients were followed up until death or July 5, 2010, whichever came first. The study included 203 consecutive cases; among them, 6 patients (2.96%) were lost to follow-up immediately after surgery and were excluded from the final analysis because we decided to include only patients who had performed at least the first outpatient visit at the Radiation Therapy Department, which was performed within 15 to 20 days after surgery for all patients. The final cohort therefore comprised 197 patients.

Postoperative infection was defined as wound/bone flap infection, cerebral abscess, or meningitis, most occurring within 2 months after surgery. The presence of infection was suspected with clinical evidence (neurological deterioration, fever, rigor nucalis, infected surgical wound) and imaging (MRI with gadolinium and diffusion images/contrast-enhanced CT scan; Figure 1). Cultures were available in almost all cases (Table 1). Ten of the 197 patients (5.08%) had postoperative bacterial infections, as described previously. Nine of 10 patients had received surgery followed by radiation therapy and chemotherapy; 1 patient refused adjuvant treatment.

Statistical Analysis

Overall survival and 95% confidence interval (95% CI) were calculated in months from the initial diagnosis to death/last follow-up visit. Survival curves were obtained by the Kaplan-Meier method; the log-rank test and the Breslow test were used to compare survival in groups identified in agreement with the following variables: age (<65 or ≥65 years), adjuvant therapy (complete, incomplete, and not received), resection extension (total, partial), KPS (≤70, >70), and postoperative infections (yes, no). Backward stepwise selection was used to choose prognostic factors for a Cox proportional hazards regression model. Only factors with a value of $P < .25$ in the univariable analyses were included in the multivariable analyses. The hazard ratio and its 95% CI were calculated for each factor in the presence of others in the final model.

The significance level was set at P .05. The statistical software used for analyses was SPSS 11.0 for Windows.

RESULTS

The sample was represented by 197 patients (116 men, 81 women; mean age, 58 years; range, 21 80 years). Among them, 75 (38.1%) were ≥65 years of age, and 71 (36.0%) had a KPS ≤70. One hundred three patients (52.3%) underwent total re section of the tumor, and 106 patients (53.8%) completed the adjuvant treatment.

Median overall survival for all 197 patients was 16 months (95% CI, 14 18 months). In total, 171 patients (86.9%) died; overall cumulative survival probabilities at 1, 2, and 3 years were 45%, 24%, and 12%, respectively.

The Kaplan Meier analyses showed a significant difference in survival between patients developing a postoperative infection and patients not developing a postoperative infection (Breslow

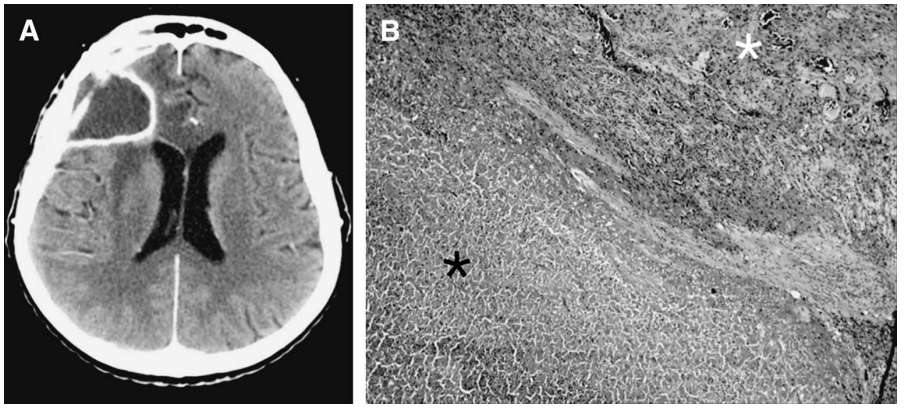

FIGURE 1. **A**, *head CT scan showing intense ring enhancement of an abscess capsule at the site of a previous surgical bed.* **B**, *surgical specimen of patient 7 (hematoxylin and eosin staining) showing the presence of inflammatory cells/purulent material (left; black asterisk) and the presence of tumor (right; white asterisk).*

TABLE 1. Summary of Patients With Postoperative Infection[a]

Patient	Age at Diagnosis, y	Site	Surgery	Adjuvant Therapy	Infection Type	Bacterium	Survival, mo
1	55	Left frontal	August 2001 (gross total resection)	RT + CT (PCV)	Abscess	Gram positive	23
			January 2002 (partial resection)				
			February 2002 (abscess)				
2	28	Right frontoparietal	April 2001 (subtotal resection)	RT (60 Gy) + CT (TMZ) + RIT Y DOTATOC	Abscess	Gram positive	30
			June 2001 (abscess)				
			April 2003 (partial resection)				
3	59	Right frontal	January 2000 (total resection)	RT (60 Gy) + CT (TMZ)	Surgical wound/ bone/abscess	*Staphylococcus* aureus, *Staphylococcus* epidermidis	103[b]
			January 2000 (abscess/wound)				
			December 2000 (radionecrosis)				
4	65	Left temporoparietal	October 2001 (total resection)	RT + CT (carmustine)	Surgical wound/bone	*S. epidermidis*	23
			October 2001 (wound revision)				
			March 2002 (radionecrosis)				
5	41	Left temporoparietal	September 2008 (total resection)	RT + CT (TMZ)	Abscess	Not available	20[b]
			September 2008 (abscess)				
6	66	Right frontoparietal	July 2008 (total resection)	None	Abscess	*S. aureus*	18
			August 2008 (abscess)				
7	33	Right frontal	August 2008 (partial resection)	RT (60 Gy) + CT (TMZ)	Surgical wound/ bone/abscess	*S. aureus*	22[b]
			October 2008 (abscess)				
8	26	Left frontal	May 2005 (total resection)	RT (8Gy) + CT (TMZ) + RIT Y DOTATOC + Cyber Knife 18 Gy	Surgical wound/ bone/abscess	*S. aureus*	54
			July 2005 (abscess)				
			October 2006 (radionecrosis)				
			February 2007 (subtotal resection)				
			April 2009 (partial resection)				
9	38	Left parietooccipital	July 2007 (total resection)	RT + CT (TMZ)	Surgical wound/bone	*S. aureus*	34[b]
			July 2007 (wound revision)				
			October 2008 (CSF shunt)				
10	56	Left parietal	September 2006 (total resection)	RT (60 Gy) + CT (TMZ 1 cycle)	Abscess	*S. aureus, Pseudomonasaeruginosa, Escherichia coli*	30
			January 2007 (partial resection)				
			February 2007 (abscess)				

[a]CT, chemotherapy; PCV, procarbazine/lomustine (CCNU)/vincristine; RIT, radioimmunotherapy; RT, radiation therapy; TMZ, temozolomide.
[b]Alive at last follow-up.

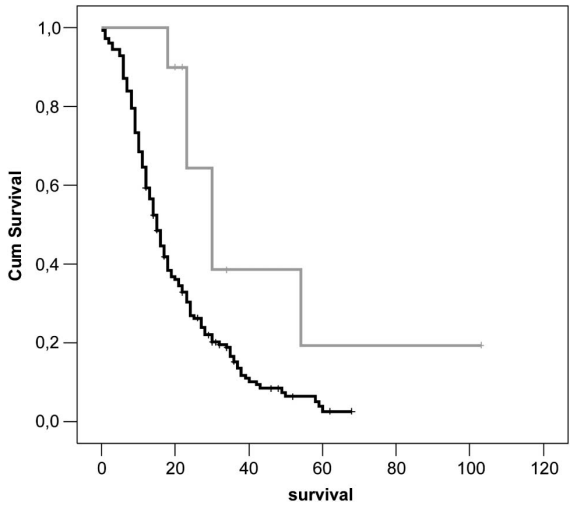

FIGURE 2. *Kaplan-Meier curves showing a significant difference of survival between patients with (gray line) and without (black line) postoperative infection (median survival time, 30 months [95% confidence interval, 21-39] vs 15 mo [95% confidence interval, 13-17]). Breslow test,* P < *.01; log-rank test,* P .01.

test, $P < .01$; log rank test, $P = .01$; Figure 2); median survival time of patients with and without infection was 30 months (95% CI, 21 39) and 15 months (95% CI, 13 17), respectively. Cu mulative survival probabilities at 1, 2, and 3 years for patients with and without postoperative infection were 100% and 42%, 67% and 22%, and 37% and 10%, respectively.

Significant differences were also shown between patients re ceiving total or partial tumor resection (Breslow test, $P < .01$; log rank test, $P < .01$; respective median survival time, 23 months [95% CI, 18 27] and 11 months [95% CI, 9 12]), between patients with KPS > 70 or ≤ 70 (Breslow test, $P < .01$; log rank test, $P < .01$; respective median survival time, 21 months [95% CI, 17 24] and 10 months [95% CI, 8 11]),

TABLE 2. Multivariable Cox Regression Analyses[ab]

	Hazard Ratio	95% CI	*P*
Subtotal/partial tumor resection	1.9	1.4 to 2.7	<.001
KPS ≤70	2.0	1.5 to 2.8	<.001
No postoperative infection	2.3	1.0 to 5.3	.04

[a]CI, confidence interval; KPS, Karnofsky Performance Score.
[b]Multivariable Cox regression analyses suggested that 3 risk factors were associated with an increased risk of mortality among 197 primary glioblastoma patients: a subtotal/partial tumor resection (reference group, total tumor resection), a KPS ≤70 (reference, KPS >70) and no postoperative infection (reference, patients without a postoperative infection).

and among patients receiving complete, incomplete, and no adjuvant treatment (Breslow test, $P < .01$; log rank test, $P < .01$; respective median survival, 21 months [95% CI, 17 25], 13 months [95% CI, 10 15], and 10 months [95% CI, 8 12]).

Younger patients (<65 years of age) had a longer survival (median survival, 17 months; 95% CI, 14 19) than patients ≥65 years of age (14 months; 95% CI, 11 17), but no significant differences were shown.

The final Cox regression model showed that patients who underwent subtotal/partial tumor resection, patients with a KPS ≤ 70, and patients not developing a postoperative infection were significantly at risk for death (Table 2).

All patients had infections during the first quarter of their overall survival period (Table 1). Organism analysis showed a high prevalence of *Staphylococcus aureus* (6 cases, 60%). Five patients presented a surgical abscess; 3 patients had both abscess and surgical wound infection; and 2 patients had surgical wound and bone flap infection requiring surgical revision. The analysis of survival by pathogen and by site of infection did not show any significant differences; the sample was too small to definitely conclude anything on the prognostic role of these variables.

DISCUSSION

In the past, infection was the major contributor to morbidity and mortality rates, occurring after almost all operations.[5] Nowadays, in neurosurgical interventions, although the use of antibiotic prophylaxis could diminish the amount of infections, 0.8% to 7% of patients receiving a clean major craniotomy still develop this complication.[6] After neurosurgical procedures, in fection most commonly presents as meningitis, empyema, wound/bone infection, or cerebral abscess. In these cases, mul tiple operations are often required.[7]

One of the myths that continues to be perpetrated in neu rosurgery relates to the observation that a postoperative infection may actually confer a survival advantage in patients with ma lignant glial tumors. Until 2009, there had been only case reports and reviews of patients undergoing surgery for primary malignant brain tumors who experienced prolonged survival after infection. Five of these tumors were grade III astrocytomas (survival: >10 years, 10.5 years, >9.75 years, 18 months, and >9 years), and only 2 cases were GBMs. Survival was 8 years among patients with grade III gliomas; the 2 patients with GBM survived 5 and 10 years, thus suggesting a strong prognostic impact of infection on survival.[8 12]

In 2009, Bohman et al[4] published a retrospective review of 17 GBM patients with postoperative infections over a 10 year period at a single institution. These patients were compared with an age matched group of 51 patients with GBM treated over the same time period. The 2 groups (cases and controls) were similar with respect to age and resection extent. However, other prognostic factors such as KPS status and type of adjuvant therapy received were not considered. Overall, no correlation between postoperative infection and prolonged average lifetime could be detected,

although subgroup analysis of patients with deep infections showed a trend toward longer survival. Thus, that retrospective single center study offered, for the first time, a higher level of evidence of this "myth," propelling its findings outside the realm of anecdote and opinion.

Similarly, we retrospectively analyzed patients affected with GBM over a long observation period and examined the potential relationship between postoperative infection and prognosis. In contrast with Bohman et al,[4] our evidence showed a significant correlation between postoperative infection and prolonged survival. Eight of 10 patients in our series had a deep infection. Comparison of outcome and patients with wound/bone infection was therefore not possible.

Many authors formulated hypotheses about the role and influence of biological mechanisms put in action by infections on the natural history of a primary brain tumor. Bohman et al[4] previously pointed out that having an infection within or near the surgical bed after tumor removal can actually stimulate the patient's immune function and promote a longer survival. A biological rationale that relates to the capacity of bacterial infections to induce increased immunity and to evoke a cascade of cytokines and chemokines, some of which also possess anticancer properties, has been provided. Another hypothetical mechanism could be pointed out as a sort of antagonistic proliferation: local competition for growth and survival between tumor cells and other replicating microorganisms such as bacteria. Therefore, either local competition for growth and survival between tumor cells and other replicating microorganisms or local stimulation of immune function resulting from infection may be detrimental to the tumor. Biotechnology is attempting to translate this rationale into a therapy by using genetically modified bacteria to treat cancer.[13] Future trials using controlled doses and applications of these microorganisms thus may be able to answer the question related to the perceived advantages of bacterial growth against tumors.

The main limitation of our study is its retrospective, nonrandomized nature (these studies may overestimate the effect of treatments because of attrition, detection, or performance bias) and the limited number of patients with a postoperative infection. In our study, many of these biases were avoided because $<3\%$ of patients were lost to follow up, the outcome and prognostic variables were standardized, and the completeness and quality of the data were carefully checked. Another problem with this study is that patients did not receive the same adjuvant treatment because standards of care varied during this long period. Most patients (from 2003 on) received temozolomide. Others received carmustine/lomustine/cisplatin, procarbazine/lomustine/vincristine, intraoperative carmustine wafers, radioimmunotherapy, and recently avastin. Moreover, patients with and without infection were temporally equally distributed, which limited differences in the treatment received and in follow up length. Another potential source of bias is treatment after recurrence, but this is a problem for all studies on the prognosis for GBM. Finally, we do not know the MGMT methylation status of most patients because this method has been available in our institution since 2007.

The take home message of this study, which can be applied to any aspect of neurosurgery, is to do everything possible to prevent a postoperative wound or cavity infection. The association between infection and prolonged survival is not definitive; we acknowledge the considerable difficulties in undertaking this type of study in a retrospective manner in view of the numerous clinical variables. A prospective randomized study on this subject is clearly not possible. Nevertheless, we believe the results of this study are important and can be used as a stimulus for further multicentric studies (to increase the number of patients) or for experimental studies using genetically modified bacteria for the treatment of GBM.

Disclosure

The authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

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Acknowledgment

We wish to acknowledge D. Petricca for preparing figures.

COMMENT

This article provides a provocative counterpoint to the study of Bohman et al,[1] which found no statistically significant survival advantage in glioblastoma patients with postoperative infection. De

Bonis et al provide a retrospective single-center analysis of a smaller patient sample (197 vs 382 in the Bohman et al study) of which 10 patients developed postoperative bacterial infection for a relatively high incidence of 5.1%. Among these 10 patients, a significant advantage was found, consisting of an overall survival of 30 months vs 15 months in the patients without infection. Because of the small patient numbers, the authors were unable to provide subgroup analyses (eg, pathogen type, time to infection, deep vs bone flap infections). This, of course, limits the reach of the authors' conclusion but is appropriately acknowledged as a limitation of their study. In the end, the relationship between postoperative infection and overall survival in GBM patients remains unclear. Although the authors make a significant contribution to the literature, major questions remain, including whether survival advantage is influenced by certain bacterial organisms or by deep infections vs bone flap infections. Ultimately, a need remains for significantly larger retrospective studies that may offer more definitive answers.

Simon Hanft
Jeffrey N. Bruce
New York, New York

1. Bohman LE, Gallardo J, Hankinson TC, et al. The survival impact of postoperative infection in patients with glioblastoma multiforme. *Neurosurgery*. 2009;64(5):828-834.

Toledo

Having been populated since the Bronze Age, Toledo, a municipality located in central Spain, grew in importance during Roman times. After the fall of the Roman Empire, Toledo served as the capital city of Visigothic Spain, beginning with Liuvigild, and was the capital of Spain until the Moors conquered Iberia in the 8th century.

After the fall of its golden age, Toledo's population turned overwhelmingly Muladi, and, because of its central location in the Iberian Peninsula, the city took a central position in the struggles between the Muslim and Christian rulers of northern Spain. On May 25, 1085, Alfonso VI of Castile took Toledo and established direct personal control over the Moorish city from which he had been exacting tribute, ending the medieval Taifa's Kingdom of Toledo. This was the first concrete step taken by the combined kingdom of Leon-Castile in the Reconquista by Christian forces. After Castilian conquest, Toledo continued to be a major cultural center; its Arab libraries were not pillaged, and a tag-team translation centre was established in which books in Arabic would be translated from Arabic or Hebrew to Spanish by Arab and Jewish scholars, and from Spanish to Latin by Castilian scholars, thus letting long-lost knowledge spread through Christian Europe again.

From: Rudolph Schrot [rudolph.schrot@ucdmc.ucdavis.edu]
Sent: Monday, October 11, 2010 9:11 AM
To: Eric Mah
Subject: Fw: [redacted]

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----- Forwarded by Rudolph Schrot/PHY/HS/UCD on 10/11/2010 09:11 AM -----

From: "Riggins, Patrick" <Patrick.Riggins@fda hhs.gov>

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Date: 06/11/2008 02:59 PM

Subject: RE: [redacted]

Dr. Schrot,
As requested in the discussion between you, me, and Dr. Przepiorka, last Friday June 6, below are the comments that I had relayed to you regarding what animal studies will be necessary prior to entering into the clinic with your proposed therapy.

"The safety of the intended clinical product should be evaluated in an appropriate preclinical study. The preclinical study design should include the following:
1. The intended clinical product should be administered into a site that mimics clinical (i.e., intracranial)

2. Several dose levels that bracket the proposed clinical dose level, should be used - this means several dose groups of animals

3. An appropriate (vehicle) control group of animal should be included.

4. The dosing schedule should mimic clinical.

5. At least 5 animals/group sacrifice interval should be included.

6. The immune status of the animals should reflect clinical, if possible.

7. Scheduled sacrifices that are 1) within 24 hours post-last dose and 2) several week post-last dose should be included.

8. Study endpoints should include clinical signs, body weights, general appetite, hematology, chemistry, gross pathology, histopathology (CNS & non-CNS tissues), measurement of presence of E. aerogenes in the brain & other target tissues.

9. Unscheduled deaths should be carefully examined (microscopically & otherwise) for cause of death.

10. Data should be collected for each animal on study for each study parameter.

11. A report should be submitted that includes study methodology, study results (animal data), and the sponsor's analysis & interpretation of the data."

Additionally, Dr. Przepiorka further pointed out that these animal studies must show not only safety, but also establish a reasonable proof of concept in order for this investigational therapy to be introduced into [redacted] patients.

If you have any further questions about any of this, please do not hesitate to call or email.

Regards,
Pat Riggins

Patrick S. Riggins, Ph.D.
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Dr. Schrott:

Thank you for providing a literature review, and product purchase information along with the pertinent clinical history of your [redacted] patient. I have spoken with Dr. Stephanie Simek, Deputy Division Director of the Office of Cellular, Tissue and Gene Therapies in CBER and also have forwarded your email to her. She is going to review the materials and provide you with regulatory guidance regarding single patient treatment IND or possibly other considerations.

Please look forward to being contacted by Dr. Simek or someone else from her staff who she may involve in this review. Also, please do not hesitate to contact me back as needed.

Again, thank you for seeking input from FDA.

Sincerely,

Sara

Sara F. Goldkind, MD, MA
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-----Original Message-----
From: Rudolph Schrot [mailto:rudolph.schrot@ucdmc.ucdavis.edu]
Sent: Wednesday, May 14, 2008 7:05 PM
To: Goldkind, Sara
Cc: Gould, Barbara; John Anderson; David Asmuth; Alexander Kon; J.paul Muizelaar; Stuart Cohen
Subject: Re: [redacted] experimental protocol

Dear Dr. Goldkind:

We have at UC Davis Medical Center in Sacramento a [redacted] with a pontine glioblastoma who has undergone resection. [redacted] tumor has rapidly grown back. The prognosis for this disease is dismal, with median survival
of about 1 year. Treatment options are limited. (Wagner 2006)

There have been some case reports of spontanous disappearance of high grade
gliomas following coincidental gram negative intracranial infections, particularly with E. aerogenes. (see the reference by Bowles 1999). This
is stimulated some interest in the antitumor effect of LPS in the bacterial
cell walls, and there has been some basic science research in this regard,
(Chicoine 2001) but no effective treatments have resulted. Current xenograft mouse models are immunocompromised, which may mitigate an immune-mediated antitumor effect.

[redacted] are very interested in exploring any possible treatment option which might offer a glimmer of hope. We propose the following protocol: PROBIOTIC INTRACRANIAL THERAPY FOR MALGNANT GLIOMA. This would involve stereotactically injecting an antibiotic-sensitive strain of Enterobacter aerogenes in the vicinity of the tumor and inducing
a controlled intra/peritumoral infection. We have proposed the protocol to
the Ethics Committee at UC Davis Medical Center who have given approval pending IRB review. My understanding from the communication from Dr. Nelson is that we would work throught CBER within the category of single patient treatment IND. The bacterial strain would be obtained from the American Type Culture Collection (ATCC).

Any assistance or guidance in this process would be greatly appreciated. I
can be reached on my cell phone at (916) 712-1706. Thank you.

Sincerely,

Rudy Schrot

(See attached file: Bowles1999.pdf)(See attached file: Wagner2006.pdf)(See
attached file: chicoine2001.pdf)

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05/14/2008 02:07 PM

cc

Subject Re: [redacted] experimental protocol

Dr. Schrot,

If the product you plan to use is available to you, I would suggest you proceed under the strategy of innovative treatment rather than research. I am not familar with the literature on what you propose. If it os only available under IND, you would need to work through CBER. There is a category of single patient treatment IND. [redacted] I am copying Sara Goldkind at FDA who could also help you negotiate the FDA process if this is the direction you decide to go.

Robert "Skip" Nelson, MD PhD
Pediatric Ethicist, Office of Pediatric Therapeutics
Office of the Commissioner, Food and Drug Administration
Tele: 301-827-1522, Fax: 301-827-1017
E-mail: Robert.Nelson@fda.hhs.gov

----- Original Message -----
From: Rudolph Schrot <rudolph.schrot@ucdmc.ucdavis.edu>
To: Nelson, Robert 'Skip' *
Sent: Wed May 14 15:57:46 2008
Subject: [redacted] experimental protocol

Dear Dr. Nelson:

I spoke with Ms Gould who referred me to you. We have at UC Davis Medical Center a [redacted] with a malignant glioma, s/p surgical resection. The tumor involves the brain stem and pons. The prognosis for this histology is dismal, with median survival of 9-12 months. There are some isolated case reports of tumors which have regressed and led to long term survival after concurrent infection with gram negative bacteria. [redacted] are aware of these reports and are looking for any therapy which might offer a glimmer of hope [redacted] We are proposing an experimental protocol for a single patient: PROBIOTIC INTRACRANAL THERAPY FOR MALIGNANT GLIOMA.

This would involve the installation of Enterobacter aerogenes in the vicinity of the tumor in a controlled fashion.

I wonder if you might be able t ome insight as to how to procede with the IRB application (what [redacted] category would this be?) We have
already had an ad hoc ethics committee meeting at UC Davis Medical Center
which has given us the thumbs up, pending IRB approval.

Thanks so much.

Rudy Schrot

Rudolph J. Schrot, MD
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[redacted] (cell)

Rudolph Schrot/PHY/HS/UCD
06/09/2008 09:07 AM

To John Anderson/PHY/HS/UCD@UCDavis
cc "ahakimelahi" <ahakimelahi@ucdavis.edu>, Elodia Tarango/SOM/HS/UCD@UCDavis, Mihaela Harris/AMB/HS/UCD@UCDavis, Derick
bcc
Subject Re: Fw: [redacted] experimental protocol

John,
I really appreciate all your help and support on this. It looks like I have my work cut out. I know there is a regulation regarding emergency use of a new drug (or biologic) when the IND requirements cannot be fulfilled. Not sure if this might apply here. The FDA people did not mention this, so it sounds like this isn't going to fly this time around.
Rudy
John Anderson
----- Original Message -----

From: John Anderson
Sent: 06/09/2008 08:54 AM PDT
To: Rudolph Schrot
Cc: ahakimelahi@ucdavis.edu; Elodia Tarango; Mihaela Harris; Derick Lau; Victoria Bradley; David Asmuth
Subject: Re: Fw: pediatric experimental protocol

Rudy,

I would interpret this to mean that the FDA (and us) won't allow this product to be used in humans without further testing. Though likely devastating for the family, there is logic to this determination. I would recommend you check with the company to see the extent of testing in animals (and humans - though this is unlikely). Conceivably, there is some information/ prior testing. You could check for an alternative vendor or other researchers that may be doing the same or similar work. It may be that you will have to consider doing some of the background work.

John

Rudolph Schrot/PHY/HS/UCD

Rudolph Schrot/PHY/HS/UCD
06/06/2008 04:12 PM

To John Anderson/PHY/HS/UCD@UCDavis
cc
Subject Re: Fw: [redacted] experimental protocol

John,
I had a phone conference today with the FDA. There are very stringent criteria for any biologic to be tested in a human, which include a list of about 9 criteria which need to be satisfied in an animal model. This would need to include the testing of Enterobacter aeogenes in a mouse model of brain tumor. There is no such data currently available, and the FDA cannot approve this trial in a human. I'm not sure where this leaves us at this point.
Rudy

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(cell)
John Anderson/PHY/HS/UCD

John Anderson/PHY/HS/UCD
06/05/2008 10:59 AM

To Elodia Tarango/SOM/HS/UCD, ahakimelahi@ucdavis.edu
cc David Asmuth/PHY/HS/UCD@UCDavis, Rudolph Schrot/PHY/HS/UCD@UCDavis
Subject Re: Fw: [redacted] experimental protocol

Lody,

I believe that this protocol will fall under a single patient treatment IND (with Rudy being the one holding the IND - it would be highly unlikely that the company providing the bacteria would have any interest in pursuing this). The time window that Rudy is working under is limited - He wants to give the agent as soon as possible and has tentatively scheduled it for next tuesday. He hasn't indicated that he has heard back from the FDA yet. It isn't clear to me that we can get it through the full committee on first pass (it is complicated, time is limited prior to the monday meeting, the reviewers won't have much time to review the protocol, Rudy will be rushed trying to complete the protocol). Given the urgency of initiating treatment - I would be inclined to have it go forward even without full committee final approval (assuming the FDA doesn't have expressed problems with this). It would still be appropriate for it to go to full committe for the purpose of additional potential patients. I have also advised Rudy that the IRB isn't the only one that needs to be notified at this institution; I would assume he also needs to get some buy off from the hospital/ med staff (ie possibly credentialing, risk management, departmental, investigational pharmacy, microbiology/epidemiology - assure agent is pure without contaminations etc). From my own informal evaluation, I know that Rudy has been doing some of this work already - ie he has had conversations with Stu Cohen and his departmental chair and senior neurosurgeons are aware of the intent to use the agent.

If the FDA gives the go ahead, and we proceed with a single patient treatment IND, and we don't have final full committee approval, we should proceed in a fashion similar to what we have with the pulmonary groups studies. Rudy will still need to have some form of consent for the experimental portion (in addition to the standard operative consent form). Additionally, I would advise him to have a written concurrence of a separate independent physician [he has had it evaluated by an ethics committee]. We (myself or Dave), would need to write a concurrence letter. It would still be advisable to proceed with the full committee evaluation - particularly for future participants.

To finally answer your question from the email - I would be happy to have it go through Committee B on monday with me as a reviewer if it is practicable (ie they are able to get a protocol to us that can be reviewed).

John

Elodia Tarango/SOM/HS/UCD

Elodia Tarango/SOM/HS/UCD

To david.asmuth@ucdmc.ucdavis.edu,

06/05/2008 09:57 AM

cc john.anderson@ucdmc.ucdavis.edu

Subject Fw: [redacted] experimental protocol

Another thought, Monday is a Committee B meeting. If the protocol is brought over to me today, soon, I can have it scanned and sent to the IRB members. I would then have Mihaela, with the help of Jeff, contact the IRB members to ask them to open their e-mails with this study.

Dr. Anderson, this is your call. I leave today at 4 PM, so does Mihaela, so I would like to work on this as early today as I can.

Lody
Elodia Tarango
Assistant Director
IRB Administration
University of California, Davis
Phone: (916) 703-9154 Fax: (916) 703-9160
E-Mail: elodia.tarango@ucdmc.ucdavis.edu
CTSC Bldg., 2921 Stockton Blvd.
Suite 1400, Rm. 1429, Sacto, CA 95817
www.research.ucdavis.edu/IRBAdmin

----- Forwarded by Elodia Tarango/SOM/HS/UCD on 06/05/2008 09:54 AM -----

Elodia Tarango/SOM/HS/UCD
06/05/2008 09:32 AM

To David Asmuth/PHY/HS/UCD

cc john.anderson@ucdmc.ucdavis.edu

Subject RE: [redacted] experimental protocol

Drs. Asmuth and Anderson: Would this qualify for emergency use concurrence in a life-threatening situation rather than submitting a full blown protocol?

Lody
Elodia Tarango
Assistant Director
IRB Administration
University of California, Davis
Phone: (916) 703-9154 Fax: (916) 703-9160
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David Asmuth/PHY/HS/UCD

David Asmuth/PHY/HS/UCD
06/04/2008 09:58 PM

To Rudolph Schrot/PHY/HS/UCD@UCDavis
cc connie.koog@ucdmc.ucdavis.edu, elodia.tarango@ucdmc.ucdavis.edu, rhildreth@ucdavis.edu, Silvana.Hughes@ucdmc.ucdavis.edu
Subject RE: [redacted] experimental protocol

yes, you will need to fill out a full committee application. I have cc'd and spoken to both Connie and Rayanne [Hildreth] asking that they assist in the preparation as you need. We have room on the agenda 2 weeks from today if the FDA has given their blessing and the application can be completed.
good luck
David

Rudolph Schrot/PHY/HS/UCD

Rudolph Schrot/PHY/HS/UCD
06/04/2008 03:54 PM

To David Asmuth/PHY/HS/UCD@UCDavis
cc connie.koog@ucdmc.ucdavis.edu, elodia.tarango@ucdmc.ucdavis.edu, rhildreth@ucdavis.edu, Silvana.Hughes@ucdmc.ucdavis.edu
Subject RE: [redacted] experimental protocol

Thanks Dr. Asmuth.

I spoke to John Anderson about this today. I am filling out a full-committee application, although I'm not sure if this is the right way to go or not, since we are just really treating a single patient for a condition in an unconventional way. I would welcome your thoughts on this. I am waiting to hear back from the FDA, CBER.

Rudy

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[redacted] (pager)
[redacted] (cell)
David Asmuth/PHY/HS/UCD

David Asmuth/PHY/HS/UCD
06/04/2008 03:46 PM

To Rudolph Schrot/PHY/HS/UCD@UCDavis
cc Silvana.Hughes@ucdmc.ucdavis.edu, connie.koog@ucdmc.ucdavis.edu, elodia.tarango@ucdmc.ucdavis.edu, rhildreth@ucdavis.edu
Subject

RE: [redacted] experimental protocol

let me know how/if we [the IRB] can help
David M. Asmuth, M.D.
Division of Infectious & Immunologic Diseases
UC Davis, Medical Center
4150 V Street, PSSB G500
Sacramento, CA 95817
(916) 734-8695 (office)
(916) 734-7766 (fax)
(916) 734-8527 (lab office)
(916) 734-8532 (lab)
david.asmuth@ucdmc.ucdavis.edu

Rudolph Schrot/PHY/HS/UCD

Rudolph Schrot/PHY/HS/UCD
06/04/2008 12:59 PM

To "Simek, Stephanie" <stephanie.simek@fda.hhs.gov>
cc "Alexander Kon" <alexander.kon@ucdmc.ucdavis.edu>, "Gould, Barbara" <barbara.gould@fda.hhs.gov>, "David Asmuth" <david.asmuth@ucdmc.ucdavis.edu>, "John Anderson" <john.anderson@ucdmc.ucdavis.edu>, "Goldkind, Sara" <sara.goldkind@fda.hhs.gov>, "J.paul Muizelaar" <j.paul.muizelaar@ucdmc.ucdavis.edu>, "Nelson, Robert 'Skip' *" <Robert.Nelson@fda.hhs.gov>, "Stuart Cohen" <stuart.cohen@ucdmc.ucdavis.edu>
Subject RE: [redacted] experimental protocol

Dear Dr. Simek:

Attached is an outline of the clinical protocol we propose for intracranial bacterial therapy for malignant glioma as well as a literature review. I have provided more detail than I previously sent.. I have been contacted by Dan Takesman as well as Pat Riggins, and if you could forward this to them, that would be great. Thanks.

[attachment "Protocol.doc" deleted by Rudolph Schrot/PHY/HS/UCD]

Sincerely,

Rudolph J. Schrot, MD
Assistant Professor
Department of Neurological Surgery
University of California at Davis

4860 Y Street, Suite 3740
Sacramento, CA 95817
916-734-8824 (office)
[redacted] (pager)
[redacted] (cell)
"Goldkind, Sara" <sara.goldkind@fda.hhs.gov>

"Goldkind, Sara" <sara.goldkind@fda.hhs.gov>

05/15/2008 05:43 AM

To "Rudolph Schrot" <rudolph.schrot@ucdmc.ucdavis.edu>

cc "Gould, Barbara" <barbara.gould@fda.hhs.gov>, "John Anderson" <john.anderson@ucdmc.ucdavis.edu>, "David Asmuth" <david.asmuth@ucdmc.ucdavis.edu>, "Alexander Kon" <alexander.kon@ucdmc.ucdavis.edu>, "J.paul Muizelaar" <j.paul.muizelaar@ucdmc.ucdavis.edu>, "Stuart Cohen" <stuart.cohen@ucdmc.ucdavis.edu>, "Simek, Stephanie" <stephanie.simek@fda.hhs.gov>, "Nelson, Robert 'Skip' *" <Robert.Nelson@fda.hhs.gov>

Subject RE: [redacted] experimental protocol

Dr. Schrott:

Thank you for providing a literature review, and product purchase information along with the pertinent clinical history of your [redacted] patient. I have spoken with Dr. Stephanie Simek, Deputy Division Director of the Office of Cellular, Tissue and Gene Therapies in CBER and also have forwarded your email to her. She is going to review the materials and provide you with regulatory guidance regarding single patient treatment IND or possibly other considerations.

Please look forward to being contacted by Dr. Simek or someone else from her staff who she may involve in this review. Also, please do not hesitate to contact me back as needed.

Again, thank you for seeking input from FDA.

Sincerely,

Sara

Sara F. Goldkind, MD, MA
Senior Bioethicist, Good Clinical Practice Program
Office of the Commissioner, Food and Drug Administration
Tel: 301-827-0428; Fax: 301-827-1169
Email: sara.goldkind@fda.hhs.gov

-----Original Message-----
From: Rudolph Schrot [mailto:rudolph.schrot@ucdmc.ucdavis.edu]
Sent: Wednesday, May 14, 2008 7:05 PM
To: Goldkind, Sara
Cc: Gould, Barbara; John Anderson; David Asmuth; Alexander Kon; J.paul Muizelaar; Stuart Cohen
Subject: Re: [redacted] experimental protocol

Dear Dr. Goldkind:

We have at UC Davis Medical Center in Sacramento a [redacted] with a pontine glioblastoma who has undergone resection. [redacted] tumor has rapidly grown back. The prognosis for this disease is dismal, with median survival
of about 1 year. Treatment options are limited. (Wagner 2006)

There have been some case reports of spontanous disappearance of high grade
gliomas following coincidental gram negative intracranial infections, particularly with E. aerogenes. (see the reference by Bowles 1999). This
is stimulated some interest in the antitumor effect of LPS in the bacterial
cell walls, and there has been some basic science research in this regard,
(Chicoine 2001) but no effective treatments have resulted. Current xenograft mouse models are immunocompromised, which may mitigate an immune-mediated antitumor effect.

[redacted] are very interested in exploring any possible treatment option which might offer a glimmer of hope. We propose the following protocol: PROBIOTIC INTRACRANIAL THERAPY FOR MALGNANT GLIOMA. This would involve stereotactically injecting an antibiotic-sensitive strain of Enterobacter aerogenes in the vicinity of the tumor and inducing
a controlled intra/peritumoral infection. We have proposed the protocol to
the Ethics Committee at UC Davis Medical Center who have given approval pending IRB review. My understanding from the communication from Dr. Nelson is that we would work throught CBER within the category of single patient treatment IND. The bacterial strain would be obtained from the American Type Culture Collection (ATCC).

Any assistance or guidance in this process would be greatly appreciated. I
can be reached on my cell phone at [redacted]. Thank you.

Sincerely,

Rudy Schrot

(See attached file: Bowles1999.pdf)(See attached file: Wagner2006.pdf)(See
attached file: chicoine2001.pdf)

Rudolph J. Schrot, MD
Assistant Professor
Department of Neurological Surgery
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916-734-8824 (office)
[redacted] (pager)
(cell)

"Nelson, Robert

'Skip' *"

<Robert.Nelson@fd

To a.hhs.gov>

<rudolph.schrot@ucdmc.ucdavis.edu>, "Gould, Barbara"

05/14/2008 02:07 <barbara.gould@fda.hhs.gov>,

PM "Goldkind, Sara"

<sara.goldkind@fda.hhs.gov>

cc

Subject Re: [redacted] experimental protocol

Dr. Schrot,

If the product you plan to use is available to you, I would suggest you proceed under the strategy of innovative treatment rather than research. I am not familar with the literature on what you propose. If it os only available under IND, you would need to work through CBER. There is a category of single patient treatment IND. [redacted] I am copying Sara Goldkind at FDA who could also help you negotiate the FDA process if this is the direction you decide to go.

Robert "Skip" Nelson, MD PhD
Pediatric Ethicist, Office of Pediatric Therapeutics
Office of the Commissioner, Food and Drug Administration
Tele: 301-827-1522, Fax: 301-827-1017
E-mail: Robert.Nelson@fda.hhs.gov

----- Original Message -----
From: Rudolph Schrot <rudolph.schrot@ucdmc.ucdavis.edu>
To: Nelson, Robert 'Skip' *
Sent: Wed May 14 15:57:46 2008
Subject: [redacted] experimental protocol

Dear Dr. Nelson:

I spoke with Ms Gould who referred me to you. We have at UC Davis Medical Center a [redacted] with a malignant glioma, s/p surgical resection. The tumor involves the brain stem and pons. The prognosis for this histology is dismal, with median survival of 9-12 months. There are some isolated case reports of tumors which have regressed and led to long term survival after concurrent infection with gram negative bacteria. [redacted] are aware of these reports and are looking for any therapy which might offer a glimmer of hope [redacted] We are proposing an experimental protocol for a single patient: PROBIOTIC INTRACRANAL THERAPY FOR MALIGNANT GLIOMA. This would involve the installation of Enterobacter aerogenes in the vicinity of the tumor in a controlled fashion.

I wonder if you might be able to offer some insight as to how to procede with the IRB application (what [redacted] category would this be?) We have already had an ad hoc ethics committee meeting at UC Davis Medical Center which has given us the thumbs up, pending IRB approval.

Thanks so much.

Rudy Schrot

Rudolph J. Schrot, MD
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[redacted] (pager)
[redacted] (cell)

PROTOCOL FOR ANIMAL USE & CARE

Protocol #: 15258
Date Expires: 5-Mar-2012

1. Contacts

Primary Investigator	
Name:	Rudolph Schrot
E-mail:	rudolph.schrot@ucdmc.ucdavis.edu
Department:	MED: Neurological Surgery
Telephone:	916-734-8824
After Hours:	[redacted]

Alternate Contact	
Name:	[redacted] *
E-mail:	[redacted]
Department:	[redacted]
Telephone:	[redacted]
After Hours:	[redacted]

***Primary contact for sick animals*

2. Title

Probiotic Intracranial Therapy for Malignant Glioma

3. Protocol Type

Research

4. Species

Common Names	Total Number for Study	Name of Source of the Animals
rat	122	Harlan

5. Procedures

Some of the animals will be injected with bacteria into the brain to cause an infection. These bacteria are normal bacteria found in the human intestine. Some of the animals will be injected with tumor cells into the brain to create brain tumors. Some of the animals will be injected with both bacteria and tumor cells.

6. Animal Location(s)

Study Area/Laboratory:

Location/Building - Room

Med Neuroscience Building - 503

Overnight Housing (vivarium):

Vivarium(s)
CLAS: Headquarters

Animals will be maintained by:

Vivarium

7. Special Husbandry Requirements

For animals with intracranial abscess, antibiotics will be added to the drinking water at doses and schedules to be determined by the veterinarian. Otherwise, there are no special requirements. Please hold dead animals for investigator. Inform Lee, 754-5160 of any dead animals.

8. Will hazardous materials be used in this study?

Yes - *Appendix A in Section 19 will be used to gather details regarding work with hazardous materials.*

Type	Material	Location
Infectious Agents	Enterobacter aerogenes	Lab, Vivarium

9. Special Procedures and/or Activities:

Anesthetics or Tranquilizers
Survival Surgical Procedures
Multiple Major Survival Surgical Procedures

Justification for Multiple Major Survival Surgical Procedures:

Tumors cells need to be allowed to grow for one week before implanting abscess-forming culture.

10. Funding and Funding Source

Department of Neurological Surgery

11. What veterinarian or veterinary service(s) will provide care for your animals?

Campus Veterinary Services

12. Objectives and Significance:

Objectives:

The object of this study is to demonstrate a therapeutic effect of intracranial infection with a gram-negative organism on a rat model of glioblastoma. These experiments will demonstrate a proof of principle that live bacteria can fight brain cancer. There is a mixed body of literature over the past century which has explored the utility of using bacteria or bacterial factors in cancer therapy, but no animal studies using live bacteria in an animal model of brain tumor are available. Clinical evidence of a therapeutic effect of bacterial infection on brain cancer has been documented. In a small but notable series, Bowles et al. (1989) documented long-term remissions in four malignant brain tumors preceded by intracranial infection; in 75% of these cases, Enterobacter aerogenes was isolated from the wound. We will test the hypothesis that intracranial infection with live Enterobacter aerogenes has a salutary effect in a syngeneic rat model of glioblastoma. Kapp enumerated four criteria necessary for a clinically useful organism in probiotic cancer therapy: "(1) Infection with the organism should inhibit tumor growth and destroy viable tumor cells. (2) The organism should not rapidly incite an immune response in the host that is immediately lethal for the organism. (3) The organism must not induce lethal infection in the host. (4) The organism should be susceptible to antimicrobial agents, so that the infection can be controlled once the desired oncolytic effect has occurred." 2 The specific aims of our experimental proposal are designed to test these with respect to E. aerogenes as applied to an intracranial syngeneic rat glioblastoma model, CNS-1/Lewis rat.

Specific Aims:

Specific Aim 1. Quantify the host response to intracranial inoculation of living cultures of E. aerogenes in the CNS-1/Lewis rat and describe the natural history of intracranial E. aerogenes infection in the Lewis rat over several inoculation doses.

Specific Aim 2: Hypothesis: The antibiotic Baytril is an effective rescue therapy after iatrogenic intracranial E. aerogenes infection in the Lewis rat.

Specific Aim 2a. Quantify treatment efficacy of Baytril in the Lewis rat with intracranial E. aerogenes infection as determined by improved survival times compared with the natural history of the disease as determined in Specific Aim 1.

Specific Aim 2b. Systematically determine the maximum time interval between inoculation and rescue therapy that leads to recovery from intracranial infection as indicated by systemic indicators of infection and survival time.

Specific Aim 3: Hypothesis: Intratumoral inoculation of E. aerogenes in the CNS-1/Lewis rat glioblastoma model followed by antibiotic rescue therapy results in improved survival times compared to uninoculated rats.

Specific Aim 3a. Measure the survival times of the Lewis rat glioblastoma model with

and without inoculation of E. aerogenes with antibiotic rescue.

Specific Aim 3b. Quantify the changes in intracranial tumor volume after inoculation through in-vivo small animal MRI imaging and through post-mortem histopathological examination.

Significance:

Over 13,000 Americans die each year from malignant gliomas. Glioblastoma is an especially rapidly growing and fatal cancer, with an incidence rate of 3 per 100,000 in the United States. Current surgical, chemo- and radiotherapies have done relatively little to halt recurrence and death from disease within 2 years in the vast majority of cases. Therapeutic responses are measured in weeks or months, and curative treatments remain elusive.

The historical and modern literature contains numerous examples of apparent therapeutic responses to bacterial infection in patients with systemic and CNS malignancy. Using pathogenic organisms to create infections in patients with cancer challenges current thinking about treatment for glioblastoma. The current standard of care for glioblastoma includes maximal safe surgical resection, using modern aseptic surgical techniques, followed by chemotherapy with temozolomide and fractionated radiotherapy. Prophylactic antibiotics are given prior to surgery and any post-surgical bacterial infections are viewed as complications and aggressively treated with antibiotics. Steroids, which are immunosuppressive, are administered in the perioperative period to control cerebral edema. Adjuvant chemotherapy and radiotherapy, which are routinely given, also suppress the immune system.

Current management of glioblastoma not only prevents bacterial infection at the time of surgery, but also suppresses the immune system with the administration of dexamethasone and chemoradiotherapy, potentially abrogating any therapeutic impact that intracranial bacterial infection may otherwise have and compromising the host's cancer fighting immune system. If certain bacterial species can be shown experimentally to induce a therapeutic response in glioblastoma, as we will attempt to demonstrate, a radical rethinking of the therapeutic management strategy for glioblastoma may ensue, revitalizing the centuries old surgical concept of "laudable pus." New avenues of treatment may be explored, including the induction of controlled intracranial infection with known pathogens or genetically modified bacteria. Promising results in our pre-clinical investigations could open the door to FDA approved phase I clinical trials in humans, offering new hope to patients whose lives are otherwise cut short by this deadly tumor.

13. Literature search for alternatives and unnecessary duplication:
a) Databases:

Database Name	Years Covered	Keywords/Search Strategy	Date
Google Scholar	1989-present	glioma infection therapy bacteria	21-Jan-2009

PubMed/Medline	1966-present	experimental brain abscess rat	21-Jan-2009
PubMed/Medline	1966-present	glioma infection therapy bacteria	21-Jan-2009

b) Results of Literature Search:

No data are available which investigate using live gram negative bacteria as therapy in malignant glioma in animal models. There is data available on experimental brain abscess, but these experiments involved a gram positive organism. (Flaris NA, Hickey WF. Development and characterization of an experimental model of brain abscess in the rat. Am J Pathol 1992;141:1299-307.) We plan to create an experimental brain abscess using a gram-negative organism, and furthermore to apply the infection as a therapy against an experimental model of glioblastoma.

Pain and distress will be minimized during surgical procedures with general anesthetic, as well as local anesthetic in the area of the incision (lidocaine). Endpoints after treatment will be when animals have lost 20 percent of starting total body weight, in order to avoid prolonged suffering. Animals will be euthanized by an injection of sodium poentobarbital (120 mg/kg intraperitoneal). This method of euthaniasia was chosen because it provides rapid action with minimal discomfort to the animal and is consistent with the recommendations of the American Veterinary Medical Association.

c) Has this study been previously conducted?

No

d) Species Rationale:

We hypothesize that the therapeutic effect of bacteria against an experimental model of brain abscess will require an intact immune system. We have selected the Lewis rat, for which a syngeneic tumor cell line exists, the CNS-1 cell line. Therefore, an experimental model of glioblastoma is created in an animal model possessing an intact immune system. Furthermore, previous work has described a procedure for creating an experimental brain abscess in the rat.

The rat brain is sufficiently large to facilitate small animal MRI imaging, which will be used during the experiments to provide correlative biology of brain tumor growth and therapeutic response to the bacterial infection.

e) Animal Numbers Justification:

Exploratory or pilot studies: This study aims to demonstrate proof of concept that intracranial bacterial infection results in improved survival in a rat model of experimental glioblastoma compared to a control group. The primary outcome is overall survival. We will require 10 animals per experimental group. This number was determined based on previously published investigations in rodent models of experimental glioblastoma. This number is an estimation of the number of data points needed to show statistical significance, since we do not know the magnitude of the

therapeutic effect.

Previous publications did not clearly indicate the statistics used, so we performed a power analysis. A logrank test with 10 animals per group will have 80% power to detect a 3 fold increase in median survival based on logrank test. 10 rats per group will show 3-fold increase in survival with a power of 80%.

In order to determine the optimum dosage for Baytril, we will conduct 4 trials of drug dosages and durations. Based on these results we can determine the efficacy of the drug and use that dosage on the following experiments.

There are four sets of experiments. In the first set, three experimental groups will be used. In the second set, four experimental groups are used, and in the third set, four experimental groups are used, for a total of 11 experimental groups of 10 animals each, or 110 animals. In the forth set of experiments, animals from Experiment 3 will be subjected to baseline + weekly MRI scans in the small animal imaging facility.

f) Study Groups and Numbers Table:

Group	Species	Number of Animals	Procedures/Treatments
Baytril Dose - 1	rat	3	Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia. On day post surgical day 4 animals are given Baytril at 10mg/kg once daily for 5 days, then euthanized and culture and sensitivities will be run on the brain tissue.
Baytril Dose - 2	rat	3	Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia. On day post surgical day 4 animals are given Baytril at 10mg/kg once daily for 10 days, then euthanized and culture and sensitivities will be run on the brain tissue.
Baytril Dose - 3	rat	3	Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia. On day post surgical day 4 animals are given Baytril at 25mg/kg once daily for 5 days, then euthanized and culture and sensitivities will be run on the brain tissue.
Baytril Dose - 4	rat	3	Animals are inoculated stereotaxically intracranially with 20 microliters of

			bacterial beads under general anesthesia. On day post surgical day 4 animals are given Baytril at 25mg/kg once daily for 10 days, then euthanized and culture and sensitivities will be run on the brain tissue.
Experiment 1 Group A	rat	10	Animals are innoculated intracranially with 20µl of bacterial-laden agarose microbeads under general anesthesia using a stereotactic frame. Two animals will have MRI's at weekly intervals.
Experiment 1 Group B	rat	10	Animals are innoculated intracranially with 40µl of bacterial-laden agarose microbeads under general anesthesia using a stereotactic frame. Two animals will have MRI's at weekly intervals.
Experiment 1 Group C	rat	10	Control group. Animals are innoculated intracranially with 40µl of sterile agarose microbeads under general anesthesia using a stereotactic frame. Two animals will have MRI's at weekly intervals.
Experiment 2 Group A	rat	10	Antibiotic rescue therapy after experimental brain abscess. Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia and brain abscess is allowed to develop. Antibiotics are given when animals are at 5% weight loss.
Experiment 2 Group B	rat	10	Antibiotic rescue therapy after experimental brain abscess. Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia and brain abscess is allowed to develop. Antibiotics are given when animals are at 10% weight loss.
Experiment 2 Group C	rat	10	Antibiotic rescue therapy after experimental brain abscess. Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia and brain abscess is allowed to develop. Antibiotics are given when animals are at 15% weight loss.

Experiment 2 Group D	rat	10	Antibiotic rescue therapy after experimental brain abscess-control. Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia and brain abscess is allowed to develop. Sterile saline will be injected when animals are at 10% weight loss.
Experiment 3 Group A	rat	10	Effect of antibiotic rescue after Enterobacter brain infection in CNS-1 Lewis rats. CNS-1 tumor (5000 cells) are injected stereotaxically into the brains of anesthetized animals. Tumors are grown for 10 days. Then Enterobacter beads (20 micrograms) are injected in the same location. Antibiotic rescue is applied when animals reach 10% weight loss (or as determined from Experiment 2). Animals will be subjected to baseline + weekly MRI (maximum of 3).
Experiment 3 Group B	rat	10	Effect of antibiotic rescue after Enterobacter brain infection in CNS-1 Lewis rats. CNS-1 tumor (5000 cells) are injected stereotaxically into the brains of anesthetized animals. Tumors are grown for 10 days. Then Enterobacter beads (20 micrograms) are injected in the same location. Vehicle (control for antibiotic rescue) is applied when animals reach 10% weight loss (or as determined from Experiment 2). Animals will be subjected to baseline + weekly MRI (maximum of 3).
Experiment 3 Group C	rat	10	Effect of antibiotic rescue after Enterobacter brain infection in CNS-1 Lewis rats. CNS-1 tumor (5000 cells) are injected stereotaxically into the brains of anesthetized animals. Tumors are grown for 10 days. Sterile beads(20 micrograms) are injected in the same location. Antibiotic rescue is applied when animals reach 10% weight loss (or as determined from Experiment 2). Animals will be subjected to baseline + weekly MRI

			(maximum of 3).
Experiment 3 Group D	rat	10	Effect of antibiotic rescue after Enterobacter brain infection in CNS-1 Lewis rats. CNS-1 tumor (5000 cells) are injected stereotaxically into the brains of anesthetized animals. Tumors are grown for 10 days. Then Sterile (20 micrograms) are injected in the same location. Vehicle is applied when animals reach 10% weight loss (or as determined from Experiment 2). Animals will be subjected to baseline + weekly MRI (maximum of 3).

14. Summary of Procedures
a) Describe the use of animals in your project.

Lewis rats in experimental groups of 10 animals are received and maintained on water and rat chow ad lib.

Design of Individual Experiments.

Baytril Effective Dose Determination: Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia. On day post surgical day 4 animals are given Baytril at 10mg/kg or 25mg/kg once daily for 5 days or 10 days, see 13f for details, then euthanized and culture and sensitivities will be run on the brain tissue.

Experiment 1. (Specific Aim 1): Quantify the host response to intracranial inoculation of living cultures of E. a rand history of intracranial E. aerogenes erogenes inthe CNS-1/Lewis at describe the natural infection in the Lewis rat over several inoculation doses.

Rationale: Clinical evidence of bacterial-mediated tumor regression has depended on the establishment of actual infection. Numerous host and pathogen-mediated mechanisms may act in concert to achieve a therapeutic benefit in malignant brain tumors. Therefore, proof of principal in a preclinical model demands that an infection be established by the therapeutic organism in the animal model. We have selected the CNS-1/Lewis rat model. The CNS-1 cell line developed in the inbred Lewis rat by induction with N-nitroso-N-methylurea. This immune-competent model has advantages over athymic rodent models for testing potentially immunomodulatory therapies. CNS-1 is syngeneic and histocompatible the Lewis rat, and bears striking histologic similarity to human glioblastoma.

Protocol: Our protocol is a modification of that described by Flaris and Hickey (1992) for experimental brain abscess in the rat. Cultures of E. aerogenes are prepared as

injectable 50-100micrometer bacteria-laden agarose beads. Sensitivity testing will be performed on all E. aerogenes cultures obtained from the American Type Culture Collection (ATCC), including sensitivity to Baytril. Experimental groups of 10 Lewis rats will be used. Bacteria-laden agarose beads are injected stereotactically into the striata in experimental groups of 10 rats, including a control group with 40 microliters of sterile beads, and injections of 20 and 40uL of bacteria-laden beads. Baseline and daily temperatures will be collected, as well as daily body weight. Animals will be euthanized when body weight falls to below 20% of baseline. Survival times between groups will be recorded and Kaplan-Meier survival curves compared by logrank test. Brains are collected and subjected to descriptive histopathological analysis to confirm the establishment of infection and correlated with clinical observations.

Potential Problems and Alternatives: It is not known what the time course is for development of an intracranial abscess in the Lewis rat, and what the mortality rate is for E.aerogenes brain abscess in the Lewis rat. The timing of animal sacrifice for histopathological brain studies needs to be further determined. The doses of Enterobacter aerogenes inocula may be either reduced or increased to produce an optimal infection. The protocol of Flaris and Hickey[42] employed Staphylococcus aureus as a pathogen. It is not known whether the protocol will work using Enterobacter aerogenes. Severe sepsis from the gram-negative organism Enterobacter aerogenes may kill the animals prior to clear establishment of an intracranial infection. In this case, lower dosages of bacterial inocula will be given. Alternatively, a different bacterial species could be employed, such as Enterobacter cloacae. An alternative strategy may also be to create a systemic infection or bacterial meningitis, as opposed to a brain abscess, and to develop this alternate approach to therapeutic iatrogenic infection. It may also be that Enterobacter aerogenes lacks sufficient virulence in the Lewis rat host, which would prompt a search or more virulent strains or species.

Experiment 2. (Specific Aim 2a, 2b): Quantify treatment efficacy of the antibiotic Baytril in Lewis rats with intracranial E. aerogenes infection and determine the maximum time interval between inoculation and rescue therapy that leads to recovery from intracranial infection.

Rationale: A probiotic therapeutic effect requires the establishment of infection, but also requires the administration of effective antibiotic rescue therapy. Treatment of animals with antibiotics too soon would preclude the therapeutic benefit of infection, whereas a prolonged delay in treatment could result in the death of the animals. The time interval for antibiotic rescue will be determined by the natural history of experimental brain abscess with E. aerogenes in Lewis rats as seen in Experiments 1 and 2.

Protocol: Experimental groups of animals are inoculated with E. aerogenes and treated with Baytril. Treatment is started when animals exhibit 5, 10, or 15 percent weight loss for each of the groups and continued over a 2 week time period or as

determined by the veterinarian. Survival times are plotted as Kaplan-Meier curves and compared by logrank test to assess the effectiveness of antibiotic rescue therapy.

Potential Problems and Alternatives: The natural history of intracranial E.aerogenes infection in the Lewis rat is unknown. Animals could rapidly become moribund, requiring early administration of antibiotics. Alternatively, animals might spontaneously resolve the infection without requiring any antibiotics. Baytril may not be an effective agent against E.aerogenes brain abscess. Alternative agents could be used. It is unknown how long antibiotics need to be administered for therapeutic effect. The time of antibiotic initiation as well as duration of antibiotics may be altered to achieve desired antibiotic rescue from infection.

Experiment 3. (Specific Aim 3a): Quantify the survival times of the CNS-1/Lewis rat glioblastoma model with and without inoculation of E. aerogenes with antibiotic rescue.

Rationale: This experiment constitutes the proof of concept for probiotic intracranial therapy with E. aerogenes. The CNS-1/Lewis rat model has been well characterized. Inoculation of E. aerogenes into the experimental brain tumor site will create an intracranial (IC) infection. A combination of mechanisms may lead to an oncolytic effect of the E. aerogenes infection on the experimental CNS-1 glioma in the Lewis rat, leading ultimately to tumor regression and increased survival compared to animals without infection. Administration of antibiotics as rescue therapy is necessary to prevent animals from dying of infection. Previous work measured survival times of 15-22 days (median 17 days) using this brain tumor model. Therefore, infection will be induced at approximately one week after tumor implantation. Animals will be treated for infection after 1 week, or as determined by Exp. 1 and 2.

Protocol: Approximately 5000 CNS-1 cells are implanted stereotactically into the striatum of syngeneic Lewis rats. Tumors will be allowed to develop for 10 days. This is based on mean survival times of 17 days in this glioblastoma experimental system. In the experimental group, an optimal volume determined in Experiments 1 and 2(e.g., 40 uL) E. aerogenes in agarose beads or sterile agarose beads will be stereotactically injected as described above. The coordinates for experimental tumor and experimental brain abscess will be the same. Control groups will include E. aerogenes + antibiotic vehicle, sterile agarose beads + antibiotic, and sterile agarose beads + vehicle. Kaplan-Meier survival curves will be plotted and survival times between the groups will be compared by logrank test for the primary hypothesis, and proportional hazards models to assess the effects of the factors in the 2x2 design.

Potential Problems and Alternatives: It is unknown what the optimal timing of rescue therapy will be. Administration of antibiotics too soon could kill the bacteria and negate any therapeutic effect of the infection. Waiting too long to administer antibiotics could result in animal death from the tumor, from the infection, or from both. The combined burden of tumor and intracranial infection could be rapidly fatal,

even with prompt rescue therapy. The number of tumor cells implanted may need to be decreased to rolong the survival of tumor-bearing animals in order to allow time for the infection to provide a herapeutic effect.

Experiment 4 (Specific Aim3b): Quantify the in intracranial tumor volume after inoculation through in vivo small animal MRI imaging and through post mortem histopathological examination.

Rationale: Correlative biology through in vivo imaging allows both tumor progression and therapeutic response to be quantified non-invasively and followed in individual animals without requiring animal sacrifice. Cerebral edema will also be monitored. Experimental and control groups will be compared.

Protocol: Animals from will receive weekly (maximum of 3) MRI scans including a baseline scan after surgery: Scans will be limited to the following three groups: (1) E. aerogenes agarose + Baytril; (2) E. aerogenes agarose + vehicle: (3) sterile agarose beads +. Both T2-weighted (TE=75 msec, TR=4000 msec, 256 X 256 matrix with 4 cm FOV) and diffusion weighted images (DWI) will be obtained from each rat to assess both tumor volume and associated brain edema and shift. Tumor volume growth curves between the groups will be described and compared using repeated measures regression models to assess the therapeutic response to treatment.

Potential Problems and Alternatives: It is not known what the optimal timing and frequency of imaging will be.

Details of rat surgical procedure: Animals are anesthetized with 4% isoflurane in a carrier gas of N2O and O2 (2:1), intubated and maintained with 2% isoflurane via a volume ventilator, and placed in a Kopf stereotaxic apparatus. The head is shaved with electric clippers and betadine solution applied to the skin. A linear sagittal scalp incision is made and the scalp is reflected laterally. The stereotaxic coordinates are -1.0 mm Bregma, 3.0 mm left lateral, 5.5 mm depth. A 2 mm burr hole is drilled and the dura opened. A 27-guage needle of a 50-um Hamilton syringe is inserted perpendicular into the brain parenchyma at the coordinates. Twenty or 40 ul of Enterobacter aerogenes agarose beads are injected over 15 seconds, and the needle is slowly withdrawn to avoid spread of infection to the CSF space. The hole is sealed with bone wax. The scalp is then sutured, bacitracin is applied over the incision, and the animal is extubated.

For creation of brain tumor model, CNS-1 tumor inoculation: About 5,000 rat CNS-1 cells are injected sterotactically into the striatum of syngeneic Lewis rats using the method described above for creation of experimental brain abscess.

Procedure for rat MRIs: Anesthetized and mechanically ventilated animals will be placed supine in a specially designed Plexiglas cradle and head-holder, with the head (e.g., brain) positioned over a Bruker singled-tuned 1H mini-surface coil with active

rf decoupling. A respiratory monitor will be positioned on the rat's chest and used to gate radio-frequency (rf) pulse sequences in order to reduce movement artifacts. Body temperature will be monitored with an MRI-compatible rectal micro-thermocouple (Physiotemp, Clifton, NJ) and maintained at 37 +/- .5º C by adjustable circulating-water heating pad under the rat. After completion of the brain imaging experiments all rats will be extubated and returned to the vivarium.

Approved Amendments	
Date Approved	**Purpose**
03-11-2011	change alt. contact

Uploaded File(s):
DocUpload-01-Schrot9176_Procedures.pdf
DocUpload-02-Schrot9176_Procedures.pdf

b) Drugs to be used (except for euthanasia) - anesthetics, analgesics, neuromuscular blocking agents, antibiotics and/or experimental compounds:

Will drugs be used in this study? **Yes**

Species	Drug	Dose	Route	When and how often will it be given?
rat	isoflurane	2% in oxygen 2L/min	Inhalation	during induction and maintenance of anesthesia
rat	Marcaine	1/2 cc of 0.25%	Intradermal	prior to skin incision during surgical procedures
rat	Baytril Injectable	10-25 mg/kg	Subcutaneous (SC)	once daily for 10 days

c) Anesthesia Monitoring:

Anesthesia is assessed using a toe pinch test. Additional inhalational agent is used as needed during surgical procedures and MRI scanning. Anesthesia is kept light enough to allow spontaneous breathing during the MRI.

d) Post-Anesthetic Monitoring:

Animals are allowed to recover from surgical procedures in the lab until reasonably alert, at which time the animals are returned to the vivarium.

e) Surgery:

i) Location(s):

Building	Room	Surgeon(s)
Med Neuroscience Building	503	Schrot, [redacted]

ii) Post-Surgical Monitoring:

a) Please identify the parameters monitored, and interval(s) and for what duration of monitoring.

After animals have recovered from the anesthetic, animals are returned to the vivarium. Daily weight will be obtained. Behavior will be noted. Wounds will be inspected for signs of infection.

b) When will analgesics be administered and at what interval(s)?

>Local anesthesia is used at the time of surgery to numb the skin. No additional analgesics will be given.

c) If post-operative analgesics cannot be given, please provide scientific justification.

No additional analgesics will be given Production of a brain abscess or brain tumor is not typically associated with pain, since the brain has no nerve endings. Acetaminophen or NSAIDS can potentially interfere with cytokine release and the therapeutic benefit from infection. A febrile response may also be beneficial in tumor therapy with bacteria, and acetaminophen can interfere with the normal febrile response The use of narcotics can depress the sensorium and cause behavioral change which may be difficult to distinguish from tumor or infection-related neurologic decline.

15. Adverse Effects:

a. Describe **all significant** adverse effects that may be encountered during the study.

Adverse events include symptoms related to increased intracranial pressure, or focal neurologic deficit from tumor or brain abscess formation, or from cerebral edema. These events could include listlessness, decreased feeding behavior, hemiparesis, or seizures. Severe sepsis with respiratory and cardiovascular failure could result from systemic gram negative bacteremia.

b. Describe criteria for monitoring the well-being of animals on the study and criteria for terminating/modifying the procedure(s) if adverse effects are observed.

Animals are monitored in the vivarium with daily weights and behavioral assessments. Some morbidity is expected from induction of experimental brain abscesses.

Some of the common symptoms of pain include not grooming, or excessive grooming. The rat could also rub the painful area or scratch it excessively. Being very quiet and not moving around are also subtle symptoms of pain. They might hide the back part of the cage away from light and/or try to bury their heads in the bedding. Not eating is the most obvious way to tell than an animal is in pain. Weighing the animal daily after surgery is the best way to determine if the rat is eating.

Rats also get a red exudate around their eyes and nostrils when they are stressed. So it is not uncommon to see this when they are in pain.

c. How will the signs listed above be ameliorated or alleviated?

Animals with experimental brain abscess with signs listed above may be administered antibiotics sooner than scheduled according to the protocol. Other options to be considered in consultation with the veterinarian include the administration of anitepileptic medications or corticosteroids to treat seizures or cerebral edema, respectively. We will follow the IACUC Policy on Humane Endpoints (http://safetyservices.ucdavis.edu/iacuc/policies/humane-endpoints).

d. Study endpoints:

The study endpoints include the overall survival times of each experimental group. Animals will be euthanized if they have shown signs discussed in the Humane Endpoints policy (like loss of >20% of body weight, etc). Additional outcome measures will include daily core temperatures (rectal) and blood glucose as measured by Accu-check (tail prick). The first animals from each of the two first experimental groups (Experiment 1 group A and B) will serve as pilot animals to assess the natural history of experimental brain abscess with Enterobacter aerogenes as measured by daily weight, temperature, and blood glucose and to work out an assessment scoring method and establish endpoints. Additional criteria for moribund animals will include core temperature less than 34 degrees Celsius and blood glucose levels less than 2 mmol/L. Animals which remain moribund for > 72 hours, despite antibiotic rescue therapy, will be euthanized.

16. Methods of Euthanasia:

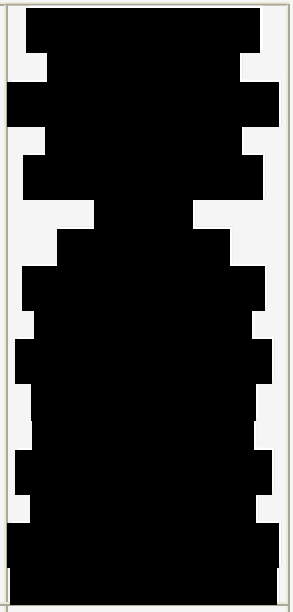
Species	Method	Drug	Dose	Route	Justification for Physical Methods
rat	Overdose	sodium pentobarbital	100mg/kg	Intraperitoneal (IP)	N/A

17. Disposition of Animals:

All animals will be euthanized.

18. Project Roster:

Name	E-mail	Occupational Health Participation	ACU 101 Training	Qualifications/Experience
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

				
Rognlie-Howes, Elizabeth L.	elrognliehowes@ucdavis.edu	7-May-2008	2-Oct-2009	9 years experience working with rodents; surgeries and behavior testing. Has completed ACU101 and lab safety class. Lab manager for Neurological Surgery.
Schrot, Rudolph	rudolph.schrot@ucdmc.ucdavis.edu	22-Dec-2005	7-Jan-2009	Neurological Surgery Assistant Professor. 8 years of residency training and 5 years of experience as an attending neurosurgeon. Laboratory experience in glioma cell culture and histopathologic techniques. Has completed ACU101 and lab safety class.

Protocol #15258 - Appendix A - Room/Lab Safety Information

1. Material(s):

Enterobacter aerogenes

2. Provide a short description of the agent:

CNS-1 tumor line is a syngenic glioblastomic tumor derived from Lewis rats. It will be maintained in cell culture and implanted in Lewis rat brains. It has no toxicity in humans. E. aerogenes - gram negative commensal organism normally found in human intestine and is known to cause opportunistic infections in immunocompromised and hospitalize patients. It is a biosafety level one organism.

3. This agent/material is hazardous for:

Humans and Animals

For which animal species?
rat

4. The agent can be spread by:

Feces/Urine

5. Describe any human health risk associated with this agent:

Risk of opportunistic infection in immunocompromised people

6. The precautions below apply to this experiment:

a. The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians:

b. Cages must be autoclaved before cleaning - No

c. Label cages and remove label after decontamination - No

d. Animal carcasses must be labeled and disposed of as follows:
Incineration

e. All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:

f. The researcher or his/her technicians are responsible for the feeding and care of these animals - No

7. Personal Protective Equipment Required:

a. The following personal protective equipment must be worn/used in the room:
Lab Coat/Coveralls
Disposable Gloves
Eye Protection/Face Shield

b. Personal protective equipment must be removed before leaving the room - Yes

c. Personal protective equipment must be discarded or decontaminated at the end of

the project - No
d. Hands and arms must be thoroughly washed upon leaving the room - Yes
e. Full shower, including washing of hair, must be taken upon leaving the room - No
f. Decontaminate Room (Inform vivarium area supervisor when cage and/or room can be returned to general use) - No

8. Provide any other information needed to safely work in this room:

Information sheets will be provided and made available to personnel on E. aerogenes.

Assurances for the Humane Care and Use of Vertebrate Animals:

I have read and agree to abide by the ***UC Davis Policy and Procedure Manual section 290-30*** (Animal Care and Use). This project will be conducted in accordance with the ***ILAR Guide for the Care and Use of Laboratory Animals***, and the **UC Davis Animal Welfare Assurance** on file with the US Public Health Service. I will abide by all Federal, State, and local laws and regulations dealing with the use of animals in research.

I will advise the IACUC in writing of any significant changes in the procedures or personnel involved in this project.

☑ I have read and agree with the above statements.

Biological Use Authorization
BUA Application

PROTOCOL FOR ANIMAL USE & CARE

Protocol #: 15258
Date Expires: 5-Mar-2012

1. Contacts

Primary Investigator		Alternate Contact	
Name:	Rudolph Schrot	**Name:**	[redacted]
E-mail:	rudolph.schrot@ucdmc.ucdavis.edu	**E-mail:**	[redacted]
Department:	MED: Neurological Surgery	**Department:**	[redacted]
Telephone:	916-734-8824	**Telephone:**	[redacted]
After Hours:	[redacted]	**After Hours:**	[redacted]

**Primary contact for sick animals*

2. Title

Probiotic Intracranial Therapy for Malignant Glioma

3. Protocol Type

Research

4. Species

Common Names	Total Number for Study	Name of Source of the Animals
rat	122	Harlan

5. Procedures

Some of the animals will be injected with bacteria into the brain to cause an infection. These bacteria are normal bacteria found in the human intestine. Some of the animals will be injected with tumor cells into the brain to create brain tumors. Some of the animals will be injected with both bacteria and tumor cells.

6. Animal Location(s)

Study Area/Laboratory:

Location/Building - Room
Med Neuroscience Building - 503

Overnight Housing (vivarium):

Vivarium(s)
CLAS: Headquarters

Animals will be maintained by:

Vivarium

7. Special Husbandry Requirements

For animals with intracranial abscess, antibiotics will be added to the drinking water at doses and schedules to be determined by the veterinarian. Otherwise, there are no special requirements. Please hold dead animals for investigator. Inform Lee, 754-5160 of any dead animals.

8. Will hazardous materials be used in this study?

Yes - *Appendix A in Section 19 will be used to gather details regarding work with hazardous materials.*

Type	Material	Location
Infectious Agents	Enterobacter aerogenes	Lab, Vivarium

9. Special Procedures and/or Activities:

Anesthetics or Tranquilizers
Survival Surgical Procedures
Multiple Major Survival Surgical Procedures

Justification for Multiple Major Survival Surgical Procedures:

Tumors cells need to be allowed to grow for one week before implanting abscess-forming culture.

10. Funding and Funding Source

Department of Neurological Surgery

11. What veterinarian or veterinary service(s) will provide care for your animals?

Campus Veterinary Services

12. Objectives and Significance:

Objectives:

The object of this study is to demonstrate a therapeutic effect of intracranial infection with a gram-negative organism on a rat model of glioblastoma. These experiments will demonstrate a proof of principle that live bacteria can fight brain cancer. There is a mixed body of literature over the past century which has explored the utility of using bacteria or bacterial factors in cancer therapy, but no animal studies using live bacteria in an animal model of brain tumor are available. Clinical evidence of a therapeutic effect of bacterial infection on brain cancer has been documented. In a small but notable series, Bowles et al. (1989) documented long-term remissions in four malignant brain tumors preceded by intracranial infection; in 75% of these cases, Enterobacter aerogenes was isolated from the wound. We will test the hypothesis that intracranial infection with live Enterobacter aerogenes has a salutary effect in a syngeneic rat model of glioblastoma. Kapp enumerated four criteria necessary for a clinically useful organism in probiotic cancer therapy: "(1) Infection with the organism should inhibit tumor growth and destroy viable tumor cells. (2) The organism should not rapidly incite an immune response in the host that is immediately lethal for the organism. (3) The organism

must not induce lethal infection in the host. (4) The organism should be susceptible to antimicrobial agents, so that the infection can be controlled once the desired oncolytic effect has occurred." 2 The specific aims of our experimental proposal are designed to test these with respect to E. aerogenes as applied to an intracranial syngeneic rat glioblastoma model, CNS-1/Lewis rat.

Specific Aims:

Specific Aim 1. Quantify the host response to intracranial inoculation of living cultures of E. aerogenes in the CNS-1/Lewis rat and describe the natural history of intracranial E. aerogenes infection in the Lewis rat over several inoculation doses.

Specific Aim 2: Hypothesis: The antibiotic Baytril is an effective rescue therapy after iatrogenic intracranial E. aerogenes infection in the Lewis rat.

Specific Aim 2a. Quantify treatment efficacy of Baytril in the Lewis rat with intracranial E. aerogenes infection as determined by improved survival times compared with the natural history of the disease as determined in Specific Aim 1.

Specific Aim 2b. Systematically determine the maximum time interval between inoculation and rescue therapy that leads to recovery from intracranial infection as indicated by systemic indicators of infection and survival time.

Specific Aim 3: Hypothesis: Intratumoral inoculation of E. aerogenes in the CNS-1/Lewis rat glioblastoma model followed by antibiotic rescue therapy results in improved survival times compared to uninoculated rats.

Specific Aim 3a. Measure the survival times of the Lewis rat glioblastoma model with and without inoculation of E. aerogenes with antibiotic rescue.

Specific Aim 3b. Quantify the changes in intracranial tumor volume after inoculation through in-vivo small animal MRI imaging and through post-mortem histopathological examination.

Significance:

Over 13,000 Americans die each year from malignant gliomas. Glioblastoma is an especially rapidly growing and fatal cancer, with an incidence rate of 3 per 100,000 in the United States. Current surgical, chemo- and radiotherapies have

done relatively little to halt recurrence and death from disease within 2 years in the vast majority of cases. Therapeutic responses are measured in weeks or months, and curative treatments remain elusive.

The historical and modern literature contains numerous examples of apparent therapeutic responses to bacterial infection in patients with systemic and CNS malignancy. Using pathogenic organisms to create infections in patients with cancer challenges current thinking about treatment for glioblastoma. The current standard of care for glioblastoma includes maximal safe surgical resection, using modern aseptic surgical techniques, followed by chemotherapy with temozolomide and fractionated radiotherapy. Prophylactic antibiotics are given prior to surgery and any post-surgical bacterial infections are viewed as complications and aggressively treated with antibiotics. Steroids, which are immunosuppressive, are administered in the perioperative period to control cerebral edema. Adjuvant chemotherapy and radiotherapy, which are routinely given, also suppress the immune system.

Current management of glioblastoma not only prevents bacterial infection at the time of surgery, but also suppresses the immune system with the administration of dexamethasone and chemoradiotherapy, potentially abrogating any therapeutic impact that intracranial bacterial infection may otherwise have and compromising the host's cancer fighting immune system. If certain bacterial species can be shown experimentally to induce a therapeutic response in glioblastoma, as we will attempt to demonstrate, a radical rethinking of the therapeutic management strategy for glioblastoma may ensue, revitalizing the centuries old surgical concept of "laudable pus." New avenues of treatment may be explored, including the induction of controlled intracranial infection with known pathogens or genetically modified bacteria. Promising results in our pre-clinical investigations could open the door to FDA approved phase I clinical trials in humans, offering new hope to patients whose lives are otherwise cut short by this deadly tumor.

13. Literature search for alternatives and unnecessary duplication:
a) Databases:

Database Name	Years Covered	Keywords/Search Strategy	Date
Google Scholar	1989-present	glioma infection therapy bacteria	21-Jan-2009
PubMed/Medline	1966-present	experimental brain abscess rat	21-Jan-2009
PubMed/Medline	1966-present	glioma infection therapy bacteria	21-Jan-2009

b) Results of Literature Search:

No data are available which investigate using live gram negative bacteria as therapy in malignant glioma in animal models. There is data available on experimental brain abscess, but these experiments involved a gram positive organism. (Flaris NA, Hickey WF. Development and characterization of an experimental model of brain abscess in the rat. Am J Pathol 1992;141:1299-307.) We plan to create an experimental brain abscess using a gram-negative organism, and furthermore to apply the infection as a therapy against an experimental model of glioblastoma.

Pain and distress will be minimized during surgical procedures with general anesthetic, as well as local anesthetic in the area of the incision (lidocaine). Endpoints after treatment will be when animals have lost 20 percent of starting total body weight, in order to avoid prolonged suffering. Animals will be euthanized by an injection of sodium poentobarbital (120 mg/kg intraperitoneal). This method of euthaniasia was chosen because it provides rapid action with minimal discomfort to the animal and is consistent with the recommendations of the American Veterinary Medical Association.

c) Has this study been previously conducted?

No

d) Species Rationale:

We hypothesize that the therapeutic effect of bacteria against an experimental model of brain abscess will require an intact immune system. We have selected the Lewis rat, for which a syngeneic tumor cell line exists, the CNS-1 cell line. Therefore, an experimental model of glioblastoma is created in an animal model possessing an intact immune system. Furthermore, previous work has described a procedure for creating an experimental brain abscess in the rat.

The rat brain is sufficiently large to facilitate small animal MRI imaging, which will be used during the experiments to provide correlative biology of brain tumor growth and therapeutic response to the bacterial infection.

e) Animal Numbers Justification:

Exploratory or pilot studies: This study aims to demonstrate proof of concept that intracranial bacterial infection results in improved survival in a rat model of experimental glioblastoma compared to a control group. The primary outcome is overall survival. We will require 10 animals per experimental group. This number was determined based on previously published investigations in rodent models of experimental glioblastoma. This number is an estimation of the number of data points needed to show statistical significance, since we do not know the magnitude of the therapeutic effect.

Previous publications did not clearly indicate the statistics used, so we performed a power analysis. A logrank test with 10 animals per group will have 80% power to detect a 3 fold increase in median survival based on logrank test. 10 rats per group will show 3-fold increase in survival with a power of 80%.

In order to determine the optimum dosage for Baytril, we will conduct 4 trials of drug dosages and durations. Based on these results we can determine the efficacy of the drug and use that dosage on the following experiments.

There are four sets of experiments. In the first set, three experimental groups will be used. In the second set, four experimental groups are used, and in the third set, four experimental groups are used, for a total of 11 experimental groups of 10 animals each, or 110 animals. In the forth set of experiments, animals from Experiment 3 will be subjected to baseline + weekly MRI scans in the small animal imaging facility.

f) Study Groups and Numbers Table:

Group	Species	Number of Animals	Procedures/Treatments
Baytril Dose - 1	rat	3	Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia. On day post surgical day 4 animals are given Baytril at 10mg/kg once daily for 5 days, then euthanized and culture and sensitivities will be run on the brain tissue.
Baytril Dose - 2	rat	3	Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia. On day post surgical day 4 animals are given Baytril at 10mg/kg once daily for 10 days, then euthanized and culture and sensitivities will be run on the brain tissue.
Baytril Dose - 3	rat	3	Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia. On day post surgical day 4 animals are given Baytril at 25mg/kg once daily for 5 days, then euthanized and culture and sensitivities will be run on the brain tissue.
Baytril Dose - 4	rat	3	Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia. On day post surgical day 4

			animals are given Baytril at 25mg/kg once daily for 10 days, then euthanized and culture and sensitivities will be run on the brain tissue.
Experiment 1 Group A	rat	10	Animals are innoculated intracranially with 20µl of bacterial-laden agarose microbeads under general anesthesia using a stereotactic frame. Two animals will have MRI's at weekly intervals.
Experiment 1 Group B	rat	10	Animals are innoculated intracranially with 40µl of bacterial-laden agarose microbeads under general anesthesia using a stereotactic frame. Two animals will have MRI's at weekly intervals.
Experiment 1 Group C	rat	10	Control group. Animals are innoculated intracranially with 40µl of sterile agarose microbeads under general anesthesia using a stereotactic frame. Two animals will have MRI's at weekly intervals.
Experiment 2 Group A	rat	10	Antibiotic rescue therapy after experimental brain abscess. Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia and brain abscess is allowed to develop. Antibiotics are given when animals are at 5% weight loss.
Experiment 2 Group B	rat	10	Antibiotic rescue therapy after experimental brain abscess. Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia and brain abscess is allowed to develop. Antibiotics are given when animals are at 10% weight loss.
Experiment 2 Group C	rat	10	Antibiotic rescue therapy after experimental brain abscess. Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia and brain abscess is allowed to develop. Antibiotics are given when animals are at 15% weight loss.
Experiment 2 Group D	rat	10	Antibiotic rescue therapy after experimental brain abscess-control. Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia and brain abscess is allowed to develop. Sterile saline will be injected when animals are at 10% weight loss.
Experiment 3 Group A	rat	10	Effect of antibiotic rescue after Enterobacter brain infection in CNS-1 Lewis rats. CNS-1 tumor (5000 cells) are injected stereotaxically into the brains of anesthetized animals. Tumors are grown for 10 days. Then

			Enterobacter beads (20 micrograms) are injected in the same location. Antibiotic rescue is applied when animals reach 10% weight loss (or as determined from Experiment 2). Animals will be subjected to baseline + weekly MRI (maximum of 3).
Experiment 3 Group B	rat	10	Effect of antibiotic rescue after Enterobacter brain infection in CNS-1 Lewis rats. CNS-1 tumor (5000 cells) are injected stereotaxically into the brains of anesthetized animals. Tumors are grown for 10 days. Then Enterobacter beads (20 micrograms) are injected in the same location. Vehicle (control for antibiotic rescue) is applied when animals reach 10% weight loss (or as determined from Experiment 2). Animals will be subjected to baseline + weekly MRI (maximum of 3).
Experiment 3 Group C	rat	10	Effect of antibiotic rescue after Enterobacter brain infection in CNS-1 Lewis rats. CNS-1 tumor (5000 cells) are injected stereotaxically into the brains of anesthetized animals. Tumors are grown for 10 days. Sterile beads(20 micrograms) are injected in the same location. Antibiotic rescue is applied when animals reach 10% weight loss (or as determined from Experiment 2). Animals will be subjected to baseline + weekly MRI (maximum of 3).
Experiment 3 Group D	rat	10	Effect of antibiotic rescue after Enterobacter brain infection in CNS-1 Lewis rats. CNS-1 tumor (5000 cells) are injected stereotaxically into the brains of anesthetized animals. Tumors are grown for 10 days. Then Sterile (20 micrograms) are injected in the same location. Vehicle is applied when animals reach 10% weight loss (or as determined from Experiment 2). Animals will be subjected to baseline + weekly MRI (maximum of 3).

14. Summary of Procedures
a) Describe the use of animals in your project.

Lewis rats in experimental groups of 10 animals are received and maintained on water and rat chow ad lib.

Design of Individual Experiments.

Baytril Effective Dose Determination: Animals are inoculated stereotaxically intracranially with 20 microliters of bacterial beads under general anesthesia. On day post surgical day 4 animals are given Baytril at 10mg/kg or 25mg/kg once daily for 5 days or 10 days, see 13f for details, then euthanized and culture and sensitivities will be run on the brain tissue.

Experiment 1. (Specific Aim 1): Quantify the host response to intracranial inoculation of living cultures of E. a rand history of intracranial E. aerogenes erogenes inthe CNS-1/Lewis at describe the natural infection in the Lewis rat over several inoculation doses.

Rationale: Clinical evidence of bacterial-mediated tumor regression has depended on the establishment of actual infection. Numerous host and pathogen-mediated mechanisms may act in concert to achieve a therapeutic benefit in malignant brain tumors. Therefore, proof of principal in a preclinical model demands that an infection be established by the therapeutic organism in the animal model. We have selected the CNS-1/Lewis rat model. The CNS-1 cell line developed in the inbred Lewis rat by induction with N-nitroso-N-methylurea. This immune-competent model has advantages over athymic rodent models for testing potentially immunomodulatory therapies. CNS-1 is syngeneic and histocompatible the Lewis rat, and bears striking histologic similarity to human glioblastoma.

Protocol: Our protocol is a modification of that described by Flaris and Hickey (1992) for experimental brain abscess in the rat. Cultures of E. aerogenes are prepared as injectable 50-100micrometer bacteria-laden agarose beads. Sensitivity testing will be performed on all E. aerogenes cultures obtained from the American Type Culture Collection (ATCC), including sensitivity to Baytril. Experimental groups of 10 Lewis rats will be used. Bacteria-laden agarose beads are injected stereotactically into the striata in experimental groups of 10 rats, including a control group with 40 microliters of sterile beads, and injections of 20 and 40uL of bacteria-laden beads. Baseline and daily temperatures will be collected, as well as daily body weight. Animals will be euthanized when body weight falls to below 20% of baseline. Survival times between groups will be recorded and Kaplan-Meier survival curves compared by logrank test. Brains are collected and subjected to descriptive histopathological analysis to confirm the establishment of infection and correlated with clinical observations.

Potential Problems and Alternatives: It is not known what the time course is for development of an intracranial abscess

in the Lewis rat, and what the mortality rate is for E.aerogenes brain abscess in the Lewis rat. The timing of animal sacrifice for histopathological brain studies needs to be further determined. The doses of Enterobacter aerogenes inocula may be either reduced or increased to produce an optimal infection. The protocol of Flaris and Hickey[42] employed Staphylococcus aureus as a pathogen. It is not known whether the protocol will work using Enterobacter aerogenes. Severe sepsis from the gram-negative organism Enterobacter aerogenes may kill the animals prior to clear establishment of an intracranial infection. In this case, lower dosages of bacterial inocula will be given. Alternatively, a different bacterial species could be employed, such as Enterobacter cloacae. An alternative strategy may also be to create a systemic infection or bacterial meningitis, as opposed to a brain abscess, and to develop this alternate approach to therapeutic iatrogenic infection. It may also be that Enterobacter aerogenes lacks sufficient virulence in the Lewis rat host, which would prompt a search or more virulent strains or species.

Experiment 2. (Specific Aim 2a, 2b): Quantify treatment efficacy of the antibiotic Baytril in Lewis rats with intracranial E. aerogenes infection and determine the maximum time interval between inoculation and rescue therapy that leads to recovery from intracranial infection.

Rationale: A probiotic therapeutic effect requires the establishment of infection, but also requires the administration of effective antibiotic rescue therapy. Treatment of animals with antibiotics too soon would preclude the therapeutic benefit of infection, whereas a prolonged delay in treatment could result in the death of the animals. The time interval for antibiotic rescue will be determined by the natural history of experimental brain abscess with E. aerogenes in Lewis rats as seen in Experiments 1 and 2.

Protocol: Experimental groups of animals are inoculated with E. aerogenes and treated with Baytril. Treatment is started when animals exhibit 5, 10, or 15 percent weight loss for each of the groups and continued over a 2 week time period or as determined by the veterinarian. Survival times are plotted as Kaplan-Meier curves and compared by logrank test to assess the effectiveness of antibiotic rescue therapy.

Potential Problems and Alternatives: The natural history of intracranial E.aerogenes infection in the Lewis rat is unknown. Animals could rapidly become moribund, requiring early administration of antibiotics. Alternatively, animals might spontaneously resolve the infection without requiring any antibiotics. Baytril may not be an effective agent against E.aerogenes brain abscess. Alternative agents could be used. It is unknown how long antibiotics need to be administered for therapeutic effect. The time of antibiotic initiation as well as duration of antibiotics may be altered to

achieve desired antibiotic rescue from infection.

Experiment 3. (Specific Aim 3a): Quantify the survival times of the CNS-1/Lewis rat glioblastoma model with and without inoculation of E. aerogenes with antibiotic rescue.

Rationale: This experiment constitutes the proof of concept for probiotic intracranial therapy with E. aerogenes. The CNS-1/Lewis rat model has been well characterized. Inoculation of E. aerogenes into the experimental brain tumor site will create an intracranial (IC) infection. A combination of mechanisms may lead to an oncolytic effect of the E. aerogenes infection on the experimental CNS-1 glioma in the Lewis rat, leading ultimately to tumor regression and increased survival compared to animals without infection. Administration of antibiotics as rescue therapy is necessary to prevent animals from dying of infection. Previous work measured survival times of 15-22 days (median 17 days) using this brain tumor model. Therefore, infection will be induced at approximately one week after tumor implantation. Animals will be treated for infection after 1 week, or as determined by Exp. 1 and 2.

Protocol: Approximately 5000 CNS-1 cells are implanted stereotactically into the striatum of syngeneic Lewis rats. Tumors will be allowed to develop for 10 days. This is based on mean survival times of 17 days in this glioblastoma experimental system. In the experimental group, an optimal volume determined in Experiments 1 and 2(e.g., 40 uL) E. aerogenes in agarose beads or sterile agarose beads will be stereotactically injected as described above. The coordinates for experimental tumor and experimental brain abscess will be the same. Control groups will include E. aerogenes + antibiotic vehicle, sterile agarose beads + antibiotic, and sterile agarose beads + vehicle. Kaplan-Meier survival curves will be plotted and survival times between the groups will be compared by logrank test for the primary hypothesis, and proportional hazards models to assess the effects of the factors in the 2x2 design.

Potential Problems and Alternatives: It is unknown what the optimal timing of rescue therapy will be. Administration of antibiotics too soon could kill the bacteria and negate any therapeutic effect of the infection. Waiting too long to administer antibiotics could result in animal death from the tumor, from the infection, or from both. The combined burden of tumor and intracranial infection could be rapidly fatal, even with prompt rescue therapy. The number of tumor cells implanted may need to be decreased to rolong the survival of tumor-bearing animals in order to allow time for the infection to provide a herapeutic effect.

Experiment 4 (Specific Aim3b): Quantify the in intracranial tumor volume after inoculation through in-vivo small

animal MRI imaging and through post-mortem histopathological examination.

Rationale: Correlative biology through in vivo imaging allows both tumor progression and therapeutic response to be quantified non-invasively and followed in individual animals without requiring animal sacrifice. Cerebral edema will also be monitored. Experimental and control groups will be compared.

Protocol: Animals from will receive weekly (maximum of 3) MRI scans including a baseline scan after surgery: Scans will be limited to the following three groups: (1) E. aerogenes agarose + Baytril; (2) E. aerogenes agarose + vehicle: (3) sterile agarose beads +. Both T2-weighted (TE=75 msec, TR=4000 msec, 256 X 256 matrix with 4 cm FOV) and diffusion weighted images (DWI) will be obtained from each rat to assess both tumor volume and associated brain edema and shift. Tumor volume growth curves between the groups will be described and compared using repeated measures regression models to assess the therapeutic response to treatment.

Potential Problems and Alternatives: It is not known what the optimal timing and frequency of imaging will be.

Details of rat surgical procedure: Animals are anesthetized with 4% isoflurane in a carrier gas of N2O and O2 (2:1), intubated and maintained with 2% isoflurane via a volume ventilator, and placed in a Kopf stereotaxic apparatus. The head is shaved with electric clippers and betadine solution applied to the skin. A linear sagittal scalp incision is made and the scalp is reflected laterally. The stereotaxic coordinates are -1.0 mm Bregma, 3.0 mm left lateral, 5.5 mm depth. A 2 mm burr hole is drilled and the dura opened. A 27-guage needle of a 50-um Hamilton syringe is inserted perpendicular into the brain parenchyma at the coordinates. Twenty or 40 ul of Enterobacter aerogenes agarose beads are injected over 15 seconds, and the needle is slowly withdrawn to avoid spread of infection to the CSF space. The hole is sealed with bone wax. The scalp is then sutured, bacitracin is applied over the incision, and the animal is extubated.

For creation of brain tumor model, CNS-1 tumor inoculation: About 5,000 rat CNS-1 cells are injected sterotactically into the striatum of syngeneic Lewis rats using the method described above for creation of experimental brain abscess.

Procedure for rat MRIs: Anesthetized and mechanically ventilated animals will be placed supine in a specially designed Plexiglas cradle and head-holder, with the head (e.g., brain) positioned over a Bruker singled-tuned 1H mini-surface coil with active rf decoupling. A respiratory monitor will be positioned on the rat's chest and used to gate radio-frequency (rf) pulse sequences in order to reduce movement artifacts. Body temperature will be monitored with an

MRI-compatible rectal micro-thermocouple (Physiotemp, Clifton, NJ) and maintained at 37 +/- .5° C by adjustable circulating-water heating pad under the rat. After completion of the brain imaging experiments all rats will be extubated and returned to the vivarium.

Approved Amendments	
Date Approved	**Purpose**
03-11-2011	change alt. contact

Uploaded File(s):
DocUpload-01-Schrot9176_Procedures.pdf
DocUpload-02-Schrot9176_Procedures.pdf

b) Drugs to be used (except for euthanasia) - anesthetics, analgesics, neuromuscular blocking agents, antibiotics and/or experimental compounds:

Will drugs be used in this study? **Yes**

Species	Drug	Dose	Route	When and how often will it be given?
rat	isoflurane	2% in oxygen 2L/min	Inhalation	during induction and maintenance of anesthesia
rat	Marcaine	1/2 cc of 0.25%	Intradermal	prior to skin incision during surgical procedures
rat	Baytril Injectable	10-25 mg/kg	Subcutaneous (SC)	once daily for 10 days

c) Anesthesia Monitoring:

Anesthesia is assessed using a toe pinch test. Additional inhalational agent is used as needed during surgical procedures and MRI scanning. Anesthesia is kept light enough to allow spontaneous breathing during the MRI.

d) Post-Anesthetic Monitoring:

Animals are allowed to recover from surgical procedures in the lab until reasonably alert, at which time the animals are returned to the vivarium.

e) Surgery:

i) Location(s):

Building	Room	Surgeon(s)
Med Neuroscience Building	503	Schrot, [redacted]

ii) Post-Surgical Monitoring:

a) Please identify the parameters monitored, and interval(s) and for what duration of monitoring.

After animals have recovered from the anesthetic, animals are returned to the vivarium. Daily weight will be obtained. Behavior will be noted. Wounds will be inspected for signs of infection.

b) When will analgesics be administered and at what interval(s)?

>Local anesthesia is used at the time of surgery to numb the skin. No additional analgesics will be given.

c) If post-operative analgesics cannot be given, please provide scientific justification.

No additional analgesics will be given. Production of a brain abscess or brain tumor is not typically associated with pain, since the brain has no nerve endings. Acetaminophen or NSAIDS can potentially interfere with cytokine release and the therapeutic benefit from infection. A febrile response may also be beneficial in tumor therapy with bacteria, and acetaminophen can interfere with the normal febrile response The use of narcotics can depress the sensorium and cause behavioral change which may be difficult to distinguish from tumor or infection-related neurologic decline.

15. Adverse Effects:

a. Describe **all significant** adverse effects that may be encountered during the study.

Adverse events include symptoms related to increased intracranial pressure, or focal neurologic deficit from tumor or brain abscess formation, or from cerebral edema. These events could include listlessness, decreased feeding behavior, hemiparesis, or seizures. Severe sepsis with respiratory and cardiovascular failure could result from systemic gram negative bacteremia.

b. Describe criteria for monitoring the well-being of animals on the study and criteria for terminating/modifying the procedure(s) if adverse effects are observed.

Animals are monitored in the vivarium with daily weights and behavioral assessments. Some morbidity is expected from induction of experimental brain abscesses.

Some of the common symptoms of pain include not grooming, or excessive grooming. The rat could also rub the

painful area or scratch it excessively. Being very quiet and not moving around are also subtle symptoms of pain. They might hide the back part of the cage away from light and/or try to bury their heads in the bedding. Not eating is the most obvious way to tell than an animal is in pain. Weighing the animal daily after surgery is the best way to determine if the rat is eating.

Rats also get a red exudate around their eyes and nostrils when they are stressed. So it is not uncommon to see this when they are in pain.

c. How will the signs listed above be ameliorated or alleviated?

Animals with experimental brain abscess with signs listed above may be administered antibiotics sooner than scheduled according to the protocol. Other options to be considered in consultation with the veterinarian include the administration of anitepileptic medications or corticosteroids to treat seizures or cerebral edema, respectively. We will follow the IACUC Policy on Humane Endpoints (http://safetyservices.ucdavis.edu/iacuc/policies/humane-endpoints).

d. Study endpoints:

The study endpoints include the overall survival times of each experimental group. Animals will be euthanized if they have shown signs discussed in the Humane Endpoints policy (like loss of >20% of body weight, etc). Additional outcome measures will include daily core temperatures (rectal) and blood glucose as measured by Accu-check (tail prick). The first animals from each of the two first experimental groups (Experiment 1 group A and B) will serve as pilot animals to assess the natural history of experimental brain abscess with Enterobacter aerogenes as measured by daily weight, temperature, and blood glucose and to work out an assessment scoring method and establish endpoints. Additional criteria for moribund animals will include core temperature less than 34 degrees Celsius and blood glucose levels less than 2 mmol/L. Animals which remain moribund for > 72 hours, despite antibiotic rescue therapy, will be euthanized.

16. Methods of Euthanasia:

Species	Method	Drug	Dose	Route	Justification for Physical Methods
rat	Overdose	sodium pentobarbital	100mg/kg	Intraperitoneal (IP)	N/A

17. Disposition of Animals:

All animals will be euthanized.

18. Project Roster:

Name	E-mail	Occupational Health Participation	ACU 101 Training	Qualifications/Experience
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Rognlie-Howes, Elizabeth L.	elrognliehowes@ucdavis.edu	7-May-2008	2-Oct-2009	9 years experience working with rodents; surgeries and behavior testing. Has completed ACU101 and lab safety class. Lab manager for Neurological Surgery.
Schrot, Rudolph	rudolph.schrot@ucdmc.ucdavis.edu	22-Dec-2005	7-Jan-2009	Neurological Surgery Assistant Professor. 8 years

				of residency training and 5 years of experience as an attending neurosurgeon. Laboratory experience in glioma cell culture and histopathologic techniques. Has completed ACU101 and lab safety class.

Protocol #15258 - Appendix A - Room/Lab Safety Information
1. Material(s):
Enterobacter aerogenes
2. Provide a short description of the agent:
CNS-1 tumor line is a syngenic glioblastomic tumor derived from Lewis rats. It will be maintained in cell culture and implanted in Lewis rat brains. It has no toxicity in humans. E. aerogenes - gram negative commensal organism normally found in human intestine and is known to cause opportunistic infections in immunocompromised and hospitalize patients. It is a biosafety level one organism.
3. This agent/material is hazardous for:
Humans and Animals *For which animal species?* rat
4. The agent can be spread by:
Feces/Urine
5. Describe any human health risk associated with this agent:

Risk of opportunistic infection in immunocompromised people
6. The precautions below apply to this experiment:
a. The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians:
b. Cages must be autoclaved before cleaning - No
c. Label cages and remove label after decontamination - No
d. Animal carcasses must be labeled and disposed of as follows: Incineration
e. All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:
f. The researcher or his/her technicians are responsible for the feeding and care of these animals - No
7. Personal Protective Equipment Required:
a. The following personal protective equipment must be worn/used in the room: Lab Coat/Coveralls Disposable Gloves Eye Protection/Face Shield
b. Personal protective equipment must be removed before leaving the room - Yes
c. Personal protective equipment must be discarded or decontaminated at the end of the project - No
d. Hands and arms must be thoroughly washed upon leaving the room - Yes
e. Full shower, including washing of hair, must be taken upon leaving the room - No
f. Decontaminate Room (Inform vivarium area supervisor when cage and/or room can be returned to general use) - No
8. Provide any other information needed to safely work in this room:
Information sheets will be provided and made available to personnel on E. aerogenes.

Assurances for the Humane Care and Use of Vertebrate Animals:

I have read and agree to abide by the ***UC Davis Policy and Procedure Manual section 290-30*** (Animal Care and Use). This project will be conducted in accordance with the ***ILAR Guide for the Care and Use of Laboratory Animals***, and the **UC Davis Animal Welfare Assurance** on file with the US Public Health Service. I will abide by all Federal, State, and local laws and regulations dealing with the use of animals in research.

I will advise the IACUC in writing of any significant changes in the procedures or personnel involved in this project.

☑ I have read and agree with the above statements.

University of California, Davis

Biological Use Authorization Application/ Recombinant DNA Project Registration

October 2008

Table of Contents

*Page numbers will change as the document text boxes expand during completion
†Sections 1 and 7 must be completed; complete other sections as applicable to your project

INSTRUCTIONS

1. Research involving recombinant DNA or infectious agents at UC Davis is governed by the **NIH Guidelines for Research Involving Recombinant DNA Molecules** and by **UC Davis Policy and Procedure 290-55** (April 2007 revision). The Biological Use Authorization (BUA) is the mechanism for documenting these types of research and securing Biological Safety Administrative Advisory Committee (BSAAC) review and approval for individual projects that involve biohazardous and other infectious or recombinant genetic materials. Acquisition, possession, or use of these materials is not permitted at UC Davis unless the principal investigator has obtained a Biological Use Authorization.

2. This BUA application/rDNA registration downloads as a MS Word fillable form. MS Word "spell check" does not work in the text boxes--complete and spell check your text in a Word document and paste into the appropriate text boxes in the Biological Use Authorization application.

3. **All applicants must complete Sections 1 (general information), 6 (health surveillance) and 7 (authorized personnel) of this form plus the sections relevant to the work to be authorized.**

4. Please refer to the Biological Use Authorization Policy and Information document for assistance in completing the application and insert that document and Appendix A of the application into your laboratory training manual along with your approved Biological Use Authorization/rDNA Registration.

5. E-mail the completed document and supporting documents as attachments to **bua@ucdavis.edu**. You will receive e-mailed information regarding the review process and the earliest possible review date for your application. Contact us at 752 1493 if you do not receive notification within two business days that we have received your application.

6. Print out the signature page, obtain all necessary signatures, and send the hard copy (just the signature page) by campus mail to "Biosafety Office, EH&S." ***We will not forward your application to the BSAAC until we have received the signed cover page.***

7. The Biosafety Office will review the application for clarity, responsiveness to the form questions, completeness of the principal investigator's risk assessment, adequacy of the proposed risk controls, and utility as a training document. We will ask that the PI revise the application if necessary before we forward the application to the BSAAC. A lab inspection within the past year is also normally required—if a new inspection is necessary the Biosafety Office will schedule a time in advance of the Committee meeting. NIH and University rules also require an annual laboratory inspection for all Biological Use Authorizations.

8. The Biological Safety Administrative Advisory Committee meets on the third Monday of the month. You may submit a BUA application at any time, but **we must receive the complete application** (including all supporting materials and the signed front page) ***by 5:00 PM on the 1st (or preceding Friday) of the month to schedule it for review during that month's meeting.***

9. If your project qualifies for rDNA project registration simultaneous with BSAAC notification as specified within Section III-E or is exempt under Section III-F of the NIH Guidelines for Research Involving Recombinant DNA Molecules the Biosafety Office will recommend your registration or exemption to the Committee before the next BSAAC meeting. You should not initiate your work until you receive confirmation from the Biosafety Office that the BSAAC has received your registration. The BSAAC may request further supporting information to qualify your project for simple registration or exemption.

10. Contact the Biosafety Office at (530) 752 1493 or visit the EH&S website if you need further information.

University of California, Davis

Biological Use Authorization

SECTION 1. Principal Investigator and Laboratory Information

Application type:	This project involves:	For EH&S use (9-24-08 rev)	
☒ New BUA ____	☐ Recombinant DNA (Sec 2)	Date received: A	BSL assigned:
☐ Renew BUA # ____	☒ Infectious Agents (Sec 3)	DSA:	ABSO Review
☐ Terminated BUA # ____	☐ Storage Only (Sec 4)	BSAAC review dates	
☐ Major change amendment, BUA # ____		First review:	Action:
☐ Storage only, BUA # ____		Second review:	Action:
		Third review:	Action:
		Approval date:	Expiration date:

Principal investigator:	Rudolph J. Schrot			Title:	Assistant Professor
Department:	Neurological Surgery		Building:	UCDMC-ACC	Room: 3740
Phone:	(916) 734-8824	Fax: (916) 703-5368	E-mail address:	rudolph.schrot@ucdmc.ucdavis.edu	
☒ Shared space owner ☒ Co-investigator	Lorena Navarro			Title:	Assistant Professor
A "Shared space owner" is the director, manager, or owner of research space or special equipment not assigned to the Principal Investigator. A "co-investigator" is a UC Davis faculty or Academic Federation member with a significant collaborative role in the research. Use additional sheets if necessary.					
Department:	Microbiology		Building:	Briggs Hall	Room: 255
Phone:	752-0260	Fax: 752-9014	E-mail address:	lonavarro@ucdavis.edu	
☒ Check here if you have listed additional shared space owners or co-investigators on a separate sheet					
Lab contact	Lee Rognlie-Howes			Title:	Lab Manager
Department:	Neurological Surgery		Building:	Neuroscience	Room: 502-B
Phone:		Fax:	E-mail address:	elrognliehowes@ucdavis.edu	

General conditions for Biological Use Authorization approval

The principal investigator agrees to:

- Ensure that personnel listed in this application have received or will receive appropriate documented training in safe laboratory practices and procedures for this protocol ***before any work begins on this project*** and at least annually thereafter
- Follow the health surveillance practices as approved for this protocol and inform those working on the protocol about appropriate emergency assistance information for their location(s).
- Inform EH&S (530 752 1493) and the occupational medicine physician (530 757 3200) of any research-related accident or illness as soon as possible after its occurrence.
- Comply with the NIH Guidelines for Research Involving Recombinant DNA Molecules, with UC Davis biosafety procedures specified in Appendix A of this form and the UC Davis Biosafety Manual, with UC Davis and UC systemwide biosafety policy and procedure, and with all other applicable laws and regulations.
- **Submit in writing (by BUA Amendment) a request for pre-approval from the Biological Safety Administrative Advisory Committee (530 752 1493) for any significant deviations from the biohazard containment or personnel protection provisions of the approved BUA , or any modifications to the study or additions or deletions of personnel, facilities, recombinant or infectious agents, or procedures.**

By signing below, I certify that I have reviewed the above conditions and agree that all project personnel will abide by those requirements and adhere to all UC Davis policies and procedures governing the use of recombinant DNA and infectious agents.

Principal investigator signature: _____ Date: _____

Shared space or co-investigator (if applicable): _____ Date: _____

Department chair (required): _____ Date: _____

BSAAC chair: _____ Date: _____

BSO (Final Appr for Cond. BUAs): _____ Date: _____

For BIOSAFETY OFFICE use:	Applicable sections of the NIH Guidelines for Research Involving Recombinant DNA Molecules: ☐ N/A (Infectious agents only)	
☐ Registration only ☐ BSAAC/IBC pre-approval required	☐ Exempt under NIH Guidelines section	BSL assigned:

Project Title Probiotic Intracranial Therapy for Malignant Glioma

Funding Agency: CRCC **Award #** **Start/End dates:**

Work and equipment locations (use additional sheets if necessary)

Specific use	Lab biosafety level	Building	Room No.	Shared space
Laboratory location	BSL 1☒ 2☐ 3☐	Neuroscience	503	☒
Laboratory location	BSL 1☒ 2☐ 3☐	Neuroscience	413	☒
Laboratory ☒ or insectary ☐ location	BSL 1☒ 2☐ 3☐	Neuroscience	412	☒
Laboratory ☒ or greenhouse ☐ location	BSL 1☐ 2☒ 3☐	Briggs Hall	255	☐
Laboratory ☒ or field planting ☐ location	BSL 1☐ 2☒ 3☐	Briggs Hall	229	☒
Housed animals	ABSL 1☒ 2☐ 3☐	Neuroscience	221	☒
Housed animals	ABSL 1☐ 2☐ 3☐			☐
Biological safety cabinet(s)				☐
Autoclave for non-medical waste		Neuroscience	400	☒
Medical waste accumulation site or approved autoclave		Neuroscience	425	☒
Storage: ☐ RT ☐ 4° ☐ -20° ☐ -80° ☐ LN_2	Locked? ☐ Yes ☐ No			☐
Storage: ☐ RT ☐ 4° ☐ -20° ☐ -80° ☐ LN_2	Locked? ☐ Yes ☐ No			☐
Storage: ☐ RT ☐ 4° ☐ -20° ☐ -80° ☐ LN_2	Locked? ☐ Yes ☐ No			☐

☐ Check here if you have no storage

☐ Check here if you have listed additional locations on a separate sheet

Biological safety cabinet information (use additional sheets if necessary)

☒ I do not plan to use a biological safety cabinet in this project

Cabinet 1	Date of most recent certification		Cabinet 2	Date of most recent certification	
Brand	Class	Type	Brand	Class	Type
Model	Width ft		Model	Width ft	
Serial number	UC No.		Serial number	UC No.	
Exhaust type (recirculating, canopy, or hard ducted)			Exhaust type (recirculating, canopy, or hard ducted)		

Cabinet 3	Date of most recent certification		Cabinet 4	Date of most recent certification	
Brand	Class	Type	Brand	Class	Type
Model	Width ft		Model	Width ft	
Serial number	UC No.		Serial number	UC No.	
Exhaust type (recirculating, canopy, or hard ducted)			Exhaust type (recirculating, canopy, or hard ducted)		

☐ Check here if you have listed additional biological safety cabinets on a separate sheet

Institutional approvals for this project (applicable to this BUA)		Protocol No.	Protocol status (submitted, pending, approved)	Approval date
Vertebrate animals*			pending	
Human Subjects protocol or exemption	☐ Exempt			

*Attach a copy of the room/lab safety sheet and the list of authorized personnel from your animal care protocol (note in Section 5 of this application). *This is mandatory even if your project is not yet IACUC-approved*

Agency permits*	*Permit type*	*Permit No.*	*Date issued*	*Expiration date*
USDA ☐				
CDFA ☐				
Other				

*Attach a copy of the permit (note in Section 5 of this application) or mail a copy to Biosafety Office, EH

Section 2: RECOMBINANT DNA

☐ Yes ☒ No	I am inserting foreign DNA or RNA into a vector or host (If you checked "yes," complete section 2A and other parts of Section 2 as appropriate)
☐	Large-scale (>10L culture) experiment or production of recombinant host or vector
☐	Introduction of a drug resistance trait into a pathogenic agent not normally known to acquire the specific resistance naturally*
☐	Introduction of a sequence that encodes a molecule that is toxic to vertebrates at LD_{50} <100ng/kg body mass*
☐	Human Gene Therapy trial protocol to be submitted to NIH Recombinant DNA Advisory Committee (RAC) for Human Gene Transfer Proposal approval and to the UC Davis Institutional Review Board (Human Subjects Committee) (***Obtain the Human Gene Therapy IBC Review Application form from the Biosafety Office at BSO@ucdavis.edu***)

*NIH Office of Biotechnology Activity approval required before BSAAC review

Section 2A. Recombinant experiment components

TABLE 1. Host, vector and gene information. *Please provide specific names including strain designations.* We will add the information you provide here to the Biological Use Authorization database so please double-check for completeness and spelling. Use additional sheets in the same format if necessary.		Risk Group (or approp. BSL)		
Component	**Name**	1	2	3
Hosts		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
Vectors		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐
Genes and related genetic material to be cloned				
DNA/RNA organismal sources (scientific names)				

☐ Check here if you have listed additional components on a separate sheet

Section 2A, continued. Further information on recombinant components.
Check the boxes as appropriate and complete only the following Section(s) indicated

Host (check all that apply for all hosts listed above in Table 1, and complete the listed Sections)

- ☐ *E. coli* K-12 (or its derivatives), *Saccharomyces cerevisiae, S. uvarum, Bacillus subtilis or B. licheniformis*
- ☐ Risk Group 2 or 3 microbial agent **(complete Sections 2D, 3, and 2E if applicable)**
- ☐ All other microbial agents **(Complete Section 2D)**
- ☐ Cultured eukaryotic cells: Primary ☐ Cell line ☐ **(Complete Section 2D and 2E if viral vectors are to be used)**
- ☐ Whole plants (including seeds) **(Compete Section 2C if appropriately at BSL1-Plant; complete Section 2D if higher level containment will be required)**
- ☐ Domestic mouse (*Mus musculus* or *M. domesticus*, including >first captive generation "wild type" (e.g., *M. spretus*) **(Complete Section 2B for pronuclear injection-generated transgenics appropriately housed at ABSL-1; otherwise complete section 2D)**
- ☐ All other animals (vertebrate and invertebrate) **(Complete Section 2D)**

Vector (check all that apply for all vectors listed above in Table 1 and complete the listed sections)

- ☐ Molecular construct (e.g., plasmid) or non-biological delivery system (e.g., nanoparticles) **(Complete Section 2D)**
- ☐ Risk Group 1 or 2 virus that infects eukaryotic cells and contains **two-thirds or less** of the viral genome **(Complete Sections 2D and 2E)**
- ☐ Risk Group 1 or 2 virus that infects eukaryotic cells and contains **more** than two-thirds of the viral genome **(Complete Sections 2D and 2E)**
- ☐ Risk Group 3 agent (Please contact the Biosafety Officer before submitting this application) **(Complete Sections 2D, 2E, and 3)**
- ☐ **Plant** transformation agent is *Agrobacterium tumefaciens* **(Complete Section 2C or 2D if containment exceeds BSL1)**
- ☐ Other viable plant transforming agent **(Complete Section 2D)**:
- ☐ Part of any type of **RNA interference system (Complete Section 2D and 2E if viral vectors are to be used)**

☐ Viral vector (Section 2D, 2E)		☐ Replication-competent	☐ Replication-defective	Generation
Vector backbone	☐ Adenovirus	☐ Lentivirus	☐ Herpes	☐ MVA
	☐ Adeno-associated virus	☐ Foamyvirus	☐ Canarypox	☐ Other:

Genetic material (check all that apply for all genes and related genetic material listed above in Table 1)

- ☐ Not believed to have biohazardous potential (human, animal, plant, or environmental) **(Complete the most appropriate Section; if unsure, please check with the Biosafety Office, 2-1493)**
- ☐ Encodes directly or encodes a unique component of a pathway that produces:
  - ☐ A known, suspected, or potential oncogene (e.g., transcription factor) **(Complete Section 2D)**
  - ☐ A virulence factor or any other gene or gene component capable of conferring or enhancing microbial pathogenicity **(Complete Section 2D)**
- ☐ Includes genetic elements from a different species than the host, vector, or gene of interest **(Complete Section 2D)**
- ☐ Includes more than two-thirds of the single strand genome of a Risk Group 2 agent **(Complete Section 2D)**
- ☐ From a Risk Group 3 or 4 agent, Select Agent, or an *animal or plant pathogen normally handled at BSL 3* **(Complete Section 2D, but please discuss this project with the Biosafety Officer (752-1493) before submitting application)**

Section 2B. Transgenic mouse development and production (BSL1/ABSL1). If your project solely involves transgenic laboratory mouse development (by pronuclear injection and implantation) with downstream phenotype evaluation, novel crosses involving transgenic parents in unrelated lines, or congenic production with at least one transgenic parent with no additional biohazardous component, please respond to the following three questions to complete your application. Insert the requested information in the box beneath each question (the box will expand automatically as necessary). The response to each question should require no more than five sentences.

- Please define all technical acronyms at the first use (universally understood acronyms (e.g. "DNA") do not require definition)
- Attach the room/lab safety sheet (if applicable) and the list of authorized personnel from your animal care and use protocol.
- **If the transgenic construct is likely to require containment above Biosafety Level 1 (e.g., significant viral genome or known or potential oncogenes) or involves animal species other than the laboratory mouse, skip this section and complete Section 2D.**

1. **Research project summary.** Describe your project objectives and experimental design and describe the known or hypothesized function of the gene(s) of interest. Describe your goals in working with transgenic lines, developing targeted gene knockouts, or breeding to produce congenic lines. Specify the mouse strain(s) to be used for transgenic production, crosses, or congenic development. Identify the facility that will produce the initial transgenic construct if applicable (e.g., Murine Targeted Genomics Laboratory or other similar facility or collaborator, or your own laboratory).

2. **Risk assessment.** Describe the potential consequences of introducing your transgenic mouse genome into wild populations of house mice and related species (e.g., *Mus spretus*) potentially already established at Davis, or inadvertently into established laboratory mouse colonies. Identify lethal combinations (if known) that can result from your breeding plan. Cite existing data or published references to support your assessment.

3. **Risk minimization.** Specify your measures to ensure that your transgenic mice do not escape containment. Discuss precautions taken during routine colony maintenance (the Biosafety Office may ask that you provide a copy of the transgenic colony management SOP) and animal transport within and between structures. Please describe your record-keeping method for tracking transgenic animals (a NIH requirement), and your methods for euthanasia and carcass and bedding disposal (per Appendices G and Q of the NIH Guidelines).

Section 2C. Recombinant plant development (BSL1-Plant). If your project solely involves recombinant plant development and downstream phenotype evaluation at BSL1-Plant (as defined in the NIH recombinant DNA Guidelines), please respond to the following three questions to complete your application. Insert the requested information in the box beneath each question (the box will expand automatically as necessary). The response to each question should require no more than five sentences.

- Please define all technical acronyms at the first use (universally understood acronyms (e.g. "DNA") do not require definition)
- **If the transgenic construct is likely to require containment above Biosafety Level 1 (e.g., noxious weeds, exotic plants, or hosts, vectors, or transformation mechanisms believed to represent a hazard to agriculture or to the environment) skip this section and complete Section 2D.**

1. **Research project summary.** Describe your project objectives and experimental design, and specify your method of plant transformation. Specify if your work involves gene discovery, and describe the general function of the genes of interest. If expression of a foreign protein is an objective of your work, describe the protein. Specify the types of facilities where transgenic plants will be grown and maintained (greenhouse, laboratory, growth chamber, field planting).

2. **Risk assessment.** Describe the potential adverse consequences of the release of your recombinant plant genome into the environment or into agricultural or wild plant populations. Cite existing data or published references to support your assessment.

3. **Risk minimization.** Specify your measures to ensure that your recombinant plants or their seeds or pollen do not escape containment. Discuss precautions taken during routine growth maintenance, sampling, and plant material transport within and between locations (the Biosafety Office may ask that you provide a copy of your SOPs). Please describe your record-keeping method for tracking recombinant plants and your method for recombinant material waste disposal.

Section 2D. All other projects that involve the production or use of recombinant constructs.

Please respond to the following questions regarding your proposed research. Insert the requested information in the box beneath each question (the box will expand automatically as necessary). This information will allow BSAAC members to evaluate the project and formulate a risk assessment. Most single projects require no more than three pages of text (including the space occupied by the questions) to describe adequately.

Important:

- The information you provide in this section should address procedures to generate and the biohazards resulting from the recombinant construct.
- Please define all technical acronyms at the first use (universally understood acronyms (e.g. "DNA") do not require definition)
- Please **double-check** for spelling errors—the Biosafety Office will not submit a BUA application to the BSAAC for review until such errors are corrected
- If the project involves vertebrate animals, attach the room/lab safety sheet and list of authorized personnel from your animal care and use protocol, and **note in Section 5 of this application.**

1. **Research project summary.** Specify your project objectives and experimental design. Include a brief statement of the known or suspected function of your genes of interest. Specify if your work involves gene discovery, and describe the general function of the genes of interest. Justify in detail any large-scale work (>10L culture). Specify any agency permits (e.g., USDA) that have been issued to cover your work.

2. **Sources of components.** Specify the sources of **all hosts, vectors, and DNA species named in Table 1 (above).** Include commercial sources and catalogue numbers, names and addresses of collaborators supplying biohazardous materials or recombinant components, and transgenic animal or plant production facilities as appropriate.

3. **Inserted sequence information.** Specify the nature of the genetic material being inserted into the host (DNA or RNA, promoter, gene, locus, etc.) If expression of a foreign protein in a host is an objective of this research **discuss the protein and identify any anticipated phenotypic changes to the host.**

4. **Vector and gene information (For work with *viral vectors* disregard this question and complete Section 2E).** Provide technical information regarding the transformed and untransformed vector, including composition and size of insert, nature and specificity of promoters and other elements, types of species targeted (*E. coli*, mammalian, legume, etc), and types of tissues targeted. **If your vector did not originate from a commercial source, cite the original reference for its development and include a genetic map of the vector that identifies all relevant elements.** If possible, provide an electronic copy of the reference. Discuss helper virus and packaging cell systems involved with your experiments.

5. **Transformation methods.** Describe your methods for transforming the vector and host. A detailed step-by-step protocol is not necessary, but please provide sufficient information on your procedures so that the committee can identify the steps that involve the greatest likelihood of worker or environmental exposure to biohazardous materials. **Indicate the steps that will be conducted in a biological safety cabinet (including reagent and construct preparation).** Specify storage conditions and labeling for the transformed vector and host.

6. **Risk assessment.** List the known or suspected biohazards of your research materials, including hazards of your gene(s) of interest, vector **(other than viral vectors, which should be described in Section 2E)**, and transformed host to healthy adult or immunocompromised people, or to pregnant women and to other animal species and to plants (where applicable). For transgenic, recombinant, and targeted gene constructs (any host), describe any significant potential environmental impacts if the host escapes containment and becomes established in agricultural or natural ecosystems, whether by hybridization or by direct colonization.

7. **Biohazard containment and risk reduction.** Identify your facilities, procedures and equipment, including personal protective equipment, to contain biohazardous materials (including transgenic animals and plants) and to prevent researcher, community, or environmental exposure to biohazardous agents and contaminated material. For large-scale projects (>10L culture) discuss in detail your provisions for complete containment and inactivation of your high-volume culture material in the event of a full-scale spill or leak.

8. **Transport and shipping.** Detail any requirement for intracampus transport of biohazardous materials, including origin and destination laboratories or other facilities, frequency of transport, and measures you will employ to prevent accidental release of biohazardous materials. Be very specific about packaging procedures. Also detail any plans for personal transport of biohazardous materials to and from campus (i. e. that do not utilize a commercial carrier), including transport to and from satellite UC Davis facilities. See Appendix A for campus policy.

9. **Terminal inactivation and waste disposal.** Specify your methods for terminal inactivation of the biological agent. Be sure to include liquid disinfectant concentrations and exposure times. Specify dry waste bag color and markings, time period between waste bag setup and closure/disposal, autoclave temperature and contact time (Appendix A), and final disposition of autoclaved waste.

10. **Medical surveillance requirements.** Describe any medical surveillance or post-exposure treatment necessary to help protect the health of individuals who work on the project or who might otherwise be exposed to biohazardous materials in your facilities. Include any animal care staff or clinical staff as appropriate, and ***attach a copy of the Room/lab Safety Sheet from your Animal Care protocol to this BUA application.*** If a standard UC Davis Employee Health Services-approved surveillance or treatment program is appropriate, include a two-sentence description of the steps in surveillance and post-exposure treatment. Check the appropriate standard protocols box in Section 6 of this application.

Section 2E. Additional information on viral vectors.

If your research involves the use of viral vectors, please respond to the following questions **in addition** to the questions in the preceding Section (2D). Note that you are not required to complete Section 3 of this application unless you are also working with infectious agents other than genetically modified viral vectors

1. **Viral vector identification and source.** Identify the viral vectors to be used in your research. Specify the types of vector (as checked off in Section 2A), the strains or other identifying designations, and the commercial sources. **Provide references and genetic maps** if the vectors are obtained from non-commercial sources.

2. **Viral genes remaining in vector.** For non-commercial vector packages, specify the native viral genes that are known to remain in the vector. This information may be included in the vector map required under question 1, above.

3. **Replication competence status.** Specify whether the vector is replication competent or defective, and specify the viral generation. If the vector is replication competent or is replication defective and used in conjunction with a helper virus, please justify its use in your experiments. If the vector is a replication defective construct **from a non-commercial source,** describe your methods and protocol for verifying replication incompetence at receipt and periodically thereafter.

4. **Viral targets.** Identify the plant or animal species and cell types known to be targeted by this vector.

5. **Biohazard risk assessment and risk minimization.** Describe the susceptibility of humans (including immunocompromised people and pregnant women) or other species to infections resulting from your vector(s). Describe the consequences of infection in susceptible individuals. Describe your methods and protocols for minimizing exposure, including engineering and administrative controls and health surveillance measures.

Section 3: INFECTIOUS AGENTS

(Viral vector use in recombinant experiments requires completion of Sections 2D and 2E but does not require completion of this section)

Yes ☐ No ☒	I am working with Human Risk Group-2 or -3 infectious, pathogenic, or toxin-producing agents, other than as RG1 or 2 recombinant vectors
Yes ☒ No ☐	I am working with Human Risk Group 1 biological agents (other than for recombinant vectors) that are infectious or pathogenic to animals or plants

If you checked Yes to either, please complete the rest of Section 3.

Table 2. Infectious agents and arthropod vector information. Please provide scientific or technical names, including strain names. Use additional sheets as necessary. The information provided here will be entered into the BUA database. Mark the Select Agent box if the agent is included in the HHS or USDA lists of select agents (Appendix B)		Risk Group	Select Agent
Agent	Enterobacter aerogenes, ATCC # 13048	☒ 1 ☐ 2 ☐ 3	☐
Agent		☐ 1 ☐ 2 ☐ 3	☐
Agent		☐ 1 ☐ 2 ☐ 3	☐
Agent		☐ 1 ☐ 2 ☐ 3	☐
Agent		☐ 1 ☐ 2 ☐ 3	☐
Agent		☐ 1 ☐ 2 ☐ 3	☐
Arthropod vector			
Arthropod vector			

☐ Check here if additional agents are listed on a separate sheet

Please respond to the following questions regarding your proposed research. Insert the requested information in the box beneath each question (the box will expand automatically as necessary) This information will allow BSAAC members to evaluate the research project and formulate a risk assessment. Most single projects require no more than three pages of text (including the space occupied by the questions) to describe adequately.

Important:

- Do not enter "N/A" in any text box (except Question 4, if it is not applicable)—all requested information must be provided.
- Please define all technical acronyms at the first use (universally understood acronyms (e.g. "DNA") do not require definition)
- If the project involves vertebrate animals, attach the room/lab safety sheet and the list of authorized personnel from your animal care and use protocol and provide details on animal use in the following sections.
- If this BUA is "storage only," skip this section and complete Section 4 only.

If you are using a Select Agent please attach the biosafety plan required under 42 CFR § 73 and note in Section 5.

1. **Research project summary.** Describe your project objectives and experimental design. For model systems or for research involving Select Agents (42 CFR § 73), briefly state the reasons that the selected infectious agents are most appropriate for your work.

Glioblastoma multiforme is a devastating malignant brain tumor for which there is no cure. Current surgery and chemoradiotherapy offers only modest benefit. Using bacteria to fight brain cancer is an unorthodox, but potentially promising novel therapeutic approach.

Evidence that infection cures cancer is old. Historical surgical literature has anecdotally reported an association between surgical wound infection in cancer operations and favorable outcome. Before modern aseptic surgical technique, surgeons routinely and deliberately produced infections after cancer surgery. "Laudable pus" (or as we now understand it, post-operative wound infection) was a firmly established concept in cancer surgery prior to the 20th century.

In a small but notable series, Bowles et al. (1989) documented long-term remissions in four malignant brain tumors preceded by intracranial infection; in 75% of these cases, Enterobacter aerogenes was isolated from the wound. We will test the hypothesis that intracranial infection with live Enterobacter aerogenes has a salutary effect in a syngeneic rat model of glioblastoma. Four criteria are necessary for a clinically useful organism in probiotic cancer therapy: "(1) Infection with the organism should inhibit tumor growth and destroy viable tumor cells. (2) The organism should not rapidly incite an immune response in the host that it immediately lethal for the organism. (3) The organism must not induce lethal infection in the host. (4) The organism should be susceptible to antimicrobial agents, so that the infection can be controlled once the desired oncolytic effect has occurred." 2 The specific aims of our experimental proposal are designed to test these criteria with respect to E. aerogenes as applied to an intracranial syngeneic rat glioblastoma model, CNS-1/Lewis rat. We plan to inject Enterobacter aerogenes via an agarose bead carrier into the brains of Lewis rats harboring experimental glioblastoma to assess the potentially therapeutic effect.

Enterobacter aerogenes is a commensal Gram-negative pathogenic bacterium that may be implicated in opportunistic infections. Several features of the Enterobacter genus may point towards its activity against tumors of the central nervous system. Enterobacter sakazakii has been shown to invade capillary endothelial cells and penetrate the blood brain barrier. Furthermore, Shigatoxin (aka verotoxin), which is elaborated by E. coli but also has been discovered in Enterobacter cloacae, is known to inhibit the growth and induce apoptosis in astrocytoma cells lines.

2. **Sources of agents.** Specify the sources of the individual biohazardous materials obtained from natural sources, from commercial vendors (include vendor names and catalogue numbers), or from collaborators (names and addresses). Include any pertinent information on required shipping permits.

Enterobacter aerogenes is obtained from the ATCC #13048, shipped freeze dried.

3. **Technical information regarding infectious agents.** Provide technical information regarding the infectious agent strain(s), including any genetic recombination or drug resistance. For antibiotic drug resistant strains provide strain name, resistance factor, and source. If drug resistance traits that are being added to infectious agents are also known to be acquired naturally, attach at least one supporting publication. If your genetically altered strains did not originate from a commercial source, cite the original references for their development or specify "developed in this laboratory" as appropriate (or specify "wild-type"). Specify if you are using clinical isolates of human or animal pathogens.

Organism:Enterobacter aerogenes Hormaeche and Edwards deposited as Aerobacter aerogenes Hormaeche and Edwards
Designations: NCDC 819-56 [Cloaca B, IFO 13534, NCTC 10006]
Isolation: sputum, South Carolina Dept. of Health and Environmental Control
Depositor: CDC
Biosafety Level: 1
Shipped: freeze-dried
Growth Conditions: ATCC medium 3: Nutrient agar or nutrient broth
Temperature: 30.0°C
Duration: aerobic

4. **Arthropod vector and insectary information.** Provide technical information regarding the host-vector-agent system you will be using, including the natural history of agent-vector infection and vector-host infection. Discuss vector-host specificity and other natural limits on disease transmission (physical, geographic, and biological). Be sure to state the natural geographic distribution of the vector and natural hosts, and note any agency permits that you have been issued or are pending. **Discuss in detail** your insectary facilities **(provide a floor plan)** and arthropod colony husbandry program, with special attention to your containment provisions.

N/A

5. **Risk assessment.** Specify the known and suspected biohazards of your infectious agents, including hazards to healthy adults, pregnant women or immunocompromised individuals, and to other species. If applicable, specify the symptoms of significant exposure or infection in humans. Include a one- or two-sentence review of laboratory acquired infections involving the agents you plan to use (statistics and outcome trends across all laboratories). If you are using an exotic arthropod vector system or any plant infectious agent, discuss the possible consequences of a release into local agricultural areas or natural ecosystems.

Enterobacter aerogenes is a Gram-negative, oxidase negative, catalase positive, rod-shaped bacterium which is a normal commensal of the human intenstine. It does not generally cause disease in healthy individuals. It has been found to live in various wastes, hygenic chemicals, and soil.

Enterobacter aerogenes is an important nosocomial pathogen responsible for various infections, including bacteremia, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections (UTIs), endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections. Enterobacter species can also cause various community-acquired infections, including UTIs, skin and soft-tissue infections, and wound infections, among others. Risk factors for nosocomial Enterobacter infections include hospitalization of greater than 2 weeks, invasive procedures in the past 72 hours, treatment with antibiotics in the past 30 days, and the presence of a central venous catheter. Specific risk factors for infection with nosocomial multidrug-resistant strains of Enterobacter species include the recent use of broad-spectrum cephalosporins or aminoglycosides and ICU care. These "ICU bugs" cause significant morbidity and mortality, and infection management is complicated by resistance to multiple antibiotics. Enterobacter species possess inducible beta-lactamases, which are undetectable in vitro but are responsible for resistance during treatment. Physicians treating patients with Enterobacter infections are advised to avoid certain antibiotics, particularly third-generation cephalosporins, because resistant mutants can quickly appear. Antibiograms must be interpreted with respect to the different resistance mechanisms and their respective frequency, as is reported for Enterobacter species, even if routine in vitro antibiotic susceptibility testing has not identified resistance.

The endocarditis model in rabbits has been used to as an experimental model of deep-seated Enterobacter aerogenes infection to test various antimicrobial regimens. Difloxacin was significantly more effective than enoxacin or cefoperazone in reducing caridac vegetations to near sterility after 10 days of treatment (Antimicrob Agents Chemother. 1987 Mar;31(3):458-60). Alternatively, a combination of a beta-lactam antibiotic (mezlocillin or ticarcillin) plus gentamicin was capable of sterilizing over half of the vegetations, even when initiated 7 days into the infection(Antimicrob Agents Chemother. 1984 Jun;25(6):683-6).

6. **Experimental procedures.** Specify the experimental procedures including isolation and culture methods and conditions that involve biohazardous materials that you will use to accomplish your objectives. A detailed step-by-step protocol is not necessary, but please provide sufficient information on your procedures so that the committee can identify the steps that involve the greatest likelihood of worker, community, or environmental exposure to biohazardous materials. Indicate the procedure steps that will be conducted in a biological safety cabinet.

Bacterial Growth Conditions:
Enterobacter aerogenes freeze-dried cultures are obtained from the ATCC and grown overnight at 30°C in Luria-Bertani broth on a rotary shaker. The bacteria are pelleted by centrifugation at 3000g for 15 minutes, washed twice in sterile, calcium-magnesium-free, Dulbecco's phosphate buffered
saline (D-PBS), pH 7.2 at 40C, and resuspended in .5cc of D-PBS before the addition to a
solution of agarose.

Preparation of Agarose Beads:
A 1.4% solution in D-PBS of low viscosity agarose is melted, then brought to 40°C. The bacteria are added to the 40°C solution of agarose and the mixture is then rapidly added to heavy mineral oil preheated to 37°C. The mineral oil is stirred on a magnetic plate in a 100-ml conical flask while the bacteria-agarose mixture is added. Stirring is continued for 1 minute, and the mixture is rapidly cooled to 0°C by adding crushed ice around the vessel while stirring is continued for 2 minutes. Standard volumes of the 1.4% agarose solution (20 ml) and mineral oil (30 ml) are used in the same vessels. The mixture of oil and agarose beads is subsequently washed in D-PBS four times. The beads are pelleted by centrifugation at 800g for 10 minutes between washings. After the last wash, the suspended beads are left to precipitate under gravity for 5 minutes to separate the smaller beads that remain longer in suspension. Beads with dimensions between 50-100 μm are obtained. A pellet containing packed 50-100 μm bacteria-laden agarose beads will be used for injection. Sterile beads will be prepared without adding bacteria. Preparation of the beads will be carried out in the Navarro lab (Briggs Hall) and transported in syringes to Neuroscience for intracranial injection.

Creation of experimental brain abscess and therapeutic intracranial injection of microbial agent:
Experimental groups of 10 male Lewis rats are used, and approximately 10 groups are used. All animal studies described will be reviewed and approved by the Committee on Animal Experimentation and by the UC Davis Veterinarian. These studies are consistent with the principles of laboratory animal care and with State of California guidelines. Adult male Lewis rats are induced with 4% isoflurane and N2O:O2 (2:1), intubated and maintained on 2% isoflurane at 0.5 l/min, and placed in a Kopf stereotactic apparatus. A small longitudinal skin incision is made lateral to midline between the ear and the eye, and a burr hole is drilled just above the temporalis muscle beside the coronal suture. A microsyringe with a 21-g needle is inserted perpendicular into the brain parenchyma 5-6 mm deep from the external surface of the calvarium; 20 or 40 μl of Enterobacter aerogenes agarose beads are injected over 15 seconds, and the needle is slowly withdrawn. The skin is sutured, and the animals are followed for signs of disease. Used needles and syringes are disposed of in appropriate biohazard medical waste facilities. The same injection procedure is used for rats with and without experimental glioblastoma.

Animal sacrifice:
After deep isofluorane inhalational anesthesia in a chamber, followed by intraperitoneal pentobarbital, transcardiac organ perfusion with 0.9%saline followed by 4% phosphate-buffered paraformaldehyde (PFO) is performed.

7. **Biohazard risk reduction.** Specify procedures and equipment, including personal protective equipment, to prevent researcher, environmental, or community exposure to infectious agents and contaminated material.

The organism used in this study is Biosafety Level I, and normally does not cause disease in healthy individuals. Our facilities include laboratory spaces in Briggs Hall (Navarro lab) and Neuroscience (Schrot, Lyeth, and Gorin lab). The Briggs Hall lab includes dedicated space in room 255 and shared space for gram-negative organism culture in room 229, which is a shared facility. Culture of Enterobacter aerogenes and preparation of bacteria-laden agarose microbeads will be conducted at the Briggs Hall facilities. We use standard microbiological techniques in this study. The laboratory procedures include cultivation of microorganisms in liquid culture using tubes or flasks, pipetting, centrifugation, the use of various organic and non-organic solutions, and the preparation of agarose microbeads. All procedures will be performed carefully to minimize the creation of splashes (no aerosol formation is expected). Employees have been trained in safety procedures for Biosafety Level I as are described in the CDC/NIH handbook. Access to laboratories will be restricted to authorized users that have been trained in Biosafety I procedures.

Subsequent procedures with vertebrate animals will be conducted in Neuroscience Rms 503 and 413. Glioblastoma cell lines are prepared in Rm 412. Animals are housed in Rm 221. All users of shared facilities will be notified of said research and the organism to be used. Employees have been trained in safety procedures for Biosafety Level I as described in the CDC/NIH handbook. Access to laboratories will be restricted to authorized users that have been trained in Biosafety Level I procedures. All persons working in the laboratory will be trained to use personal protective equipment (PPE) and all persons entering the laboratory will be required to wear appropriate PPE (lab coat). When performing experiments with potentially infectious agents, persons will be required to wear appropriate PPE (lab coat, gloves). When performing procedures where splashes may occur, personnel will be required to wear additional PPE (safety glasses). Personnel are required to wash their hands after handling organisms, removing gloves or before leaving the laboratory. Containment of microorganisms will be maintained through the use of aseptic procedures and routine disinfection of laboratory work surfaces (at least once a day and after any spill of viable material) with 10% Alconox solution, followed by ddH20, and finally by 70% ethanol solution. Liquid cultures are decontaminated in a final concentration of 10% household bleach solutions for > 30 minutes to prevent community or environmental exposure. Contaminated plastic waste will be collected and disposed within a week of use. No food, drinking or smoking is allowed in the laboratory. Mouth pipetting is prohibited.

Although it is extremely unusual, some procedures do present the opportunity for accidental secondary contamination. The affected area will then be disinfected with a solution of 10% (final concentration) household bleach for 30 minutes or by spraying with 70% (final concentration) ethanol. Solutions are made fresh to ensure the proper concentrations. Decontamination solution will be collected by absorption with paper towels, which will be placed in a red biohazard waste bag and disposed of by the UC Davis Medical School waste removal service.

8. **Transport and shipping.** Detail any requirement for intracampus transport of biohazardous materials, including origin and destination laboratories or other facilities, frequency of transport, and measures you will employ to prevent accidental release of biohazardous materials. Also detail any plans for **personal transport** of biohazardous materials to and from campus (i. e. that do not utilize a commercial carrier), including transport between campus and satellite UC Davis facilities. Be very specific about packaging procedures and compliance with IATA and DOT training requirements (See Appendix A for campus policy).

Transportation procedures: The bacteria used in this study will be transported in such a way that liquids are sealed (tubes with snap caps) and placed in a secondary container if they are in flasks. These leak-proof secondary containers will contain absorbent material, will be clearly labeled on the outside "Infectious Substance, Biohazard," and will be disinfected on the outside by spraying with 70% ethanol. The name and telephone of the PI or other responsible person(s) will be included on the outside of the outer container. Bacteria-laden agarose microbeads prepared at the designated Briggs Hall facilities will be transported by authorized personel to the Neurosciences facilities for intracranial injection in the animal model in said leak-proof primary and secondary containers. Dispensation and disposition of biological materials will be documented.

9. **Terminal inactivation and waste disposal.** Specify your methods for terminal inactivation of the biological agent. Be sure to include liquid disinfectant concentrations and exposure times (Appendix A). Specify dry waste bag color and markings, time period between waste bag setup and closure, autoclave temperature and contact time (Appendix A), and final disposition of autoclaved waste. Specify if you are generating medical waste.

Methods of terminal inactivation of Biological Agent: Chemical disinfectants to be used will include 10% household bleach and 70% ethanol (each made fresh daily).

1. Hard surfaces (bench tops, etc) will be sanitized with 70% ethanol at least once a day. After any spill of viable material, hard surfaces will be sanitized using 10% bleach (contact time 30 minutes).
2. All solid waste, including disposable plastic ware that comes in contact with potentially infectious material, will be disposed of in double-layered red medical waste bags contained within labeled, hard-walled, leak proof secondary waste containers. Small bench top waste containers are located inside Rm 255 Briggs. These containers are labeled with biohazard markings and are hard-walled, leak proof. Laboratory workers use these bench top containers for pipette tips and disposable centrifuge tubes. At the conclusion of a work session, or when the bench top waste container is full (whichever occurs first), the double-layered waste bags lining the bench top container are removed, twisted-tied closed, and placed inside a large, free-standing secondary waste container located on the floor. These large, freestanding containers are also labeled with biohazard markings, and are hard-walled, leak proof and have tight fitting covers. They are lined with a double layer of large medical waste disposal bags. Once per week the UC Davis Med Center personnel come and collect the large waste containers and take them for disposal. Once at the medical waste collection area, the large waste bags are transferred to the medical waste dumpster and each bag is logged into the medical waste logbook. All bags are labeled with the room number and the Principal Investigator's last name. The medical waste manifest describing the method of treatment and disposal of waste is returned to our lab and filed in a binder for future reference. All employees are trained in accordance with our campus medical waste management plan. Disposal of medical waste from surgical procedures is further provided in Neuroscience Rm 425.
3. All liquid waste within disposable plastic or non-disposable contaminated glassware/labware will be treated with household bleach (10% final concentration) for at least 30 minutes. Disinfection takes place in glass waste containers with a lid, which are kept in Rm 255 Briggs. After disinfection, the bleach solution is then disposed of with copious amounts of water in the sink in Rm 255 Briggs, in accordance with the UCD Medical Waste Management Plan. Laboratory workers are responsible for removing all liquid waste in a timely manner: after a period of no more than 7 days, or when the waste container becomes full, whichever comes first. If liquid waste is kept for more than 1 day, fresh bleach will be added upon the addition of more liquid waste. At the end of each workday, the work area is inspected, any remaining contaminated liquid is placed in the liquid waste container and the container is securely closed. Contaminated glassware is soaked in 10% bleach for at least 30 minutes before being washed with soapy water and returned to general lab use.

Limited infectious waste will be generated during the procedures carried out at Neuroscience Building, Newton Court. This waste will include used syringes and needles generated by surgical procedures on rats. The pathogenic organism is part of the normal human intestinal flora and is in Risk Category 1. Therefore, infectious waste, including needles and syringes, will be placed in red medical waste disposal containers and appropriate medical waste sharps containers. A room for disposal of medical waste is provided in Neuroscience room 425. Autoclaves for non-medical waste disposal are available in Neuroscience Rm 400.

10. Medical surveillance. Describe any medical surveillance or post-exposure treatment necessary to help protect the health of individuals who work on the project or who might otherwise be exposed to biohazardous materials in your facilities. Include lab and animal care staff, and if vertebrate animals are a component of your project ***attach a copy of the Room/Lab Safety Sheet from your Animal Care and Use protocol to this BUA application.*** If a standard UC Davis Employee Health Services-approved surveillance or treatment program is appropriate, include a one- or two-sentence description of the steps in surveillance and post-exposure treatment. Check the appropriate standard protocols box in Section 6 of this application.

The organism used is a normal intestinal commensal in humans and infection is extremely unlikely. Exposure to the organism through surface contact, splashes, or injestion is unlikely to result in illness and routine medical surveillance will not be performed. Lab personnel developing symptoms such as fever, chills, gastrointenstinal disturbance, cough, or neck stiffness will be referred immediately to the Employee Health Center. Enterobacter aerogenes infections are effectively treated with antibiotics. Laboratory personnel developing infection with Enterobacter aerogenes will be treated with appropriate medical care at no cost.

Section 4. Storage only. When a project covered under an approved BUA is completed and you have no plans to continue that investigative path you should transfer or destroy and dispose the biohazardous components or products of the work. To transfer biohazardous materials to another user, submit a BUA Amendment form that identifies your transfer destinations and transport methods. ***Biohazardous materials may only be transferred to on-campus users who have current Biological Use Authorizations that allow the possession of the specific biohazardous materials. They may not be transferred to a co-investigator or an "authorized user" on your BUA without prior BSAAC approval.*** Biohazardous materials may be transferred to non-UC Davis locations but you must first obtain a letter from the director of the receiving facility. The letter must authorize the transfer and acknowledge that the receiving institution will assume permanent ownership of the materials. The BSAAC will review your transfer plan and you may initiate the transfer upon notification of Committee approval. If you choose instead to inactivate and dispose your biohazardous materials, use the BUA amendment form to notify the Committee.

If you wish to store viable or usable stocks of biohazardous materials from completed experiments in your own laboratory and you do not anticipate using them for planned work, file an amendment to your existing BUA and indicate "storage only." Attach a list of all biohazardous materials (***including laboratory identification codes***) in storage that were previously approved in your BUA, and indicate the storage conditions and the ***precise*** locations of the stored materials (e.g., freezer shelf number and location on the shelf). To prepare biohazardous materials for storage, label the materials accurately (use general common names such as "lentiviral vector" in addition to laboratory codes), package them securely, identify the Principal Investigator, department, and storage date, and affix the universal biohazard label for Human Risk Group 2 or 3 agents including genetic material and recombinant constructs if appropriate. When the BUA that originally provided for use of those materials expires, you must renew the BUA, check "storage only" on the front page, and obtain all required signatures. Should you decide to resume work with "storage only" biohazardous materials, you must amend your BUA to detail the new work for BSAAC review. Use the regular BUA form and check "Major Change Amendment" if the same project covered in an earlier BUA is to be resumed, or check "New" if it is a new project with your stored materials. This system enables the Biosafety Office to track stored biohazardous materials that may otherwise eventually be forgotten.

Storage of biohazardous materials. Attach a complete list of biohazardous materials to be held in storage. Use the following format. Alternatively, attach a spreadsheet that provides the required information in a tabular format
Recombinant description or infectious agent species and strain Enterobacter aerogenes ATCC#13048 Laboratory identification codes TBD Risk Group (consult the Biosafety Office) 1 BUA for which the biohazardous materials were obtained or produced (if applicable) Storage conditions (Room temperature, 4°, -20 °, -80 °, LN_2) 4C Building Briggs Room TBD Location in laboratory and/or freezer (i.e. shelf #, box #, etc) TBD Locked? yes

Section 5. List of attachments to this application

- ☐ References on vector design (for vectors from non-commercial sources)
- ☐ Vector genetic map (for vectors from non-commercial sources)
- ☒ References on infectious agent naturally acquired drug resistance
- ☒ Animal Care and Use protocol Room/lab Safety Sheet (MANDATORY if the project includes vertebrate animal use)
- ☒ Animal Care and Use protocol list of authorized personnel (MANDATORY if the project includes vertebrate animal use)
- ☐ Facility floor plan (required only for insectaries)
- ☐ Select Agent required Biosafety plan
- ☐ USDA or other permit
- ☐ Letter from off-campus facility director authorizing permanent transfer of biohazardous materials to the off-campus site
- ☐ Other documents:

Section 6. Health protection, surveillance and post-exposure treatment programs to be used in this project

Consult Employee Health Services BEFORE completing the Biological Use Authorization application. All surveillance, vaccination, post-exposure treatment, and PPE clearance and fit-testing services are to be provided at no cost to the employee. Check all that apply:

☒ Health surveillance is not planned for this project

- ☐ **Bloodborne pathogens:** HBV vaccination (or declination), post-exposure follow-up and treatment, vaccination record retention by principal investigator, initial BBP training and annual retraining, and universal precautions
- ☐ **HIV post-exposure prophylaxis:** Post-exposure treatment with anti-retroviral drugs within two hours, with medical follow-up
- ☐ **Q-Fever:** Annual medical exams, serologic testing, vaccine use when available, respiratory protection and training
- ☐ **Orthopoxviruses (vaccinia and others):** Medical screening, vaccination and contraindication awareness, and training
- ☐ **Prion research:** Training and special procedures for exposure reporting, decontamination and records handling
- ☐ **Cercopithecine herpesvirus-1 (Herpesvirus simiae, Herpes B):** Initial training and post potential exposure follow-up and treatment
- ☐ **Serum sample banking:** Consult with Employee Health Services
- ☐ **Health history form:** Consult with Employee Health Services
- ☐ **Respirator clearance and fit-testing:** Clearance from Employee Health Services and fit-testing at Environmental Health and Safety
- ☐ **Custom health surveillance/immunization program will be adopted:** Please describe the plan in the appropriate headings of Section 2C, 2D, or 3. Be sure to obtain Employee Health Services approval before submitting the plan to the BSAAC.

This page is not subject to F.O.I.A. requests

(Freedom of Information Act)

Section 7. Authorized users (use additional sheets if necessary)

Note: Complete this page for ***all*** personnel involved with your project. If the project includes vertebrate animal use, *attach the list of authorized personnel from your Animal Care and Use protocol* to the BUA application. The Biosafety Office and the BSAAC will keep the information on this page and on the animal care protocol page confidential.

				Documented training			
Name	UCD ID[1]	Telephone	e-mail address (text will wrap)	Bio-safety[2]	BSC	BBP	MW
Principal Investigator Rudolph Schrot	753728112	(916)734-8824	rudolph.schrot@ucdmc.ucdavis.edu	☐	☐	☐	☐
Lab Contact Lorena Navarro	454936931	752-0260	lonavarro@ucdavis.edu	☒	☒	☒	☒
[redacted]			[redacted]	☐	☐	☐	☐
Ivy Jose	352243620	752-3641	irjose@ucdavis.edu	☒	☒	☒	☒
				☐	☐	☐	☐
				☐	☐	☐	☐
				☐	☐	☐	☐
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				☐	☐	☐	☐
				☐	☐	☐	☐

☐ **Check here if additional users are listed on a separate sheet**

[1] If not a UC Davis affiliate, specify "non-UC" or "non-UC Davis" but include requested ***local*** contact information

[2] **Biosafety**=Formal EH&S-provided or approved training in the principles and practices of biological safety, mandatory before beginning work

BSC=Biological Safety Cabinet training (mandatory for users of Biological Safety Cabinets)

BBP=Bloodborne pathogen exposure control training (if applicable, mandatory before beginning work, must be repeated annually)

MW=Medical Waste management training (if applicable, mandatory before beginning work, must be repeated annually)

Appendix A. UC Davis Policies and Standard Operating Procedures for work with biohazardous agents

Note: Your signature on Page 1 of the BUA Application affirms that you have reviewed the material in this appendix and agree to abide by all of its provisions.

1. **Training.** Only those persons who are adequately trained may work with biohazardous materials (human, animal, plant, environment) at UC Davis or its satellite laboratories. This standard includes all students, staff, and faculty involved with the project including the principal investigator, and includes visiting scholars and volunteers. Basic biosafety, bloodborne pathogen control, and medical waste management training is available from UC Davis Environmental Health and Safety (enroll at http://ehs.ucdavis.edu/train/classes/ index.cfm#bloodborne or call 530 752 1493). Training in these topics must be renewed annually. The EH&S class "Safe Work Practices in the Biological Safety Cabinet" is required for all users of these devices. More specialized training such as safe work practices in the Biosafety Level 3 laboratory and the Select Agent system may be available from the Campus Biosafety Office (530 752 1493). The principal investigator is responsible for all lab- and project-specific training, including experimental methods and techniques, specific hazards associated with the project components, methods employed to reduce the risks to an acceptable level, available project-specific medical surveillance and treatment, and training to use lab equipment. The training record should include a list of topics covered and materials used (such as the approved BUA), and the trainer and trainee should both sign the training record sheet. ***All training must be documented.*** A brief written quiz or some other method of evaluating trainee comprehension should also be included in the training and training documentation.

2. **Shipping and Receiving.** All shipments (domestic and international) of biological materials must follow university policy and all applicable federal and international regulations and permitting requirements. **Biohazardous materials may not be personally transported to or from campus unless specifically authorized by the BSAAC in your approved BUA and packaged to comply with current DOT, IATA, and public health agency guidelines, standards and regulations.** ***Biohazardous materials may not be transported in private vehicles.***

3. **Local Transport of Infectious Materials.** Intracampus transport of infectious materials must be specifically authorized by the BSAAC as part of your approved BUA protocol. Biohazardous materials transported between laboratories or to other on-campus facilities must be packaged in absorbent material (enough to absorb the entire liquid volume of the biohazardous material) in a primary leak-proof container with a sealed lid or top, which is enclosed in a secondary leak-proof, non-breakable container (e.g., a Coleman cooler) appropriately labeled with the biohazard symbol (for human biohazards). ***Biohazardous materials may not be transported in private vehicles.*** Any biohazardous or potentially biohazardous materials transported between campus and UC Davis satellite facilities will be escorted by a responsible staff or faculty member. Packaging and labeling must comply with the IATA dangerous goods guidelines or DOT hazardous materials regulations, and shippers must have documented current required training. Transport of Select Agents must comply with special provisions indicated in approved biosafety and security plans as well as current CDC, USDA-APHIS, and DOT rules.

4. **Personal Protective Equipment (PPE).** Wear appropriate PPE such as gloves, safety glasses and a laboratory coat whenever you work with biohazardous materials. Specific PPE requirements are determined by the risk assessment for the research.

5. **Footwear.** No open-toed or open-heeled shoes or sandals are allowed in the laboratory. A good practice is to keep a set of lab-dedicated closed-toed, closed-heeled shoes at the laboratory work site. Change into them when you arrive at the lab, and change back to street shoes when you are ready to leave the building.

6. **Hand washing.** After working with biohazardous materials remove your gloves immediately and wash your hands with soap and water. If soap and water are not available (such as in field work locations), use disinfectant hand wipes.

7. **Use of sharps.** Minimize the use of sharps with biohazardous materials. Never recap, bend or shear needles—use only hard-walled sharps containers and do not overfill. Wherever possible, replace glassware with plasticware. Keep sharps containers readily available in all locations where sharps waste may be generated. Any programmatic use of sharps in a biological safety

cabinet should be documented by a risk assessment that shows that no other alternative is acceptable and that details additional training to safeguard the users.

8. **Plastic sharps.** The Biological Safety Office recommends strongly that plastic pipettes and pipette tips contaminated with agents biohazardous to humans be disposed in a hard walled red sharps container. Other contaminated pipette tips may be disposed into secondary containers which can then be sealed and disposed into clear autoclave bags (for tips contaminated with other infectious agents or "biotechnology waste"). Medical waste bags used for pipette tip disposal must conform to the current Cal-OSHA bag color and strength standard. Medical waste must be disposed in the approved medical waste stream, and biotechnology waste (with no Medical Waste component as defined in the California Health and Safety Code) must be autoclaved before being disposed in the landfill.

9. **Food and Beverage.** Eating, drinking, storing food and drink for human consumption, smoking, applying cosmetics or lip balm and handling contact lenses in the laboratory are prohibited in all UC Davis laboratories.

10. **Aerosol Generation.** Procedures that could generate biohazardous aerosols must be performed in a certified biological safety cabinet. Experimental systems at BSL1 containment (no demonstrable biohazard) are exempted from this requirement.

11. **Safe use of biological safety cabinets.** Specific training in the safe use of biological safety cabinets is required for all users of these protective devices. EH&S offers a 1.5-hour class that satisfies this training requirement. Biological safety cabinets should be sited as far from doorways and common use walkways as possible. All biological safety cabinets must be certified under the NSF49 standard before first use, annually thereafter, and after the cabinet has been relocated or repaired. Biological safety cabinets used for containment of Risk Group 2 or 3 microbiological agents must be gas-decontaminated before being relocated or decommissioned. Only one person at a time may use a biological safety cabinet. Open flames are prohibited in biological safety cabinets. UV light may only be used in a biological safety cabinet if the user removes and cleans the UV tube weekly with 70% ethanol and replaces the UV tube annually. All such maintenance must be documented. Never work in a biological safety cabinet when the UV light is energized, and never operate the UV light with the sash open. Best practice: use the UV light only when the laboratory is unoccupied. Best practice of all: do not use the UV light—it is a mediocre to ineffective method of decontaminating biological safety cabinets and presents significant hazards to the laboratory occupants.

12. **Proper Labeling.** Place a universal biohazard label adjacent to the doorway of a lab where biohazardous materials that are infectious to humans are used. Also label work areas, containment cabinets, and equipment including freezers, refrigerators, incubators, centrifuges, shakers, etc. with the biohazard label.

13. **Decontamination Procedures.** Use a spray bottle of 10% solution of household bleach in water (made fresh daily) to decontaminate equipment and work surfaces. Where bleach could cause corrosion (stainless steel surfaces), use an iodophor such as Wescodyne., or wipe away the sprayed bleach and spray 70% ethanol on the surface. Decontaminate liquids by adding bleach to a final concentration of 10%, with a 30 minute contact time.

14. **Spills.**

    a. **Risk Group 2 or 3 agent spill outside of a biological safety cabinet:** Let the spill "settle" for at least 30 minutes—evacuate the laboratory and post signs on the doors to prevent re-entry before it is safe. Wear lab coat or Tyvek gown, gloves, goggles, and at least a surgical mask to clean biohazardous spills outside of a biological safety cabinet. Wear a properly fit-tested respirator (at least N95) to clean Risk Group 3 agent spills and Risk Group 2 agent spills if aerosol infection is possible (including plasmids with oncogenes). Distribute paper towels around the periphery of the spill, then towards the center. When the spill is fully contained, spray 10% bleach or other approved disinfectant on the paper towels, allow 30 minutes contact time, and clean up the paper towels with large forceps. Change gloves, and spray 10% bleach or other approved disinfectant on the surface residue. Wipe up the residue with paper towels and repeat at least once. Dispose all of the paper towel waste in a medical waste bag. Always maintain a biological spill kit in the laboratory that includes the items described in this section.
    b. **Risk Group 2 or 3 agent spill inside of a biological safety cabinet:** Always ensure that the bottom drain is closed before working at a biological safety cabinet. Use the same techniques described above regarding paper towel placement and disinfectant use, but a 30 minute wait for the initial spill to settle is usually not necessary unless some of the spill occurred

outside of the biological safety cabinet. If 10% bleach is used to decontaminate the spill on a stainless steel surface, follow with sprayed water or 70% ethanol and wipe dry with paper towels.

15. **Mouth Pipetting.** Mouth pipetting may lead to accidental ingestion of biohazardous material and is strictly prohibited.

16. **Storage.** Store all biohazardous materials in containers clearly labeled with the universal biohazard symbol. Label permanently stored biohazardous material with common names wherever possible, in addition to lab-specific codes.

17. **Waste.** If your work results in the production of medical waste (materials in contact with human and non-human primate tissue, other waste known or suspected to harbor human infectious agents or potentially harbor such agents naturally), you must adopt a Medical Waste Management plan that includes the use of sturdy red plastic bags imprinted with the universal biohazard symbol and hard-walled red sharps containers. The red-bagged waste must be autoclaved in a unit certified to handle medical waste or must be transported to a medical waste accumulation site for disposal in the medical waste stream. **Research lab dry waste known or suspected to harbor other types of infectious agents (animal and plant) must also be accumulated in sturdy (non-red) bags and autoclaved (autoclave maintained at the medical waste standard is recommended) before disposing to the landfill.** Deface all biohazard symbols before disposing autoclaved biohazardous waste to the landfill. "Biotechnology waste" (with NIH-exempted cloning hosts such as *E. coli* K-12 or *Saccharomyces cerevisiae* but free of all infectious agents) can be accumulated in clear autoclave bags (deface any imprinted biohazard symbols) and must be autoclaved before disposal to the landfill. As discussed above, liquid waste of any kind that is contaminated or potentially contaminated with any viable agent (infectious or non-infectious) must be decontaminated by 30 minutes exposure to 10% (final concentration) household bleach before it is disposed in the sanitary sewer.

18. **Autoclaves and autoclave safety.** The standard autoclave sterilization process is 30 minutes exposure at 121°C (250° F). Large volumes of material require longer exposure times. Always use a sterilization indicator such as autoclave tape. Prion inactivation requires longer exposure at higher temperature, usually preceded by exposure to strong alkali. Always wear heat-resistant gloves, goggles or safety glasses, and a laboratory coat when opening an autoclave. Be sure to allow the superheated steam to dissipate before attempting to remove the autoclave contents.

19. **Injuries and health emergencies.** Report all injuries and accidental autoinoculation, ingestion or inhalation of infectious agents to the lab director or supervisor, EH&S (530 752 1493) and the UC Davis Occupational Health physician for evaluation and possible treatment. Dial 911 (530 752 1230 from cell phones) immediately for any medical emergency. After normal business hours and on weekends Sutter Davis Hospital handles UC Davis employee health emergencies (call 911 or 530 752 1230 from cell phones).

20. **Emergencies.** During natural disasters, fires, power failures, bomb threats, major biohazardous spills, or other emergencies, take the following precautions and evacuate the lab by posted or ordered evacuation routes
    a. Secure infectious materials as quickly as possible. If a biological safety cabinet is being used, close all containers and if possible close the sash.
    b. Call 911 (or 530 752 1230 from cell phones) and request emergency response.
    c. When the incident is resolved, if the building is safe to enter (at the direction of the incident commander), proceed to the lab, don appropriate PPE, and assess the lab for the disaster-related release of infectious material. Use the above spill control procedures to contain released material.

Appendix B. Select Agents and Toxins

HHS SELECT AGENTS AND TOXINS

Abrin
Cercopithecine herpesvirus 1 (Herpes B Virus)
Coccidioides posadasii
Conotoxins
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Ebola virus
Lassa fever virus
Marburg virus
Monkeypox virus
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
Ricin
Rickettsia prowazekii
Rickettsia rickettsii
Saxitoxin
Shiga-like ribosome inactivating proteins
South American Haemorrhagic Fever viruses
- Flexal
- Guanarito
- Junin
- Machupo
- Sabia

Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses
- Central European Tick-borne encephalitis
- Far Eastern Tick-borne encephalitis
- Kyasanur Forest disease
- Omsk Hemorrhagic Fever
- Russian Spring and Summer encephalitis

Variola major virus (Smallpox virus) and
Variola minor virus (Alastrim)
Yersinia pestis

OVERLAP SELECT AGENTS AND TOXINS

Bacillus anthracis
Botulinum neurotoxins
Botulinum neurotoxin producing species of *Clostridium*
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei (formerly *Pseudomonas mallei*)
Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*)
Clostridium perfringens epsilon toxin
Coccidioides immitis
Coxiella burnetii
Eastern Equine Encephalitis virus
Francisella tularensis
Hendra virus
Nipah virus
Rift Valley fever virus
Shigatoxin
Staphyloccal enterotoxins
T-2 toxin
Venezuelan Equine Encephalitis virus

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus
Akabane virus
Avian influenza virus (highly pathogenic)
Bluetongue virus (Exotic)
Bovine spongiform encephalopathy agent
Camel pox virus
Classical swine fever virus
Cowdria ruminatium (Heartwater)
Foot-and-mouth-disease virus
Goat pox virus
Japanese encephalitis virus
Lumpy skin disease virus
Malignant catarrhal fever virus
(Alcephaline herpesvirus type 1)
Menangle virus
Mycoplasma capricolum/M.F38/M. mycoides Capri
(contagious caprine pleuropneumonia)
Mycoplasma mycoides mycoides
(contagious bovine pleuropneumonia)
Newcastle disease virus (velogenic)
Peste des petit ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Vesicular stomatitis virus (Exotic)

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS

Candidatus Liberobacter africanus
Candidatus Liberobacter asiaticus
Peronosclerospora philippinensis
Ralstonia solanacearum race 3, biovar 2
Schlerophthora rayssiae var *zeae*
Synchytrium endobioticum
Xanthomonas oryzae pv. *oryzicola*
Xylella fastidiosa (citrus variegated chorosis strain)

Genetic elements, recombinant nucleic acids, and recombinant organisms listed below are regulated as Select Agents:

- Select Agent viral nucleic acids (synthetic or naturally derived, contiguous or fragmented, in host chromosomes or in expression vectors) that can encode infectious and/or replication competent forms of any of the Select Agent viruses.
- Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the listed toxins if the nucleic acids: a) are in a vector or host chromosome, b) can be expressed in vivo or in vitro; or c) are in a vector or host chromosome and can be expressed in vivo or in vitro.
- Listed viruses, bacteria, fungi, and toxins that have been genetically modified, with the exemptions noted below.

Exemptions:

- Medical use of pharmaceutical preparations of select toxins for patient treatment is exempt.
- Other exempted agents and toxins include:
  - Any agent or toxin that is in its naturally occurring environment provided it has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.
  - Non-viable Select Agent organisms or nonfunctional toxins.
  - Exempt vaccine and attenuated strains--consult the exempted select agent web site: http://www.cdc.gov/od/sap/sap/exclusion.htm

Exempt quantities of Select Toxins registration

Investigators who plan to use "exempt" quantities of Select Toxins (Table 3) for research at UC Davis must do the following:

1. Notify your Environmental Health and Safety Department Safety Adviser by e-mail, and include the following information:
Principal investigator/lab information:

- Principal investigator name
- Principal investigator department
- Principal investigator lab location (building, room)
- Principal investigator contact information (day, night, emergency)
- List of authorized users, with UC Davis ID, e-mail and telephone
- Date of most recent training in safe handling of the toxin in that experimental system for each user
- Name of the person who provided the training

Toxin information:

- Toxin name
- Maximum exempt possession limit under the Select Toxin rules
- Maximum quantity to be possessed
- Commercial source of the toxin. If the toxin is to be received from a non-commercial source, the name, address, and contact information for the source must be specified.
- Brief outline of the intended use of the toxin (2-3 sentences)
- Safety equipment available for the safe handling of the toxin (fume hood, glove box, etc)
- Required personal protective equipment (gloves, lab coat, etc)
- Symptoms of significant exposure to the toxin
- Toxin storage site
- Method of inactivation and disposal

NOTE: separate registration is required for each Select Toxin.

2. The principal investigator must report the maximum quantity in possession annually in the Chemical Inventory System (CIS) for that lab.

The Department Safety Adviser forwards the information to the Biosafety Office, and the Biosafety Officer notifies the Biological Safety Administrative Advisory Committee of the toxin registration during the next meeting. The Biosafety Officer will communicate any Committee questions to the principal investigator and will restate the information provided originally by the principal investigator in a registration letter to the Principal investigator. The principal investigator is responsible for updating the information on file with the Biosafety Office (through the DSA), and the principal investigator's inventory is subject to on-scene audit by the Biosafety Officer. Principal investigators are not currently required to keep special inventory records. Registration expires when the principal investigator permanently ceases working with select toxins and inactivates remaining stocks by an approved process (toxins may not be transferred to another user). **These rules do not permit the culture of select toxin-secreting agents for the purposes of obtaining select toxins, nor do they permit extraction of select toxins from natural sources.** Those activities require Biological Safety Administrative Advisory Committee approval and may also require registration with the Select Agent program.

HHS Toxins	Amount
Abrin	100 mg
Conotoxin	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Shiga-like ribosome inactivating proteins	100 mg
Tetrodotoxin	100 mg
HHS/USDA Overlap Toxins	**Amount**
Botulinum neurotoxins	0.5 mg
Staphylococcal enterotoxins	5.0 mg
Clostridium perfringens epsilon toxin	100 mg
Shigatoxin	100 mg
T-2 toxin	1000 mg

Table 3. Exempt possession limits of HHS and HHS/USDA overlap select toxins

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Please submit completed form, organization profile and Material Transfer Agreement to:

sales@atcc.org

P.O. Box 1549
Manassas, VA 20108-1549 USA

Fax: 703-365-2750

New Account Application BSL 1

Print Form

New Accounts are subject to approval. Print or type all information to be legible.

All information gathered will be used for ATCC purposes only. Your information will not be shared with any outside organization. Please allow 3 to 5 business days after receipt of completed application for account approval.

***Required Information**

Organization/Institution Information			
Organization Name*		Employer Identification Number (EIN)*	
Department		Web Site Address	
Check type of organization:* ☐ University/Education ☐ Research Foundation ☐ U.S. Government ☐ Hospital/Clinic	☐ Diagnostic Lab ☐ Pharmaceutical/Drug Discovery ☐ Biotechnology/Life Science ☐ Contract Laboratory	☐ Industrial ☐ Manufacturing ☐ Food Processing/Agriculture ☐ Environmental	☐ International ☐ Government Purchasing Agent (**billing only**)
☐ **If you work at a private, nonprofit organization, attach a copy of the Federal 501(c)(3) Form**			
If your organization is exempt from state and local sales and use tax – please provide ATCC a copy of your Tax Exempt Certificate issued by the state.			
Billing Address (Invoices will be sent to this address) **Please verify this information with the accounts payable department for your organization.**			
Contact Name (**Complete first & last name**)			
Department	Building	Room Number	
Street Address/P.O. Box*		City*	
State/Province*	Zip/Postal Code*	Country*	
Telephone (including Country Code)*	Fax (including Country Code)*	E-mail (of contact name)*	
Shipping Address (Complete street address; PO Boxes are not acceptable)			
Department	Building	Room Number	
Street Address (PO Boxes cannot be accepted)*			
State/Province*	Zip/Postal Code*	City*	
Telephone (including Country Code)	Fax (including Country Code)	E-mail*	
Applicant Information (Primary End User)			
First Name*	Last Name*	Title	
Department	Building	Room Number	
Street Address*		City*	
State/Province*	Zip/Postal Code*	Country*	
Telephone (including Country Code)*	Fax (including Country Code)*	E-mail*	
Additional End User Information			
First Name	Last Name	Title	
Department	Building	Room Number	
Street Address		City	
State/Province	Zip/Postal Code	Country	
Telephone (including Country code)	Fax (including Country code)	E-mail	

Additional End User Information		
First Name	Last Name	Title
Department	Building	Room Number
Street Address		City
State/Province	Zip/Postal Code	Country
Telephone (including Country code)	Fax (including Country code)	E-mail

Product Use
Please provide a scope of use for materials.*

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. The following standard practices apply to BSL-1 (Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009).

- Access to the laboratory is controlled and enforced.
- Laboratory personnel are trained on the hazards associated with handling the material and on standard microbiological practices prior to beginning work and at least annually thereafter.
- Laboratory personnel are supervised by a scientist with training inn microbiology or a related science.
- Personal protective equipment is provided and use is enforced.
- Decontamination procedures are in place and enforced for work surfaces, spills and biohazardous waste.

I acknowledge that these policies apply to this facility and are enforced by our institutional policies and procedures.

X_____
Biosafety Officer or Environmental Officer (Print)

X_____
Biosafety Officer or Environmental Officer's Signature and Date

Additional Required Information*

- **Material Transfer Agreement (MTA)***
  The MTA must be completed and signed by a duly authorized individual of your organization. The MTA can be found on our website at www.atcc.org. See attached, dated 7/30/08.
- **Organization Profile***
  On company letterhead, include a brief description of your organization, such as the mission statement or 'About Us' page from your organization's website, along with your completed application.
- **Curriculum Vitae**
  A Curriculum Vitae may be required.

ATCC | THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED

Internal use only
Account #:_____
EIN:_____
Date:_____

Credit References*

US Trade References only. Do not include banks, insurance companies, customs brokers or shipping companies. Do not use Thermo-Fisher Scientific or Sigma Aldrich- they do not provide references. Credit References are only necessary if your institution does not have an ATCC account number. (Academic institutions only require one credit reference. US Government agencies are exempt from this requirement.)

Credit Reference 1*			
Organization Name	Contact Person		Account No.
Street Address			
City	State	Zip Code	Country
Telephone (including Country Code)	Fax (including Country Code)		E-mail
Credit Reference 2*			
Organization Name	Contact Person		Account No.
Street Address			
City	State	Zip Code	Country
Telephone (including Country Code)	Fax (including Country Code)		E-mail
Credit Reference 3*			
Organization Name	Contact Person		Account No.
Street Address			
City	State	Zip Code	Country
Telephone (including Country Code)	Fax (including Country Code)		E-mail

See attached Credit References sheet

Orders are subject to the ATCC Material Transfer Agreement. See our website for more information at www.atcc.org. * as attached
Credit Terms Net 30. To ensure proper credit of your payment, please include a purchase order number for reference. If the total of your initial order exceeds $1,500.00, the order must be prepaid. Payments may be made by check, wire transfer, credit card or payment in advance as requested. Purchaser is responsible for all taxes, duties, tariffs and permit fees assessed in connection with ATCC Material. ATCC will package the ATCC Material for shipping in accordance with applicable laws and regulations. Purchaser is responsible for ensuring that all permits required for Purchaser to receive its order are obtained and that sufficient proof of such permits is provided to ATCC. A processing fee will be charged if special processing or packaging is necessary. All ATCC Materials are shipped Freight on Board (FOB) Manassas, freight prepaid via carrier of ATCC's choice and added to Purchaser's invoice. If the ATCC Material is lost or damaged during shipment, ATCC will replace such ATCC Material at no additional charge, provided that Purchase has reported lost or damaged shipments to the applicable carrier and notified ATCC's Customer Service Department or exclusive distributor within fourteen (14) days from invoice date. Each invoice will be mailed the following day after ATCC Material is shipped from the point of shipment.

ATCC accepts:

1) Visa, MasterCard, or American Express. The account number, expiration date, name as it appears on card, and signature are required.
2) U.S. Currency by check drawn on any U.S. bank or international money order made payable to:
   American Type Culture Collection, 5779 Collections Center Dr., Chicago, IL 60693
3) Money wire transfer to our account #003933990352, ABA#052001633 at Bank of America, N.A., Baltimore, MD.

We authorize the above listed to provide a credit reference to ATCC. We also acknowledge that in the event our account becomes delinquent, ATCC may report to credit agencies and/or forward our account to a collections service.

Authorized By: (please print/type) X_____

Title: X_____

Signature: X_____ **Date:** _____

Application Completed By: (please print/type) **X**_____

Title: X_____

Signature: X_____ **Date:** _____

If you have questions regarding the status of your application, contact us by phone at 800-638-6597 or 703-365-2700, or by e-mail at sales@atcc.org.

UNIVERSITY OF CALIFORNIA, DAVIS

BERKELEY • DAVIS • IRVINE • LOS ANGELES • MERCED • RIVERSIDE • SAN DIEGO • SAN FRANCISCO SANTA BARBARA • SANTA CRUZ

ACCOUNTING AND FINANCIAL SERVICES
ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8504
(530) 757-8501

CREDIT REFERENCES

Purchasing Department
202 Cousteau Place, Suite 205, Davis, CA 95618
Telephone: (530) 752-0370
Fax: (530) 757-8720

Business Name: University of California, Davis
Corporate Name: The Regents of the University of California
Business Type: Governmental Corporation – State of California
Date Business Started: 1868
Dunn & Bradstreet Number: 04-712-0084
Federal Taxpayer Identification Number: 94-6036494

Principal Officers:

Mark G. Yudof
President
University of California
1111 Franklin, 12th Floor
Oakland, CA 94607-5200

Linda Katehi
Chancellor
University of California
Davis, CA 95616
(530) 752-2065

John Meyer
Vice Chancellor – Administration
University of California
Davis, CA 95616
(530) 752-7941

Bank References:

Wells Fargo Bank
Government, Education & Labor Division
MAC A0112-102
550 California Street; 10th Floor
San Francisco, CA 94104
Account: 4759606791
(877) 275-7852

Bank of America
Credit Inquiry Services
P.O. Box 34893
Seattle, WA 98124
Account 12337-14115
(415) 343-9301

Trade References:

OfficeMax
1555 River Park Drive, #211
Sacramento, CA 95815
Contact – Jennifer Chalumeau
jenniferchalumeau@officemax.com
Phone - (916) 207-3740
Fax - (910) 922-3219

Fisher Scientific
2000 Park Lane Drive
Pittsburg, PA 15275
Contact – Renae Swanson
Renae.Swanson@thermofisher.com
Phone - (412) 490-8429
Fax - (412) 490-5752

Merry X-Ray
1045 National Drive, Suite 9
Sacramento, CA 95834
Contact – Ashley Eyster
Phone - (916) 928-0450
Fax - (916) 928-0453

Office of the Treasurer of the Regents authorizes credit term agreements for the University of California. Vendor requests for completion of credit applications or agreements must be forwarded to the Purchasing Department, University of California, Davis, Telephone (530) 752-0370. The above information is herewith submitted for the purpose of opening an account, and I do hereby certify this information to be accurate and true.

J. Michael Allred
Associate Vice Chancellor, Finance/Controller

May 2010

MATERIAL TRANSFER AGREEMENT ("MTA")

ATCC

Last updated July 30, 2008

IMPORTANT! PLEASE READ CAREFULLY BEFORE SUBMITTING AN ORDER. THIS IS A CONTRACT.

This Material Transfer Agreement ("MTA") is between The Regents of the University of California, as represented by its Davis campus having its statewide administrative offices at UC Davis Innovation Access – Technology Transfer Services; 1850 Research Park Drive, Suite 100; Davis CA 95618-6134 ("Purchaser") and the American Type Culture Collection, a not-for-profit organization, having its principal place of business at 10801 University Boulevard, Manassas, VA 20110-2209 ("ATCC"). Purchaser must have an approved, current ATCC account to place an order. This MTA is effective for a period of five (5) years as of the last date of execution by the parties and governs the purchase and use of all ATCC Materials under the terms and conditions set forth below.

TERMS AND CONDITIONS

Definitions

"**ATCC Material(s)**" means materials acquired from ATCC as documented on an ATCC Sales Order.

"**ATCC Sales Order**" means an order submitted for ATCC Materials in a form and format as determined by ATCC from time to time.

"**Biological Material(s)**" means ATCC Materials, Progeny, Unmodified Derivatives and Modifications, either individually or jointly.

"**Commercial Use**" means the sale, license, lease, export, transfer or other distribution of the Biological Materials to a third party for financial gain or other commercial purposes and/or the use of the Biological Material: (a) to provide a service to a third party for financial gain; (b) to produce or manufacture products for general sale or products for use in the manufacture of products ultimately intended for general sale (c) in connection with ADME (Absorption, Distribution, Metabolism and Excretion) testing; (d) in connection with drug potency or toxicity testing which does not include either screening multiple cell lines for potential inclusion in a screening assay system or screening multiple compounds in a system for internal research purposes only; (e) in connection with proficiency testing service(s), including but not limited to, providing the service of determining laboratory performance by means of comparing and evaluating calibrations or tests on the same or similar items or materials in accordance with predetermined conditions; or (f) for research conducted under an agreement wherein a for-profit entity receives a right whether actual or contingent to the results of the research. Commercial Use specifically does not include Industry Sponsored Academic Research.

"**Contributor(s)**" means an organization(s) and/or individual(s) providing original material to ATCC for deposit.

"**Industry Sponsored Academic Research**" means research sponsored by a for-profit organization carried out at a non-profit organization and by the non-profit organization's employees.

"**Investigator**" means the Purchaser's principal scientist or researcher using the Biological Material(s).

"**Modification(s)**" mean substances created by Purchaser which contain and/or incorporate a significant or substantial portion of ATCC Material.

"**Progeny**" means an unmodified descendant from the ATCC Materials, such as virus from virus, cell from cell, or organism from organism.

"**Purchaser(s)**" means the organization purchasing and receiving ATCC Material pursuant to this MTA.

"**Unmodified Derivative(s)**" mean substances created by Purchaser that constitute an unmodified functional sub-unit or product not changed in form or character and expressed by the ATCC Material provided by ATCC. Unmodified Derivatives include, but are not limited to, subclones of unmodified cell lines, purified or fractionated subsets of materials provided by ATCC, proteins expressed by DNA/RNA supplied by ATCC, or monoclonal antibodies secreted by a hybridoma cell line.

Scope of Use

Subject to the terms of this MTA, Purchaser's Investigator may make and use the Biological Materials provided to Purchaser by ATCC *for research purposes in Purchaser's Investigator's laboratory only*. The Biological Materials are not intended for use in humans. Purchaser agrees that Biological Materials designated as biosafety level 2 or 3 constitute known pathogens and that other

Biological Materials not so designated may be pathogenic under certain conditions. Purchaser assumes all risk and responsibility resulting from the Purchaser's receipt, handling, storage, disposal, transfer and Purchaser's use of the Biological Materials including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. Purchaser agrees that any activity undertaken with the Biological Materials will be conducted in compliance with all applicable guidelines, laws and regulations, and that Purchaser will obtain all permits, licenses or other approvals required by any governmental authority in connection with Purchaser's receipt, handling, storage, disposal, transfer and use of the Biological Materials.

Purchaser shall not distribute, sell, lend or otherwise transfer to a third party the Biological Material, as defined above, for any reason, without ATCC's prior written agreement.

Any Commercial Use of the Biological Material is strictly prohibited without ATCC's prior written consent. Purchaser acknowledges and agrees that Purchaser's use of certain Biological Material may require a license from a third party, or be subject to third party restrictions ("Third Party Terms"). ATCC's catalog of ATCC Materials and/or customer service representative will identify any Biological Material that is subject to such Third Party Terms, and ATCC shall make such Third Party Terms reasonably available for review by Purchaser upon request. Purchaser expressly acknowledges that if there is a conflict between this MTA and the Third Party Terms, the Third Party Terms shall govern. Use of the Biological Materials may be subject to the intellectual property rights of a third party not listed in the ATCC catalog or website, and ATCC makes no representation or warranty that such rights do not exist. Purchaser shall have the sole responsibility for obtaining any intellectual property licenses necessitated by its possession and use of the Biological Materials. Notwithstanding anything in this paragraph, all distributions of ATCC patent deposits are made pursuant to, and in compliance with, all Budapest Treaty or 37 C.F.R. requirements.

The use permitted under this Agreement for Industry Sponsored Academic Research extends only to the academic research carried out at the non-profit organization and the non-profit organization's employees. Any non-profit Purchaser using the Biological Materials in connection with Industry Sponsored Academic Research shall notify the industry sponsor that any use of the Biological Materials by the industry sponsor will require a separate license from ATCC and/or its Contributors and that ATCC and/or its Contributors are under no obligation hereunder to license any Biological Materials to any such industry sponsor.

Warranty; Warranty Disclaimer

ATCC warrants that (a) cells and microorganisms included in the ATCC Material shall be viable upon initiation of culture for a period of thirty (30) days after shipment thereof from ATCC and (b) any ATCC Material other than cells and microorganisms shall meet the specifications on the applicable ATCC Material product information sheet, certificate of analysis, and/or catalog description until the expiration date on the applicable ATCC Material's product label (such thirty (30) day period, or period until the expiration date, referred to herein as the "Warranty Period"). Purchaser's exclusive remedy, and ATCC's sole liability, for breach of the warranties set forth in this paragraph is for ATCC to, at ATCC's sole option, either (i) refund the fee paid to ATCC for such ATCC Material (exclusive of shipping and handling charges), or (ii) replace the ATCC Material. The warranties set forth in this paragraph apply only if Purchaser handles and stores the ATCC Material as described in the applicable ATCC Material product information sheet. To obtain the exclusive remedy, Purchaser must report the lack of viability or non-conformation to specifications to ATCC's Technical Service Department within the applicable Warranty Period. Any expiration date specified on the ATCC Material shipment documentation states the expected remaining useful life, but does not constitute a warranty or extend any applicable Warranty Period. **Except as expressly provided above, the ATCC Material and any technical information and assistance provided by ATCC are provided as-is, without warranties of any kind, express or implied, including but not limited to any implied warranties of merchantability, fitness for a particular purpose, typicality, safety, accuracy and/or non-infringement.**

Compliance With Laws

Purchaser is solely responsible for compliance with all foreign and domestic, federal, state and local statutes, ordinances and regulations applicable to use of the Biological Materials. Without limiting the generality of the foregoing, any shipment of Biological Materials to countries outside the United States

must comply with all applicable foreign and U.S. laws, including the U.S. export control laws and related regulations.

Indemnification

If Purchaser is a for-profit or private non-profit organization:
Purchaser hereby agrees to indemnify, defend and hold harmless ATCC and its Contributors against all third party claims, losses, expenses and damages, including reasonable attorneys' fees (collectively "Claims") caused by Purchaser's use, receipt, handling, storage, transfer, disposal and other activities, provided that Purchaser's liability shall be limited to the extent that any such Claim arises out of ATCC's gross negligence or willful misconduct. All non-monetary settlements of any such Claims are subject to ATCC's prior written consent, such consent not to be unreasonably withheld.

If Purchaser is a Federal or State non-profit organization or a foreign organization that is prohibited by law from entering into the indemnification obligation set forth in the above paragraph:
Purchaser assumes all liability for any and all third party claims, losses, expenses and damages, including reasonable attorneys' fees (collectively "Claims") arising out of or relating to Purchaser's use, receipt, handling, storage, transfer, disposal and other activities relating to Biological Materials, provided that Purchaser's liability shall be limited to the extent that any such Claim arises out of ATCC's gross negligence or willful misconduct, and provided further that if the Purchaser is the U.S. federal government or a state institution such Purchaser assumes such liability only to the extent provided under the Federal Tort Claims Act, 28 U.S.C. §§ 2671 et seq. or under equivalent applicable State or foreign law.

Limitation of Liability

In no event will ATCC or its Contributors be liable for any indirect, special, incidental or consequential damages of any kind in connection with or arising out of the MTA or Purchaser's use of Biological Materials (whether in contract, tort, negligence, strict liability, statute or otherwise) even if ATCC has been advised of the possibility of such damages. In no event shall ATCC's cumulative liability exceed the fees paid by Purchaser under this MTA for the twelve (12) month period preceding the date of the event giving rise to the claim. Purchaser agrees that the limitations of liability set forth in this MTA shall apply even if a limited remedy provided hereunder fails of its essential purpose.

Intellectual Property; Identification

As between the parties, ATCC and/or its Contributors shall retain ownership of all right, title and interest in the ATCC Materials, Progeny, Unmodified Derivatives and ATCC Materials contained or incorporated in Modifications. Purchaser retains ownership of: (a) Modifications (except that, as between the parties, ATCC retains ownership rights to ATCC Material included therein) and (b) those substances created through the use of ATCC Material, but which do not contain ATCC Material. Notwithstanding the foregoing, Purchaser acknowledges and agrees that the Biological Materials are subject to the restrictions noted in the "Scope of Use" section above. Purchaser agrees to acknowledge ATCC and any Contributor indicated by ATCC as the source of the Biological Material in all research, academic or scholarly publications and in patent applications that reference the Biological Material. If required by the Contributor of the ATCC Material, ATCC may inform the Contributor of Purchaser's identity. Purchaser explicitly acknowledges that ATCC retains all right, title and interest in the ATCC trademarks, trade-names, logos, ATCC catalog numbers and ATCC specific designations of ATCC Materials sold by ATCC (including but not limited to UNIPLUS™, YOUR DISCOVERIES BEGIN WITH US®, THE GLOBAL BIORESOURCE CENTER™, Authenticult™, SafeTsource™, ATCC CULTURES™, ATCC BIOPRODUCTS™, ATCC SPECIAL COLLECTIONS™, ATCC SERVICES™, ATCC Genuine Cultures®, ATCC Licensed Derivative®, BioEscrow®, ATCC Standards Resource®, ATCC Proficiency Standard®, ATCC Standard Reference Material™). Except as maybe required or professional expected in a publication or a research of a patent filing, Purchaser expressly agrees not to use the ATCC trademarks, trade-names, logos, ATCC catalog numbers or ATCC specific designations of ATCC Materials sold by ATCC in any way without ATCC's prior written agreement.

Payment; Taxes; Shipping

Payments may be made by check, wire transfer or credit card. Unless payment in advance is required by ATCC or its exclusive distributors, payments due to ATCC or its exclusive distributors shall be invoiced to Purchaser and due within thirty (30) days after the date of invoice. In the absence of payment of outstanding invoices sixty (60) days after the date of receipt of invoice by Purchaser, ATCC will place Purchaser's accounts on hold until payment of such invoices. Purchaser is responsible for all taxes, duties,

tariffs and permit fees assessed in connection with this MTA and the ATCC Material. Purchaser shall, upon demand, pay to ATCC or its exclusive distributors an amount equal to any such tax(es), duties, tariffs and permit fees actually paid or required to be collected or paid by ATCC or its exclusive distributors. ATCC and/or its exclusive distributors shall have no obligation hereunder to accept an order from Purchaser unless Purchaser has satisfied the requirements of ATCC's applicable credit approval process and has satisfied any additional credit requirements imposed by ATCC, which may include providing ATCC with a deposit, letter of credit, or payment in advance, as requested.

ATCC will package the ATCC Material for shipping in accordance with applicable laws and regulations. Purchaser is responsible for ensuring that all permits required for Purchaser to receive its order are obtained and that sufficient proof of such permits is provided to ATCC. ATCC will notify Purchaser when orders are submitted without the necessary permits, and Purchaser will have a two (2) month period after such notification to supply proof of the necessary permit(s) before an order will be cancelled. A processing fee will be charged if special processing or packaging is necessary. All ATCC Materials are shipped Freight on Board (FOB) point of shipment, freight prepaid via carrier of ATCC's choice and added to Purchaser's invoice. If the ATCC Material is lost or damaged during shipment, ATCC will replace such ATCC Material at no additional charge, provided that Purchaser has reported lost or damaged shipments to the applicable carrier and notified ATCC's Customer Service Department or exclusive distributor within fourteen (14) days from invoice date. Each invoice will be mailed the following day after ATCC Material is shipped from the point of shipment.

Miscellaneous

Any disputes arising under this Agreement shall be tried exclusively in a court of competent jurisdiction.

Purchaser agrees that any breach of this contract, including but not limited to any breach of the scope of use provisions of this contract, will entitle ATCC to immediately cease without notice to Purchaser further shipments of Biological Materials and may create such irreparable injury as to entitle ATCC to temporary restraining orders and other preliminary or permanent injunctive relief in addition to all other equitable and legal remedies that may be afforded under U.S. or foreign laws.

Purchaser may not assign or otherwise transfer this MTA or any rights or obligations under this MTA, whether by operation of law or otherwise. Any such attempted assignment or transfer will be void and of no force or effect. This MTA, including all documents incorporated herein by reference, constitutes the entire agreement between ATCC and Purchaser with respect to the Biological Material and supersedes all previous agreements or representations (whether written or oral) between ATCC and Purchaser relating to the same subject matter. This MTA may not be modified, waived or terminated except in writing and signed by the parties hereto. No term or provision contained herein shall be deemed waived and no breach excused unless such waiver or consent shall be in writing and signed by the parties. If any provision of this MTA is for any reason found to be unenforceable, the remainder of this Agreement will continue in full force and effect. None of the provisions of this MTA are intended to create, nor shall be deemed or construed to create, any relationship between ATCC or Purchaser other than that of independent entities contracting with each other hereunder solely for the purpose of effecting the provisions of this Agreement.

SIGNATURES ON FOLLOWING PAGE

For ATCC

Digitally signed by Gary D. Meyer
Date: 2008.07.30 11:31:23 -04'00'

By: _____

Name: Gary D. Meyer, MBA

Title: Director, IP, Licensing and Services

Date: July, 30 2008

For PURCHASER

By: _____

Name: Rafael A. Gacel

Title: Associate Director
Technology Transfer Services

Date: July 30, 2008

Any correspondence concerning the ATCC Material Transfer Agreement should be addressed to ATCC, Attention: Office of IP, Licensing and Services, P.O. Box 1549, Manassas, VA 20108, Phone: (703) 365-2700 or contact us at licensing@atcc.org

MICROBIOLOGY
Order Request

P.O.# 3BMICB1075
FACULTY Navarro
DOC# 0135496O2
For office Use Only

PLEASE WRITE LEGIBLY

VENDOR: (Please give complete information)
ATCC
10801 Univ. Blvd
Manassas, VA 20110-2209
(city) (state) (zip)
Phone 800-638-6597
Fax#

YOUR NAME Lorena Navarro Date 4/2/09
LAB PHONE 2-0260 If Rush Date Needed By
ACCOUNT# 4973103-
5SEED
Will pick up from Vendor ☐
Check if radioactive ☐ RUA#
PROJECT

IS THIS A REQUEST FOR EQUIPMENT? _____ SOLE SOURCE? IF YES AN ADDITIONAL FORM IS REQUIRED.

IF THIS IS AN ADD-ON TO EXISTING EQUIPMENT, PLEASE LIST EQUIPMENT NAME, UC PROPERTY #, AND SERIAL #:

4/2 TP

Quan	Unit	Catalog no.	Description	pg#	Cost Unit	Cost Total	B/O
1	EA	13048	E. aerogenes		33.30	33.30	

Subtotal	33.30
Tax	6.79
Shipping	49.00
Total	89.09

pd

For Office Use Only Date 4/2/09
Phone in to David By Ellen
Priced by David
Faxed to
Emailed By
Mailed By FOB
Comments

Shipping date 4/3
Ship VIA
Delivery 4/6
Confirmation # 50927707

3BMICB1075
Navarro

Ellen Vick

S-4973103-SSEED

From: Lorena Navarro [lonavarro@ucdavis.edu]
Sent: Wednesday, March 18, 2009 4:59 PM
To: Ellen Vick
Subject: Fwd: Probiotic intracranial therapy

Hi Ellen,

Here is the information we discussed.

I need these items ordered:

E. aerogenes ATCC #13048 $33.30 S/H 49.00

Low viscosity agarose (Type XII, Sigma) A7299-100G

Heavy mineral oil (330760-1L, Sigma)

BD PrecisionGlide Needles, 21 Gauge (14-826C, Fisher)

BD Luer-Lok 1 ml Disposable Syringe (14-823-30, Fisher)

As far as materials costs, I am funding this through an account with the Department of Neurological Surgery. The recharge number is SHRT. The Account number is S4973103.
Rudy

sub SSEED per Pauline

Rudolph J. Schrot, MD
Assistant Professor
Department of Neurological Surgery
University of California at Davis
4860 Y Street, Suite 3740
Sacramento, CA 95817
916-734-8824 (office)
[redacted] (pager)
(cell)

Pauline Stewart
734-8365

Send order copy and invoice copy to Pauline

David @ ATCC ships 4/3
delivers 4/6

Conf# 50927707

order

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA

S A L E S O R D E R

Order Number: SO927707 Revision: 0
Order Date: 04/02/09 Page: 1
Print Date: 04/02/09

Bill-To: 134328

University of California at Davis
Microbiology
One Shields Ave
Davis, CA 95616
USA

Ship-To: 00156593

University of California at Davis
Microbiology
354 Briggs Hall
Davis, CA 95616
USA

Attention: Vick, Ellen
Telephone: 530-752-7199
Fax/Telex: 530-752-9014

Attention: Navarro, Lorena
Telephone: 530-752-0260
Fax/Telex: 530-754-8973

Salesperson 1: dsaylor

Credit Terms: 30
Due 30 days from invoice
Resale:
Remarks:

Purchase Order: 3BMICB1075
Ship Via: FedEx
FOB Point: Manassas, VA

Ln	Item Number	Due Date	Qty Open	UM	Price	Extended Price
1	13048 Enterobacter aerogenes	04/03/09	1.0	EA	33.30	33.30

Non-Taxable: 82.30
Taxable: 0.00
Tax Date: 04/03/09
Containers: 0.00
Line Charges: 0.00

Currency: USD
0.00%

Line Total:	33.30
Discount:	0.00
Prepaid Shipping 05 :	0.00
Shipping & Handling 20 :	49.00
Special Handling 25 :	0.00
Total Tax:	0.00
Total:	82.30

♀

Page 1

04/03/2009

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BILL-TO:

134328
University of California at Davis
Microbiology
One Shields Ave
Davis, CA 95616
USA

ATTN: Vick, Ellen
TEL #: 530-752-7199 FAX#: 530-752-9014

SHIP-TO: SALES ORDER #: SO927707

00156593
University of California at Davis
Microbiology
354 Briggs Hall
Davis, CA 95616
USA

ATTN:Navarro, Lorena
TEL #:530-752-0260 FAX#: 530-754-8973

CUSTOMER P.O. #: 3BMICB1075 4/6/09 Navarro

SOLD TO: 134328 ORDER DATE: 04/02/09 SALESPERSON: 100	FOB: Manassas, VA BOL:	SHIPPED VIA: FedEx SHIP DATE: 04/03/09	FREIGHT LIST Standard

#	BSL	ITEM NUMBER	UM	QTY SHIP	QTY B.O.	DESCRIPTION		LOT NUMBER
001	1	13048	EA	1	0	Enterobacter aerogenes		57675415

Page # 001

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INVOICE NUMBER/DATE
[redacted] 134328
[redacted] 04/06/09

BILL- TO:

134328
University of California at Davis
ATTN: Vick, Ellen
Microbiology
One Shields Ave
Davis, CA 95616
USA

Phone: 530- 752- 7199 FAX: 530- 752- 9014

SHIP- TO:

00156593
University of California at Davis
Microbiology
354 Briggs Hall
Davis, CA 95616
USA

ATTN: Navarro, Lorena
Phone: 530- 752- 0260 FAX: 530- 754- 8973

Due 30 days from invoice

SALES ORDER #:	SO927707	INVOICE #:	IV770677	PO #: [redacted] Navarro	SHIPPED VIA: FedEx
ORDER DATE:	04/02/09	INVOICE DATE:	04/06/09	FOB: Manassas, VA	SHIP DATE 04/03/09
SALESPERSON:	100	SOLD TO:	134328	BOL:	

#	ITEM NO	UM	QTY SHIP	QTY B.O.	TAX	DESCRIPTION	PRICE	NET PRICE
001	13048	EA	1.0		No	Enterobacter aerogenes	33.30	33.30

DEPARTMENT OF MICROBIOLOGY
DATE INVOICE REC'D 4/13
DATE MDSE. REC'D 4/6/09

DaFIS Document #:01- 013572359

Non- Taxable:	82.30	Line Total:	33.30
Taxable Total:	0.00	Total Tax:	0.00
		Prepaid Shipping:	
		Shipping & Handling:	49.00
		Special Handling:	
		00.00% Discount:	0.00
		USD Grand Total:	**82.30**
		Payments Applied:	0.00
		USD Balance Due:	[redacted]

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Page # 1

Proposed innovative treatment with patient
Eric Mah to: Rudolph Schrot 10/11/2010 09:57 AM

History: This message has been replied to and forwarded.

Dear Dr. Schrot,

As a follow up to our phone conversations today, I understand you intend to perform a nonstandard clinical procedure on single patient in the course of clinical care and in the best interests of the patient. Based on our conversation, I do not believe this requires IRB review as it does not qualify as human subjects research. Furthermore, the procedure does not appear to fall under the auspices of the FDA's authority because you are treating a single patient in the course of clinical care and not as part of research and are not trying to obtain the drug/biologic from an outside source.

If your intention changes to conduct human subjects research, IRB review and an IND application to the FDA may be required.

Please keep in mind that you should properly consent the patient in accordance with UCDHS policies and procedures and document that the patient and/or family, as appropriate, understands the risks of your innovative treatment. I have consulted with Chief Compliance Officer Teresa Porter and she recommends you seek guidance/approval from Chief Medical Officer Al Siefkin.

If I can facilitate this process in any other way, please do not hesitate to contact me.

Best,
Eric

Eric C. Mah
Director, IRB Administration
Acting Director, Compliance & Integrity
Office of Research
University of California, Davis
916.703.9157
ecmah@ucdavis.edu

Re: Fw: consent [redacted] for treatment with live bacteria.

Allan Siefkin to: J.paul Muizelaar [redacted] 2010 09:26 AM

Cc: Rudolph Schrot, anna.orlowski

History: This message has been replied to.

Paul,

Risk: Thank you for putting together this draft. As we discussed very briefly Weds, the only thing I would add to the consent is to make sure you put into the consent and chart that you or Rudy did discuss with Human Subjects Chair as a compassionate single trial (or whatever the appropriate language is to discribe what you are going to do) . I have sent to Anna Orlowki in her role as Risk Manager so she knows you are planning this very rare (unproven) trial of one as a compassionate attempt to prolong life and that the patient (family) are aware there is no controlled scientific evidence but only some limited animal experience and observational human cases that may or many not show this treatment would help. Please document in writing your conversation with the Human Subjects Chair who said "you can proceed as long as you talk with the CMO".

Since you stopped me briefly after the Council of Chairs with the description of this case I really only responded to making sure the patient and their family was informed about the lack of proven scientific basis for the treatment and the fact that you might get worse and the treatment might cause [redacted] death. However with alittle more time I have thought of three other items that need addressing:

#1 Complication from the treatment: I did not think of this in our 5 mins conversation after Council of Chairs Weds AM. Will the patient be a full code if they arrest/have respiratory or cardiac problems immediately associated with your treatment? Will we put this patient in the ICU, intubate, etc? I think this needs to be discussed with the family before you proceed. What will be the end point of treatment. I know these are difficult questions since you don't know what will happen when the patient develops [redacted] meningitis/encephalitis, but this is exactly the reason you must determine what you will do ahead of time. I assume antibiotics will be used? Blood transfusions? Tube feeding? TPN? Invasive procedures? Will you take to the OR to drain a brain abscess? ICU care/ Code? I really would not proceed until you and the family agree on the scope of treatment and the end point in care.

#2 Ethics: I don't know if the ethics committee would be a resource or not in this case, but I think we should have one anyway. I did not think of this issue when we discussed this very briefly Weds AM. When patients and their family are dying and have been given no hope for treatment (as appears to be the case here from what you told me), they may turn to any thing in desperation. We want to make sure when we do the one time unproven interventions that there is some reason to believe there may be (abiet a small chance) that the patient will live longer or have an improved quality of life. Since we are not gathering data in any scientific manner, the most this can contribute to advancement of treatment for other patients is a single case report. If you are intending on writing the results (good or bad) so others can use this knowledge to develop trials, etc, then saying in the case report that an ethics consult concurred that this is an ethical treatment is needed. I think you should ask for a Ethics consult. What happens if the patient and family want to proceed and you still want to proceed and the ethics consult believes you should not? I would not proceed.

#3. Costs: The other issue I did not discuss (again I did not think of it in our brief conversation Weds AM) was the funding of this "trial of one". I did not ask what the patient's payer is, but commercial payers and CMS have specific regulations/rules regarding what they will pay for and not pay for. I think the patient's family needs to know if they will be obligated for a large bill that their insurance will not cover. We also need to know how much UCDMC and the SOM will be contributing for the care of this patient should we determine that this treatment is not covered by insurance. I think we need to do a financial screen that Ann Frankel's folks can do quickly for you and identify the payer and assess who will be responsible for the cost. If we determine the family is responsible and they cannot pay, then you should ask for CTS funding if you believe this is an appropriate teaching/learning case. You are aware their is a process

through the CFO office for this for the hospital and through Jim Goodnight for the SOM compensation plan. Obviously if one of our own managed care patients you will need to contact Gibbe Parsons, Medical Director of Managed Care. What happens if the payer states they won't pay, the family states the won't/can't pay, and you cannot get internal approval for CTS of other hospital/School assumption of the costs of care? I would not proceed.

Paul and Rudy, I hope you will not view these additional thoughts as putting up road blocks in your attempt to help this patient. However since you have raised this issue with me (and it appears to have be punted to me from Human Subjects) I feel obligated to ask that you now address these other issues also. I only cc'd Anna on the risk issue, but if you want to proceed please contact Scott Christiansen chair of Ethics consult service and Ann Frankel for the other financial clearance issues. Also remember to put into the medical records your conversation with the chair of the Human Subjects committee including the name of the person, date, and time of the conversation.

I appreciate this is alot of work for end of life care for this single case....but I think we all agree this is not a usual case.

Al

Allan D. Siefkin, M.D.
Chief Medical Officer, UC Davis Health System
916/734-1166 (Phone)
916/734-3234 (Fax)
allan.siefkin@ucdmc.ucdavis.edu

J.paul Muizelaar ----- Forwarded by J.paul Muizelaar/PHY/HS/UC... [redacted]/2010 10:47:04 PM

From: J.paul Muizelaar/PHY/HS/UCD
To: Rudolph Schrot/PHY/HS/UCD@UCDavis, Allan Siefkin/PHY/HS/UCD@UCDavis
Date: [redacted]/2010 10:47 PM
Subject: Fw: consent Patient #1 for treatment with live bacteria.

----- Forwarded by J.paul Muizelaar/PHY/HS/UCD on [redacted]/2010 10:43 PM -----

From: [redacted]
To: j.paul.muizelaar@ucdmc.ucdavis.edu
Date: [redacted]/2010 10:38 PM
Subject: [spam] consent Patient #1

Please find attached the extra consent we will have signed by the patient, [redacted] and the surgeons for the proposed treatment of a brain stem Glioblastome Multoforme with live gram negative bacteria.

[attachment "Additional Consent Surgery for Patient #1 .doc" deleted by Allan Siefkin/PHY/HS/UCD]

[redacted]

Additional Consent Surgery for [redacted]

We, the undersigned [redacted] (patient) and [redacted] are aware that if during the proposed frontotemporal craniotomy the diagnosis of malignant primary brain tumor (glioblastoma multiforme or similar) can be confirmed, the surgeons intent to implant live gram negative bacteria into the tumor bed and around the bone flap.

There is no proof that such treatment might be beneficial, nor are there animal data to support it. The reason to try this as a last resort treatment are case reports in the literature and personal experiences of the surgeons Drs Schrot and Muizelaar that patients with GBM or other malignant brain tumors with an unexpectedly long survival have suffered from infections in the tumor bed and/or the bone flap, in particular with gram negative bacteria.

We acknowledge that [redacted] has been under treatment for [redacted] GBM [redacted], and that after review of [redacted] last MRI scan there, [redacted] was advised on [redacted] 2010 not to have any surgery or other (chemotherapy) treatment anymore, but rather seek "hospice care", due to the very poor prognosis and lack of response to exhaustive previous treatment, with an estimated life expectancy of approximately three months.

We are fully aware that treatment with live bacteria has never been tried before, that the surgeons have no experience with it, and can not predict exactly when to start treatment with antibiotics, but will do so based on clinical and laboratory data. The ensuing infection ("meningitis" and/or "brain abscess") may be totally ineffective in treatment of the tumor. However, the infection might be or become violent causing further neurological deterioration such as paralysis, inability to speak or understand speech, inability to swallow, vegetative state, coma or death.

We are aware that the bacteria to be implanted come from the brain tumor animal laboratory of Dr Schrot, and have not been tested or certified by the FDA or any other Federal or State agency.

Signed and dated/timed

[redacted] (patient) [redacted]

[redacted]

Rudolph Schrot MD (surgeon) [redacted] 1410

J. Paul Muizelaar MD, PhD (surgeon) [redacted]

RE: Proposed innovative treatment with patient
Eric Mah to: Rudolph Schrot 11/14/2010 05:31 PM
Cc: "J.paul Muizelaar"

Rudy,

I know the FDA has become more aggressive with IND and investigational devices. For that reason, I think an IND is sensible or at very minimum, you obtained documentation from the FDA that no IND is needed. I believe a treatment IND is appropriate here.

In a separate email, I will introduce you to Kate Marisuna of the CTSC to assist you with an IND application.

If you have any other questions or concerns in the meantime, please do not hesitate to contact me.

Best,
Eric

Eric C. Mah
Director, IRB Administration
Acting Director, Compliance & Integrity
Office of Research
University of California, Davis
916.703.9157
ecmah@ucdavis.edu

From: Rudolph Schrot [mailto:rudolph.schrot@ucdmc.ucdavis.edu]
Sent: Sunday, November 14, 2010 1:13 PM
To: Eric Mah
Cc: J.paul Muizelaar
Subject: RE: Proposed innovative treatment with patient

Hi Eric,

Thanks so much for your helpful reply. We completely agree that continued treatment should involve IRB approval in the context of a clinical trial, and we would absolutely like to move forward with conducting a pilot study. I reviewed the draft FDA guidelines you referenced.

Two circumstances of the use of live organisms are mentioned the FDA draft, including, "pathogenesis studies using modified organisms", and "studies using wild-type organisms in challenge models". The relevant language is copied below:

An IND is required for challenge studies in which a live organism (e.g., virus, bacteria, or fungi that is modified or wild-type) is administered to subjects to study the pathogenesis of disease or the host response to the organism (see part 312). Although the challenge organism is not intended to have a therapeutic purpose, there is intent to affect the structure or function of the body. Thus, the organism is a biological product (see 21 CFR 600.3(h)(1)) and a drug, and an IND is required for the clinical investigation, unless the criteria for exemption in 21 CFR 312.2 are met. Similarly, an IND is required for a clinical investigation designed to evaluate whether a product colonized with a strain of bacteria and then administered to subjects can treat or prevent disease in patients with a chronic immune disorder.

Is an IND required if a product containing attenuated microorganisms is evaluated for amelioration of symptoms of a disease or prevention of the disease? Even when a microorganism is attenuated with the intention to increase safety of a product, a clinical investigation that evaluates the potential for that microorganism to relieve symptoms of a disease or prevent the disease requires an IND under part 312, unless the study meets the criteria for an exemption under 21 CFR 312.2(b).

Our non-standard treatment method does use live microorganisms, but we are using neither attenuated microorganism, nor wild-type organisms in a "challenge study", and contrary to the language of the FDA guidelines, our use of the microorganisms IS intended to have a therapeutic purpose.

One way of looking at what we are doing would be to say that we area altering a surgical method by relaxing the standard aseptic technique and promoting wound infection by enlisting the help of a microbiology laboratory to produce a controlled infection. We are not implanting an engineered strain of bacteria. The surgery we are performing is otherwise routinely performed for recurrent glioblastoma. We are altering our technique in such a way that the wound becomes contaminated, both by introducing wild type bacteria and by not giving pre-operative antibiotics.

Nevertheless, it could be that, by extension, our proposed study would be considered to be a challenge study by virtue of "altering the stucture or function of the body" and, as such, would be regulated. Is your understanding that we would still need to proceed with an FDA IDE treatment application? If so, what is the next step and what resources are available at UCD to assist with this process?

Thanks!

Rudy

Rudolph J. Schrot, M.D., M.A.S.
Assistant Professor of Clinical Neurosurgery

Phone Numbers:
916-734-8824 (Academic Office)
916-734-6511 (Robert Dillman, Administrative Assistant)
916-734-7463 (Appointments)
[redacted] (pager)
[redacted] (cell)

From: Eric Mah <ecmah@ucdavis.edu>

To: Rudolph Schrot <rudolph.schrot@ucdmc.ucdavis.edu>
Cc: "j.paul.muizelaar@ucdmc.ucdavis.edu" <j.paul.muizelaar@ucdmc.ucdavis.edu>
Date: 11/12/2010 06:11 PM
Subject: RE: Proposed innovative treatment with patient

Dear Rudy,

When we initially spoke, I understood the innovative/unconventional treatment was an extremely rare event with a terminally ill patient who was rapidly declining. Previously, as a single patient experience, the procedure would not qualify as human research under federal definitions. In addition, you described the use of bacteria as a form of treatment in the course of your practice of medicine (not a clinical investigation or experiment).

As you increase that number of patients, however, your activity could appear to be human research.

What's more, the FDA has recently issued new guidance since our last discussion pertinent to this issue. Although the guidance is labeled "draft", FDA generally considers the draft document effective with the same weight and consideration as a "final" version.
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM229175.pdf

This new guidance specifies Live Organisms (page 9) and defines in clear terms what FDA considers a drug and it appears the substance you intend to use would qualify as a drug. With this new information, I believe the use of the bacteria would (in FDA's eyes) be subject to their regulation, purview and authority. (None of this addresses potential California state issues.)

So what does all of this mean? Because it is FDA guidance and not regulation, I am going to repeat the advice I offered you previously with one important caveat. If you choose to administer the locally-grown bacteria to this second patient in the course of your practice of medicine and in your best judgment as the patient's treating physician, I would again obtain surgical consent from the subject and/or family for the procedure with ad-hoc language referencing the unconventional/innovative methods. As with the first patient, I recommend you consult with Chief Medical Officer Al Siefkin prior to performing the procedure. **Finally, the caveat: if you anticipate another future patient will need this unconventional/innovative treatment, I recommend a treatment IND application be submitted to FDA and the protocol undergo IRB review, as appropriate, prior to the next procedure.**
Furthermore, I am happy to direct you to UCDMC resources to assist you with any such IND application.

Please let me know your thoughts, and if you have any additional questions or concerns.

Best,
Eric

Eric C. Mah
Director, IRB Administration
Acting Director, Compliance & Integrity
Office of Research
University of California, Davis
916.703.9157
ecmah@ucdavis.edu

Re: More beads

[redacted] to: Rudolph Schrot [redacted]2010 07:07 PM

History: This message has been replied to.

Hi Rudy,

I can have the beads made up for Friday morning - I'll make them Thursday afternoon, then store them overnight at 4C to make sure they settle correctly and any remaining oil from the preparation is washed off. Let me know when you set a time, in case it's early, so I can let Lorena know when I need to get them from her lab.

[redacted]

On Sun, [redacted] 2010 at 10:37 AM, Rudolph Schrot <rudolph.schrot@ucdmc.ucdavis.edu> wrote:

Hi [redacted]

We have another patient with a recurrent glioblastoma in whom we are planning to implant bacteria. the case will be done in Friday. This tumor is in the right frontal lobe. I have discussed the case with the director of the UC Davis IRB and he has given us cautious clearance to do this. After this, we will need an IND from the FDA to further proceed with clinical work. The rat data will be crucial in making such an application to the FDA.

Can you get a similar preparation ready for Friday morning? I don't yet know what time the case will go.

Rudy

Rudolph J. Schrot, M.D., M.A.S.
Assistant Professor of Clinical Neurosurgery

Phone Numbers:
916-734-8824 (Academic Office)
916-734-6511 (Robert Dillman, Administrative Assistant)
916-734-7463 (Appointments)
[redacted] (pager)
[redacted] (cell)

Re: IND application assistance
Kate Marusina to: Eric Mah 11/15/2010 09:43 AM
Cc: Rudolph Schrot

History: This message has been replied to.

Dear Dr.Schrot,

would be happy to be of assistance. We also have a website with a series of presentations and basic documents for IND preparation. Please feel free to contact me anytime.

http://www.ucdmc.ucdavis.edu/ctsc/investigators/IND/

k.

Kate Marusina, Ph.D., MBA
Manager,
Research Facilitation and Industry Alliance,
Clinical and Translational Science Center
UC Davis School of Medicine
TEL: (916)703-9177
CEL: [redacted]
EMAIL: kate.marusina@ucdmc.ucdavis.edu

http://www.ucdmc.ucdavis.edu/ctsc/areas/partnering/index.html

From: Eric Mah <ecmah@ucdavis.edu>
To: "Kate Marusina (kate.marusina@ucdmc.ucdavis.edu)" <kate.marusina@ucdmc.ucdavis.edu>
Cc: Rudolph Schrot <rudolph.schrot@ucdmc.ucdavis.edu>
Date: 11/14/2010 05:37 PM
Subject: IND application assistance

Dear Kate,

Dr. Rudy Schrot has a project which will require an IND submission. It's possible that during the pre-IND process, the FDA could determine no IND is needed. Notwithstanding, I was hopeful that you could provide him guidance as to how to utilize the services of the CTSC in this regard. This is investigator-initiated and for a treatment IND.

I have copied him on this email.

Please let me know if there is anything I can do to facilitate.

Thank you,
Eric

Eric C. Mah
Director, IRB Administration
Acting Director, Compliance & Integrity
Office of Research
University of California, Davis
916.703.9157
ecmah@ucdavis.edu

[redacted] MRN Stamp

Additional Consent Surgery for [redacted] Patient #2

I, the undersigned [redacted] Patient #2 (patient), am aware that if during the proposed frontotemporal craniotomy the diagnosis of malignant primary brain tumor (glioblastoma multiforme or similar) can be confirmed, the surgeons intent to implant live gram negative bacteria into the tumor bed and around the bone flap.

There is no proof that such treatment might be beneficial, nor are there animal data to support it. The reason to try this as a last resort treatment are case reports in the literature and personal experiences of the surgeons Drs. Schrot and Muizelaar that patients with GBM or other malignant brain tumors with an unexpectedly long survival have suffered from infections in the tumor bed and/or the bone flap, in particular with gram negative bacteria.

I acknowledge that I have undergone standard treatment for glioblastoma with evidence of recurrence on the last MRI. I understand that I am in hospice, meaning that I am not expected to survive very long with the status of my current disease, despite the best available standard treatment.

I am fully aware that treatment with live bacteria is not clinically proven, that the surgeons have little experience with it, and cannot predict exactly when to start treatment with antibiotics, but will do so based on clinical and laboratory data. The ensuing infection ("meningitis" and/or "brain abscess") may be totally ineffective in treatment of the tumor. However, the infection might be or become violent causing further neurological deterioration such as paralysis, inability to speak or understand speech, inability to swallow, vegetative state, coma or death.

We are aware that the bacteria to be implanted come from the brain tumor animal laboratory of Dr Schrot, and have not been tested or certified by the FDA or any other Federal or State agency.

Signed and dated/timed

[redacted] Patient #2 (patient) [redacted]

[redacted]

Rudolph Schrot MD (surgeon)

[redacted]

J. Paul Muizelaar MD, PhD (surgeon)

[redacted] MRN Stamp

Additional Consent Surgery for [redacted] Patient #3

We, the undersigned [redacted] Patient #3 (patient) and [redacted] e are aware that if during the proposed right frontotemporal craniotomy the diagnosis of malignant primary brain tumor (glioblastoma multiforme or similar) can be confirmed, the surgeons intent to implant live gram negative bacteria into the tumor bed and in the bone flap.

There is no proof that such treatment might be beneficial, nor are there animal data to support it. The reason to try this as a last resort treatment are case reports in the literature and personal experiences of the surgeons Drs Schrot and Muizelaar that patients with GBM or other malignant brain tumors with an unexpectedly long survival have suffered from infections in the tumor bed and/or the bone flap, in particular with gram negative bacteria. Furthermore, we have previously treated 2 patients with recurrent glioblastoma with the therapy, and one of the patients seems to have responded favorably, although it is too early to make a definite determination.

We are aware that standard therapy for a newly diagnosed glioblastoma involves maximal safe resection of the enhancing part of the tumor, followed by 6 weeks of radiation therapy and temozolomide chemotherapy pills. We are also aware that the patient may qualify for a clinical trial (CORE trial) to test a new drug, cilengitide, for glioblastoma, and that the proposed probiotic treatment would disqualify the patient from the chance of entry into the trial. We are aware that even with standard therapy, the life expectancy after a diagnosis of GBM is 12 to 15 months.

We are aware that treatment with live bacteria has been tried by the surgeons twice in the past year, and that one patient died from disease progression, and that the other patient seems to be improving with signs of tumor regression. We are aware that the surgeons may need to treat the ensuing infection with antibiotics, and that the surgeons may need to perform additional surgery, such as removal of the bone flap or drainage of an abscess, in order to treat the infection. We are aware that the proposed treatment may lengthen the hospital course. The ensuing infection ("meningitis" and/or "brain abscess") may be totally ineffective in treatment of the tumor. However, the infection might be or become violent causing further neurological deterioration such as paralysis, inability to speak or understand speech, inability to swallow, vegetative state, coma or death.

We are aware that the bacteria to be implanted come from the brain tumor animal laboratory of Dr Schrot, and have not been tested or certified by the FDA or any other Federal or State agency.

We grant permission for Drs. Muizelaar and Schrot to publish any information learned in the course of this clinical treatment option. We understand that this treatment option is not currently part of a clinical trial, and that this treatment method has not been subject to FDA approval or IRB review, but represents the best clinical judgment of Drs Muizelaar and Schrot, who are solely responsible for its use in this particular case.

Signed and dated/timed

[redacted] Patient #3 [redacted] Patient #3 (patient)s _____

[redacted] Patient #3 [redacted] _____

Rudolph Schrot MD (surgeon) _____

J. Paul Muizelaar MD, PhD (surgeon) _____

TO:

Karen Smith, RN
Robert O'Donnell, MD, PhD
Stuart H. Cohen, MD
Praveen Prasad, MD

March 9, 2011

RE: Treatment of newly diagnosed and recurrent glioblastoma using an intentional wound infection (Probiotic Intracranial Therapy) surgical technique

Dear Committee member:

Thank you for agreeing to serve on this *ad hoc* Ethics Committee at the behest of Fred Meyers, MD, MACP, Executive Associate Dean of the UC Davis School of Medicine. You are asked to review the proposed clinical activity within the Department of Neurological Surgery. We request to temporarily continue our informal clinical activities with intentional wound infection according to our best clinical judgment and current experience with the method, with the understanding that we will work to develop an IRB-approved formal protocol.

Sincerely,

Rudolph J. Schrot, MD, MAS
J. Paul Muizelaar, MD, PhD

Prospectus

Whereas a newly diagnosed glioblastoma patient with an especially poor prognosis has specifically requested to come to UC Davis Medical Center to receive urgent probiotic intracranial therapy; whereas Drs. Muizelaar and Schrot already have experience with this method and have demonstrated a positive response; and whereas Drs. Muizelaar and Schrot have treated the first 3 patients with the understanding that special permission was granted by the hospital administration to apply this technique, this *ad hoc* Ethics Committee has been assembled to make a recommendation to the Dean and/or the IRB administration regarding the ethics of urgently treating this patient in the absence of a formal IRB protocol and also, by extension, treating several other glioblastoma patients (no more than 5) who request the treatment. We ask that you consider the information contained herein.

Proposed treatment: We plan to intentionally inoculate the tumor cavity with live bacterial cultures of *Enterobacter aerogenes* at the time of operation or reoperation and withhold antibiotic treatment when clinically feasible. In this way, we are enlisting *Enterobacter aerogenes* in the creation of a chronic surgical wound infection. We have preliminary experience with this method in 3 patients deemed to have a very poor prognosis, and we propose to use the same method in a limited number of future patients until such time as a formal IRB approved research protocol is in place.

Method: Purified cultures of *E. aerogenes* are maintained in the laboratory of Lorena Navarro, PhD, Assistant Professor of Microbiology. *Enterobacter aerogenes* (*E. aero*) were obtained from ATCC (Manassas, VA) and stored at -80ºC. 48 hours prior to experimental use, a clean pipette tip is used to scrape the frozen stock, streak it across an LB agar plate, and incubate the plate at 30ºC overnight. A single colony from the plate is used to inoculate an autoclaved flask of 200 mL LB broth. The flask is shaken overnight at 30ºC and approximate bacterial numbers determined the following day by reading the optical density at 600 nm and using the relation: 2.943 AU (absorbance units) = 2.75 x 10^9 CFU (colony forming units) / mL. The *E. aerogenes* suspension is then transferred to sterile 50 mL conical tubes, sealed, and transported to the surgical site. At the time of surgery, Gelfoam® implants (2x2 cm) and the bone flap are soaked in the bacterial suspension and implanted at the time of surgery. The bone flap is replaced. Antibiotics are withheld until such time that they are needed as rescue therapy. Additional supportive measures following surgery include the use of glucocorticoids and mineralocorticoids, and the use of GM-CSF to stimulate white blood cell production in clinical situations of relative leucopenia. The *E. aerogenes* cultures are of known antibiotic sensitivity and pan-sensitive to antibiotics effective against gram negative organisms.

Background:

Glioblastoma is a devastating diagnosis. Little progress has been made in stemming the relentless progression of disease. Optimal management includes maximal safe surgical resection, followed by 6 weeks of radiation therapy with adjuvant temozolomide chemotherapy.[1] Even with optimal treatment, the median survival is only 12-15 months, hardly a therapeutic victory. For patients with very suboptimal resections, such as those tumors that cross the midline, are multifocal, or involve eloquent speech and motor areas, survival time is even shorter.[2] Not much time passes before a patient's status changes from newly diagnosed to recurrent. Those patients that do beat the odds and survive over 2 years face progressive cognitive decline from the late term effects of radiation therapy.[3]

Neurosurgeons have long been aware of anecdotal long-term survivors of glioblastoma who had coincidentally developed wound infections, and in fact this beneficial effect of wound infection in prolonging survival is widely accepted.[4] An association between infection and tumor regression has been known for hundreds of years.[5, 6] Prior to modern aseptic surgical technique, "laudable pus" was heralded as clinically favorable following surgical procedures for cancer, and surgical textbooks antedating the antibiotic era even recommended intentional wound infection in cancer operations.[6] Dr. William Coley, a surgeon at the New York Hospital, pioneered the use of probiotic agents against cancer at the turn of the 20th century. He noted regression of soft tissue sarcomas in patients who developed erysipelas, which led to the development of Coley's toxin, a cocktail of heat-killed Staphylococcus and Serratia.[5] The antibiotic era, with the proliferation of antimicrobial agents between 1945 and 1970, as well as refinements surgical technique, ushered in war against infectious agents by the medical establishment. However, some have suggested that microorganisms normally help eradicate cancer and that efforts at infection treatment and antisepsis have brought about a progressive rise in cancer rates.[7]

In 1999, Bowles *et al* created a stir when they reported a series of infection-related brain tumor cures.[8] In this series, there was 1 malignant meningioma, 1 glioblastoma, and 2 anaplastic astrocytomas. All had all developed post-surgical infection, confirming what many neurosurgeons have suspected all along. Three cases involved bone flap infection overlying the tumor and one case involved meningitis and ventriculitis. In 3 of the 4 cases, *Enterobacter aerogenes* was isolated from

the wound. Additional anecdotal reports support this phenomenon[6, 9-11], prompting a retrospective study at Columbia University to examine the survival of glioblastoma patients who developed post-operative infections (N=18).[4] Although no statistically significant difference was seen in the overall group, a subgroup analysis revealed improved survival in those patients with deep infections. (p=0.08) The study was flawed in that all of the infections encountered in the subjects were aggressively treated, unlike the chronic infections described anecdotally. Furthermore, 94% of the infections were gram positive. Gram-negative wound infections are much less common, yet have been associated more commonly with cancer regression.

It is significant that Bowles *et al* isolated *Enterobacter aerogenes* from 75% of the cases in their series. Gram-negative organisms contain lipopolysaccharide in their cell walls, which has been shown to result in experimental regression of gliomas in a mouse model.[12] Furthermore, some Enterobacteriacea elaborate exotoxins that have been shown to selectively inhibit the growth of astrocytoma cells.[13] Lind and colleagues note that certain gram-negative bacteria can invade both endothelial cells and macrophages and they suggest that a predilection for tumor-associated neovascularity among gram-negative organisms may explain this tropism.[14] Another important feature of infection-related cancer remission is the chronicity of the infections. Few clinically encountered infections are left untreated today.

Work by Francis Conley provides the most direct pre-clinical model of the effect of intracranial infection in a brain tumor model, albeit for a different tumor histology and organism. Intracranial tumor was modeled in a mouse using a syngeneic sarcoma cell line. Mice injected intracranially with *Corynebacterium parvum*, a gram positive bacteria, had significantly increased survival compared to uninfected mice.[15] Hepnner and Mose described the intracarotid injections of the spores of non-pathogenic Clostridium, a gram-positive rod, in 49 glioblastoma patients, producing a putrid abscess which they then rapidly treated. They report that no survival benefit was conferred.[16] There is no pre-clinical data on the use of gram-negative organisms to create chronic infectious states.

<u>Clinical Experience at UC Davis Medical Center:</u>

We have used the intentional wound infection surgical technique (a.k.a. Probiotic Intracranial Therapy) in the course of clinical treatment of three patients with glioblastoma at UC Davis within the past year. The details of these cases are presented below.

Case 1. A [redacted] was diagnosed with GBM in [redacted] 2010. [redacted] underwent a resection of a left frontal mass with placement of Gliadel wafers, followed by adjuvant chemo and radiation therapy. In [redacted] 2010, [redacted] developed a recurrence in the pons that was treated with Avastin, but the treatment was complicated by an M.I. [redacted] was offered hospice in the face of rapidly progressing hemiparesis. [redacted] presented to our clinic in [redacted] 2010. After a discussion with IRB staff, it was felt that the use of the probiotic surgical technique of intentional wound infection with E. aerogenes would fall outside the purview of IRB for the purposes of treating this patient according to the best clinical judgment of the treating surgeons.

On [redacted]10, after extensive informed consent, as well as discussions with hospital administrative staff, the patient was taken to the operating room for left subtemporal craniotomy and implantation of E. aerogenes cultures (3.6 x 10^9 CFU/ml) in the pontine tumor focus, in addition to contamination and reimplantation of the bone flap. The frozen section revealed glioblastoma. Four hours after surgery the patient developed an acute febrile response, with tachycardia and a need for re-

intubation. Antibiotic rescue therapy was initiated, and stopped after 4 days. Mineralocorticoid support was added, as well as GM-CSF to counter leucopenia. An MRS three weeks after surgery revealed the apparent absence of tumor signal within the pontine focus and replacement with an abscess. An indium-111 tagged white blood cell study was done on [redacted]/10 and revealed accumulation of white cells

intracranially and in the pons, consistent with the probiotic treatment. The patient had a waxing and waning course, but eventually stopped following commands, after which time full-scale antibiotic rescue therapy was re-started. Stereotactic aspiration of the pons on [redacted]/10, one month after a, but no growth on culture. The patient failed to improve and a new MRI showed apparent tumor progression. The family opted to withdraw support on [redacted]/10 and consented to an autopsy. Post-mortem exam revealed progressive glioblastoma, however in the area of the pons where the probiotic was placed, the tumor cells were oddly negative for GFAP, a hallmark of glioblastoma, suggesting a phenotypic change to a more differentiated state in this subpopulation of cells.

Case 2. A [redacted] was diagnosed with glioblastoma after [redacted] presented in [redacted] 2010 with a right frontal intracerebral hemorrhage. [redacted] underwent gross total resection and implantation of Gliadel wafers on [redacted]/10, followed after a 4-month delay by a course of standard IMRT fractionated radiation to a 2 cm margin with adjuvant temozolomide, completed on [redacted]/10. During and immediately after [redacted] radiation [redacted] noted a decline in cognitive function with memory loss and word finding problems and progressive left hemiparesis. Post-treatment. Imaging revealed tumor progression in a "butterfly" pattern extension across the corpus callosum and multifocal involvement with a contralateral satellite lesion of the posterior temporal lobe. The oncology team felt that [redacted] had failed standard treatment and [redacted] was placed on hospice, under the assumption that no additional therapy would be effective in light of [redacted] rapidly progressive and multifocal disease and that [redacted] survival was expected to be six months or less.

At the time of follow-up in our clinic on [redacted]/2011, [redacted] was offered the option of probiotic intracranial therapy with *Enterobacter aerogenes*. Additional discussion with IRB and hospital administrative staff was carried out, and detailed informed consent with the patient and family was accomplished. On [redacted]/10 [redacted] underwent re-do right frontal craniotomy, partial tumor resection, and implantation of Gelfoam soaked in *E. aerogenes* suspension (3.3×10^9 CFU/ml), as well as re-implantation of the bone flap which was also soaked in bacterial solution. The frozen section revealed glioblastoma. Following the procedure, [redacted] developed an acute febrile response to 39.9 at 4 hours post-op, as observed in Case 1, however antibiotic rescue therapy was not administered. [redacted] immune response was supported with GM-CSF until [redacted] WBC normalized, and [redacted] was weaned from steroids. [redacted] developed an expressive aphasia post-operatively, which improved over time. [redacted] baseline right hemiparesis was unchanged. Percutaneous aspiration a subgaleal fluid collection on [redacted]/10 revealed positive cultures of Enterobacter aerogenes, indicating an active infection. No antibiotic therapy has been necessary in this patient. [redacted] was discharged to a nursing home on [redacted]/10, one month after [redacted] probiotic therapy surgery. After discharge, the cranial wound

developed external signs of infection with some purulent drainage from the wound, but antibiotics were still withheld. [redacted] very gradually began to improve at the nursing home, with increased participation in physical therapy, and communicative ability, and was noted to be using [redacted] phone to send text messages. [redacted] spirits were excellent. Because [redacted] had improved from a dependent assistance/maximum assistance level to moderate assistance-minimum assistance, [redacted] was transferred on [redacted]/11 to UC Davis for inpatient rehab in anticipation of going home to live with [redacted]

A follow-up MRI/MRS was done on [redacted]/11 and revealed a marked therapeutic response, with greatly iminished involvement of the corpus callosum, decreased mass effect on the lateral ventricle, and disappearance of the contralateral satellite lesion. MRS showed areas consistent both with persistent tumor and with abscess. Indium-111 WBC to the resection cavity.

Case 3. A [redacted] with h/o HTN, depression, and asthma presented to the ED with stabbing neck pain which has been going down [redacted] right arm and with tingling in [redacted] right hand for 3 weeks, two episodes of LE weakness resulting in falls, and nausea and vomiting. [redacted] clinical history was consistent with new onset seizure. The patient was neurologically intact on exam. Imaging revealed a large right temporal tumor consistent with a glioblastoma on imaging. The likely diagnosis and expected prognosis was discussed extensively with the patient and [redacted] family, and the standard treatment was discussed, which involves resection and adjuvant chemo/radiation. The option of intentional wound contamination with *E. aerogenes*, and our previous clinical experience with Case 1 and 2, was also discussed. The patient and family were interested in having the wound contaminated at the time of surgery. After discussion with hospital administrative staff, an extensive informed consent was accomplished with the patient and [redacted] family.

On [redacted]11, the patient underwent right temporal craniotomy and gross total resection of the tumor via a right temporal lobectomy. An Ommaya reservoir was implanted in the resection cavity in order to allow percutaneous aspiration of an abscess in the resection cavity, if necessary. The frozen section diagnosis was glioblastoma. At the conclusion of the case, Gelfoam implants soaked in *E. aerogenes* suspension (3.3×10^9 CFU/ml) were implanted along the posterior resection cavity margin. The bone flap was soaked in bacterial suspension and re-implanted. Following surgery, the patient developed a predictable febrile response 4 hours after surgery. Antibiotics have been withheld. The patient became encephalopathic post-operatively for several days. [redacted] has been supported with blood transfusions, GM-CSF, and steroids. By POD#5, [redacted] exam has greatly improved, and [redacted] is now alert, communicative, and briskly following commands. Although [redacted] has good spontaneous breathing, [redacted] has remained intubated because of concern for airway edema. [redacted] has remained hemodynamically stable.It is too early to draw any conclusion about the effectiveness or risks of probiotic intracranial therapy in Case 3.

Regulatory Issues: Probiotics are live microorganisms that are felt to be beneficial to the host organism. The current definition was adopted by the Food and Agriculture Organization and the World Health Organization of the United Nations: “Live microorganisms which when administered in adequate amounts confer a health benefit on the host".[17] Probiotics in this sense commonly refer

to live microorganisms used as food additives, commonly referred to as "friendly bacteria". These microorganisms are endogenous human gastrointestinal flora and are unaltered in any way. Similarly, the surgical technique of intentional wound infection we employ utilizes cultures of an endogenous species that is ubiquitous in the human gastrointestinal tract and non-pathogenic in healthy subjects. The organism has not been attenuated or genetically altered in any way. The FDA considers live biotherapeutic products as biological products regulated under Section 351 of the Public Health Service Act 41 U.S.C. 262, and as drugs that are "intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animal (Federal Food, Drug and Cosmetic Act of 1938).[18] Live biotherapeutic products therefore require an Investigational New Drug Application (IND) for use in research subjects. Use of biologics is furthermore regulated by interstate commerce under the Public Health Service Act, Section 351. Production of live biotherapeutic products occur in a GMP facility according to high standards of purity.

We feel however that enlisting the endogenous organism *E. aerogenes* cultures to create an intentional surgical wound infection lies in a grey area in terms of FDA regulation. It is difficult to accurately quantify the dosing of the bacteria, especially in the case of soaking a bone flap in solution prior to re-implantation, and varying numbers of CFUs will be implanted. Our treatment method, unlike the paradigm of targeted drug therapy, is essentially non-mechanistic and uses an endogenous organism to produce a beneficial health effect (boosting the immune system to fight cancer), similar to the claims promoted for probiotic food additives, which are not currently considered drugs according to the FDA nor regulated as such. Nevertheless, we are using *E. aerogenes* in a specific way to treat malignant glioma, and we will develop a formal clinical research trial protocol in the near future. We will explore the need for an IND application to accompany a formal IRB-approved clinical trial at that time.

Conclusion:

Probiotic Intracranial Therapy with an endogenous gram-negative organism, *Enterobacter aerogenes*, is a promising but unproven therapeutic option for patients with malignant glioma. The rational for intentional wound infection is based on anecdotal case reports in the literature, supported by limited mechanistic and pre-clinical data. If intentional wound infections are similar to the accidental infections that have been reported in long-term survivors, complete cure of the disease is a distinct possibility. For all we do to prevent infection in the operating room, this technique is relatively simple to perform and largely involves, in addition to bacterial inoculation, suppressing the urge to treat the infection. Importantly, we have at UC Davis the only experience in the world, to our knowledge, of the use of this technique. The creation of intentional wound infections is a high-risk prospect. Nevertheless, the overwhelmingly poor prognosis of the glioblastoma diagnosis warrants drastic and high-risk measures, which, if successful, hold the promise of a cure. Furthermore, our clinical experience thus far has demonstrated an limited imaging and histopathologic response in Case 1, and a robust imaging and clinical response in Case 2. In Case 3 it is too early to draw conclusions, however the patient is currently doing very well and rapidly recovering from the treatment stress. None of the patients have developed sepsis or meningitis. We propose that on ethical grounds, in light of the dismal prognosis for glioblastoma, additional judicious use of the method in a limited number of patients is warranted.

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From: Rudolph Schrot [rudolph.schrot@ucdmc.ucdavis.edu]
Sent: Thursday, March 17, 2011 10:41 AM
To: Eric Mah
Cc: David Asmuth; John Anderson (john.anderson@ucdmc.ucdavis.edu); j.paul.muizelaar@ucdmc.ucdavis.edu
Subject: Re: Cease and Desist: Probiotic Treatment

Dear Eric:

I am in receipt of this email and I confirm that we will cease and desist our clinical use of probiotic treatment pending further IRB review.

Rudolph J. Schrot, M.D., M.A.S.
Assistant Professor of Clinical Neurosurgery

From: Eric Mah <ecmah@ucdavis.edu>
To: "Rudolph Schrot (rudolph.schrot@ucdmc.ucdavis.edu)" <rudolph.schrot@ucdmc.ucdavis.edu>
Cc: "j.paul.muizelaar@ucdmc.ucdavis.edu" <j.paul.muizelaar@ucdmc.ucdavis.edu>, David Asmuth <david.asmuth@ucdmc.ucdavis.edu>, "John Anderson (john.anderson@ucdmc.ucdavis.edu)" <john.anderson@ucdmc.ucdavis.edu>
Date: 03/17/2011 10:18 AM
Subject: Cease and Desist: Probiotic Treatment

Dear Dr. Schrot,

As a follow up to our meeting yesterday, please confirm (by replying to this email) your agreement to cease all activities using the probiotic treatment. Any additional activity will require review by the IRB and/or IRB Chair.

Thank you.

Eric C. Mah
Director, IRB Administration
Acting Director, Research Compliance & Integrity
Office of Research
University of California, Davis
916.703.9157
ecmah@ucdavis.edu

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SACRAMENTO, CALIFORNIA 95817

CONFIDENTIAL

September 16, 2011

Via electronic and campus mail

J. PAUL MUIZELAAR, MD
NEUROLOGICAL SURGERY

RUDOLPH SCHROT, MD
NEUROLOGICAL SURGERY

Re: "Probiotic Intracranial Therapy for Malignant Glioma"

Dear Drs. Muizelaar and Schrot:

The Institutional Review Board (IRB) reviewed the August 31, 2011 report on Investigation Findings related to the three surgical procedures performed under the activity "Probiotic Intracranial Therapy for Malignant Glioma" from October 2010 through March 2011. In addition, the IRB reviewed your undated written response to the Investigation Findings and your Prospectus dated March 9, 2011.

In accordance with University Policy 240, the IRB is responsible for reviewing all research conducted at the institution involving human subjects and as required by state and federal regulations. The main issue before the IRB is whether IRB review and approval should have been obtained by you prior to performing the procedures.

The IRB understands that for Patient #1, you indicated to then-IRB Director Eric Mah that the procedure was only performed in the course of the practice of medicine as an innovative/unconventional treatment for a patient facing certain death and in urgent need. In addition, it was a one-time event using bacteria which was locally obtained.

While the treatment of Patient #1 with the bacteria in the course of the practice of medicine alone may not have constituted human subjects research, the IRB investigation revealed that the bacteria was not locally derived but originated from ATCC of Manassas, Virginia. In addition, ATCC had specifically instructed that it was not intended for human use. The IRB has determined that providing inaccurate information to IRB staff led to an incorrect determination that no IRB review was needed for this one patient.

Furthermore, the IRB determined that administering the bacteria to Patient #2 and Patient #3 constituted human research without IRB review and approval in violation of Policy 240 and other established procedures and institutional requirements. The IRB believes the activity performed possibly increased the risk to patient-subjects but the IRB cannot fully evaluate the specific risks because the IRB was never presented with a protocol for these three patients treated prior to the surgeries.

While the IRB recognizes that you believed you were acting in the best interests of your patients, the IRB has concluded that serious and continuing noncompliance occurred and the procedures you performed required IRB review and approval.

The IRB requires the following:

1) You not use this bacteria or perform this procedure with similar aims using any other unapproved substance without IRB and FDA review and authorization.

2) On all human research for which you serve as Principal Investigators (PIs) or co-Principal Investigators, you must immediately halt all enrollment of new subjects.

    - Please report within 10 days of the date of this letter in a tabular format, the project title, IRB protocol number (as applicable), the current subject enrollment, whether the research is a treatment or non-treatment protocol, and whether ongoing participation by subjects is in the clinical best interests of the subjects and why.

3) Within 10 days of the date of this letter, please provide a report to the IRB on all human research for which you serve as study personnel and/or co-investigators, as applicable.

    - In this report, referencing the project title and IRB protocol number (as applicable), please clarify your specific roles in the research, the current subject enrollments, whether the research is a treatment or non-treatment protocol, and whether ongoing participation by subjects is in the clinical best interests of the subjects and why.

Based on your report and review of the individual protocols, the IRB will make study-by-study determinations on the appropriateness of your continued involvement in these research projects and whether your involvement minimizes risks to subjects in accordance with University policy and federal regulations.

In addition, the use of an unapproved agent may also have violated other University policies, which fall outside of the purview of the IRB. Consequently, this case will also be referred to:

- The Committee on Biosafety regarding the use of the bacteria in humans
- The UC Davis Health System for allowing non-approved products in a surgical setting

- Campus Academic Personnel as a potential violation of Academic Personnel Manual 015 (Faculty Code of Conduct) for conducting human research without prior IRB review and approval, in violation of Policy 240.

We are also required to report IRB findings to certain external entities, including the Food & Drug Administration. You will be copied on any such correspondence.

Finally, because you did not obtain IRB approval, the IRB reminds you that most medical journals require IRB approval when reviewing manuscripts involving human participants; your activity required IRB approval, and use of identifiable data requires current IRB approval. From the IRB's perspective, this unsanctioned research activity should not be submitted for dissemination or publication.

If there is additional information you wish to share with the IRB, which you would like considered before the above actions are taken, please submit that to us within seven (7) business days.

If you have any questions about this process, you may contact us at david.asmuth@ucdmc.ucdavis.edu and john.anderson@ucdmc.ucdavis.edu. Please copy both of us on all correspondence.

Sincerely,

David Asmuth, MD
Chair
Clinical Committee-A

John Anderson, MD
Chair
Clinical Committee-B

cc: Ralph Hexter, Provost & Executive Vice Chancellor
Harris Lewin, Vice Chancellor for Research
Claire Pomeroy, Vice Chancellor for Health Sciences and Dean, School of Medicine
John Meyer, Vice Chancellor for Administrative & Resource Management

CONFIDENTIAL

Response to IRB Communication Re: "Probiotic Intracranial Therapy for Malignant Glioma"

September 22, 2011

Dear Drs. Asmuth and Anderson:

I have received the confidential communication from the IRB dated September 16, 2011. I will provide the required information to the IRB as requested and I have halted all enrollment of new subjects in any study protocols for which I serve as the PI pending further review of these protocols by the IRB.

Based on my initial and subsequent communications with the Director, my understanding was that we were cautiously advised to proceed in the treatment of both patients #1 *and* #2 with bacteria, and that FDA approval or IRB review was not necessary for these 2 patients (see Emails #1 and #2). Email #2 states "Because it is FDA guidance and not regulation, I am going to repeat the advice I offered you previously… [i.e. to proceed without FDA approval or IRB review]… with one important caveat…if you anticipate another *future* patient will need this unconventional/innovative treatment, I *recommend* a treatment IND application be submitted to FDA and the protocol undergo IRB review prior to the next procedure [i.e. >2 procedures, my italics]." My reading of Email # 2 was that FDA approval and IRB review was strongly advised if the treatment of more than 2 patients, but that in fact we had clearance to treat these 2 anecdotal patients without FDA clearance and IRB review.

We made the clinical judgment that we had some latitude to act in the treatment of patient #3 (especially since a precedent had been set in treating the first 2 patients and because the second patient did so well). Furthermore, we were not acting in isolation. I discussed our plan for treatment of each patient with Dr. Muizelaar, who is himself a seasoned senior clinical researcher, having served as PI on numerous clinical trials and as the director for numerous DSMBs for the NIH and the Veterans' Administration. Dr. Muizelaar had in turn discussed the proposed treatment with the leadership of the medical center and the medical school, prior to treating both patient's #2 and #3, and he communicated to me that we should proceed. We provided full informed consent to all the patients and families. My intention was not to defy the recommendations of the Director or to flaunt the policies of the IRB. It seems to me now based on the findings communicated by the IRB that it was not the intention of the Director that more than even a single patient would be treated. Obviously a serious miscommunication occurred.

I did not intend to supply inaccurate information to the IRB regarding the source of the biologic. My understanding at the time was that the bacteria were, in fact, "locally-grown". Dr Lorena Navarro, Assistant Professor of Microbiology, ordered the bacteria over 3 years ago and maintained the cultures in her laboratory in Briggs Hall in Davis. Dr. Navarro collaborated with us in the preclinical work, and provided the cultures that were used to prepare the bacteria for human use in her laboratory, and was aware of any and all clinical use of the bacteria. I understood that the bacteria were locally obtained, coming from Dr. Navarro's lab. I did not explore the importance of the ultimate source of the seed cultures with the Director at the time that I sought guidance from the IRB in proceeding with clinical use. I regret that this technical issue was not further discussed from the outset and it reflects my incomplete understanding of the definition of a locally obtained drug or biologic.

Our use of the bacteria was not part of any defined research protocol, and our clinical use of the bacteria was purely anecdotal. Our motivation was to help these patients who were faced with a rapidly fatal disease, glioblastoma, for which there is no cure. Nevertheless, because this clinical use is being considered research, we agree that publication of these patients as case reports is not appropriate without the full review and approval of the UC Davis IRB. Of note, patient #2 has survived up to 4 times [redacted] estimated prognosis at the time of treatment. We recognize an ethical dilemma in withholding information about a potentially life-saving treatment from the world, and we hope that the IRB shares our enthusiasm for properly practiced pre-clinical and clinical research in this area.

To ensure that I have no role in such non-compliance issues in the future, I am motivated to deepen my understanding and compliance with good clinical practice in human subjects research. I am aware of a "2 day boot camp" sponsored by the NIH, and will ask Dr. Muizelaar for protected time to pursue such supplemental education.

Sincerely,

Rudolph J. Schrot

J. Paul Muizelaar, MD, PhD

IRB Protocol #	Title	Role	Number of subjects currently enrolled to study	Treatment or Non-Treatment	Ongoing Enrollment/ Participation by subjects?
233486-1	Pediatric Traumatic Brain Injury Consortium: Hypothermia	Co-Principal Investigator	13	Treatment	Yes, one patient in long term follow up. Study closed to enrollment.
200917517-2	Analysis of Traumatic Brain Injury Outcomes and External Validation of Published Prognostic Models	Co-Principal Investigator	1254	Non-treatment	N/A
201118682-1	Retrospective Analysis of EVD-related infections	Co-Principal Investigator	2000	Non-treatment	N/A
216551-1	A Randomized, Double-blind, Placebo-controlled, Dose-escalation study of NNZ-2566 in Patients with Traumatic Brain Injury (INTREPID-2566 Study)	Local Safety Monitor	7	Treatment	No patients currently being followed, but study still open for enrollment.
200614296-5 LDR Spine	Mobi-C Artificial Disc vs ACDF in Treatment of DDD of cervical Spine at 1 or 2 levels	Sub-Investigator	53 (total) 4 (Muizelaar) Enrolled four subjects on to this trial. Three patients continue to be followed; one patient is lost to follow-up at this time.	Treatment	Study closed to accrual March of 2008. Study is in long-term follow-up at this time. It is imperative that these subjects continue to be followed, since they may have an investigational device implanted, depending on their randomized treatment assignment.

IRB Protocol #	Title	Role	Number of subjects currently enrolled to study	Treatment or Non-Treatment	Ongoing Enrollment/ Participation by subjects?
200816020-2 Medtronics	Post-Approval Investigation of the PRESTIGE® Cervical Disc Device at a Single Level	Sub-Investigator	23 (total) 0 (Muizelaar)	Post-Market study. Did not enroll any subjects on pivotal or post-market study	Study just completed 7-year follow-up and will close after data has been submitted to FDA.
200816483 Blackstone Medical, Inc.	Blackstone Advent ™ Cervical Disc vs. ACDF for Treatment of One Level Degenerative Disc Disease	Sub-Investigator	10 (total) 0 (Muizelaar)	Treatment	Accrual stopped 07/30/2009 by the Sponsor due to safety issues with the device. We are still following the subjects enrolled. Two of the three subjects that were randomized to the investigational device have subsequently had it removed. Despite recommendation that the device be removed, one subject refuses removal of the device at this time.
20091776-2 Lanx, Inc.	Randomized study of ASPEN™ Spinous Process System vs Pedicle screws in ALIF	Sub-Investigator	9 (total) 0 (Muizelaar)	Treatment	Yes. It is imperative that subjects be followed because they have a device implanted. (No Muizelaar patients).
215132-2 Integra LifeSciences	Efficacy and Safety of Integra Accell EVO3 Demineralized Bone Matrix in Instrumented Lumbar Spine Fusion: Comparison to rh-BMP2	Sub-Investigator	0 (total)	Treatment	Yes. This study has just started enrollment. This is an outcomes study looking at the fusion rates between BMP-2 and EVO3.
215274-2 Globus	Does the type of procedure affect the incidence of dysphagia and dysphonia in ACDF patients?	Sub-Investigator	6 (total) 0 (Muizelaar)	Treatment	Yes. This is an outcome study looking at dysphagia.

IRB Protocol #	Title	Role	Number of subjects currently enrolled to study	Treatment or Non-Treatment	Ongoing Enrollment/ Participation by subjects?
Medical, Inc.					
220609-1 Mesoblast, Inc.	A Prospective, Multi-Center, Randomized, Single-Blinded, Controlled Study Evaluating Safety and Preliminary Efficacy of NeoFuse™ when Comparing with MasterGraft ™ Matrix in Subjects Undergoing Multi-Level ACDF with Anterior Cervical Plate Fixation.	Sub-Investigator	2 (total) 0 (Muizelaar)	Treatment	Yes. Phase I study just began enrollment. It is imperative that subjects who received investigational product be followed. (No Muizelaar patients).
226065-2 Medtronics (formerly Kyphon, Inc.)	Treatment of Lumbar Spinal Stenosis with XSTOP®PK Interspinous Process Decompression (IPD) System in Moderately Symptomatic Patients.	Sub-Investigator	10 (total) 0 (Muizelaar)	Treatment	No. Study was closed for new patients at UCD due to large number of revision surgeries. Patients are being followed per protocol for 5 years.

Rudolph Schrot, MD

IRB Protocol #	Title	Role	Number of subjects currently enrolled to study	Treatment or Non-Treatment	Ongoing Enrollment/ Participation by subjects?
214797-3	Cilengitide in subjects with newly diagnosed glioblastoma multiforme and methylated MGMT gene promoter - a multicenter, open-label, controlled Phase III study, testing cilengitide in combination with standard treatment (temozolomide with concomitant radiation therapy, followed by temozolomide maintenance therapy) versus standard treatment alone. (Cilengitide in Newly Diagnosed GBM Patients with Methylated MGMT Gene Promoter). [CLINICAL STUDY EMD 121974-011]	Principal Investigator	1	Treatment	No, closed to enrollment, no patients in long-term follow up
223610-2	Cilengitide in subjects with newly diagnosed glioblastoma multiforme and unmethylated MGMT gene promoter - a multicenter, open-label, Phase II study, investigating two cilengitide regimens in combination with standard treatment (temozolomide with concomitant radiation therapy, followed by temozolomide maintenance therapy)-CORE (Cilengitide in Newly Diagnosed GBM Patients with Unmethylated MGMT Gene Promoter) [CLINICAL STUDY EMD 121974-012]	Principal Investigator	3	Treatment	Yes, one patient in long-term follow up for safety. Study is closed to enrollment.
201018007-1	Immunohistochemical Markers of Brain Metastasis	Principal Investigator	168	Non-treatment	No
201017966-1	Clinical findings and urodynamic measurement before and after surgery in patients with symptomatic Tarlov cysts	Principal Investigator	49	Non-treatment	No
200816263-3	Time resolved laser-induced fluorescence for guided therapy of brain tumors	Principal Investigator	9	Non-treatment	No

IRB Protocol #	Title	Role	Number of subjects currently enrolled to study	Treatment or Non-Treatment	Ongoing Enrollment/ Participation by subjects?
200715889-5	Intraoperative specimen harvest for ex-vivo analysis and study	Principal Investigator	52	Non-treatment	No
201018249-1	Diffusion tensor imaging in normal pressure hydrocephalus: Changes in periventricular white matter anisotrophy before and after ventriculoperitoneal shunting	Principal Investigator	0	Non-treatment	N/A
200816483 Blackstone Medical, Inc.	Blackstone Advent ™ Cervical Disc vs. ACDF for Treatment of One Level Degenerative Disc Disease	Principal Investigator	10 (total) 4 (Schrot)	Treatment	Accrual stopped 07/30/2009 by the Sponsor due to safety issues with the device. We are still following the subjects enrolled. Two of the three subjects that were randomized to the investigational device have subsequently had it removed. Despite recommendation that the device be removed, one subject refuses removal of the device at this time.
200917003-1	The Role of Cervical Spinal Stenosis in the production of Cervicogenic Headache	Principal Investigator	Retrospec-tive chart review - 400+ reviewed from all participants	Non-Treatment	No. Chart review data is currently being analyzed.
200614296-5 LDR Spine	Mobi-C Artificial Disc vs ACDF in Treatment of DDD of cervical Spine at 1 or 2 levels	Sub-Investigator	53 (total) 14 (Schrot) Eleven	Treatment	Study closed to accrual March of 2008. Study is in long-term follow-up at this time. It is imperative

IRB Protocol #	Title	Role	Number of subjects currently enrolled to study	Treatment or Non-Treatment	Ongoing Enrollment/ Participation by subjects?
			subjects currently being followed. Three subjects lost to follow-up at this time.		that these subjects continue to be followed, since they may have an investigational device implanted, depending on their randomized treatment assignment.
200816020-2 Medtronics	Post-Approval Investigation of the PRESTIGE® Cervical Disc Device at a Single Level	Sub-Investigator	23 (total) 0 (Schrot)	Post-Market study. Did not enroll any subjects on pivotal or post-market study.	Study just completed 7-year follow-up and will close after data has been submitted to FDA.
20091776-2 Lanx, Inc.	Randomized study of ASPEN™ Spinous Process System vs Pedicle screws in ALIF	Sub-Investigator	9 (total) 3 (Schrot)	Treatment	Yes. It is imperative that these subjects be followed because they have a device implanted.
215132-2 Integra LifeSciences	Efficacy and Safety of Integra Accell EVO3 Demineralized Bone Matrix in Instrumented Lumbar Spine Fusion: Comparison to rh-BMP2	Sub-Investigator	0	Treatment	Yes. This study has just started enrollment. This is an outcomes study looking at the fusion rates between BMP-2 and EVO3.
215274-2 Globus Medical, Inc.	Does the type of procedure affect the incidence of dysphagia and dysphonia in ACDF patients?	Sub-Investigator	6 (total) 0 (Schrot)	Treatment	Yes. This is an outcome study looking at dysphagia.
220609-1 Mesoblast, Inc.	A Prospective, Multi-Center, Randomized, Single-Blinded, Controlled Study Evaluating Safety and Preliminary Efficacy of NeoFuse™	Sub-Investigator	2 (total) 1 (Schrot)	Treatment	Yes. It is imperative that this subject be followed as the subject has received investigational.

IRB Protocol #	Title	Role	Number of subjects currently enrolled to study	Treatment or Non-Treatment	Ongoing Enrollment/ Participation by subjects?
	when Comparing with MasterGraft ™ Matrix in Subjects Undergoing Multi-Level ACDF with Anterior Cervical Plate Fixation.				product.
226065-2 Medtronics (formerly Kyphon, Inc.)	Treatment of Lumbar Spinal Stenosis with XSTOP®PK Interspinous Process Decompression (IPD) System in Moderately Symptomatic Patients.	Sub-Investigator	10 (total) 4 (Schrot)	Treatment	No. Accrual has stopped at UCD due to high number of revisions on our patients. It is imperative that these subjects be followed as they have a device implanted.
217916-2	Visual Field Changes in Patients with Pseudotumor Cerebri after VP or LP Shunt	Co-investigator	88	Non-treatment	Retrospective chart review. No subject enrollment.

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OFFICE OF RESEARCH
IRB Administration
Phone (916) 703-9151
FAX: (916) 703-9160

SACRAMENTO, CALIFORNIA 95817

CONFIDENTIAL

October 14, 2011

Via electronic mail & campus mail

J. PAUL MUIZELAAR, MD
NEUROLOGICAL SURGERY

RUDOLPH SCHROT, MD
NEUROLOGICAL SURGERY

RE: "Probiotic Intracranial Therapy for Malignant Glioma"

Dear Drs. Muizelaar and Schrot:

The Institutional Review Board (IRB) reviewed your response letter dated September 22, 2011, and the two undated documents you provided to us via email on September 26, 2011, reflecting your involvement in currently approved human studies at the University.

After due consideration by each of the IRB biomedical committees, the IRB reaffirms its initial determination that the surgical procedures to treat glioblastoma with intentional wound infection were undertaken without IRB review and approval and that these activities constitute serious and continuing non-compliance with University policies and federal regulations.

As to your participation in IRB-approved research protocols, the IRB determined the following:

1. You are to immediately cease and desist from any research activity in studies for which you serve as Principal Investigator or co-Principal Investigator. If you desire any of these studies to continue, you must identify other faculty members who will assume your roles and responsibilities. Please submit their names (with corresponding IRB protocol number and study title) for consideration by the IRB. No new subjects may be enrolled in any of these studies without IRB review and approval. Please work with the contracting office to assure that any required notifications are submitted to sponsors.

2. For studies where you serve as co-investigator, you must cease participation on any human research activity. You must inform the Principal Investigators of those projects and the PI must remove you from the study research personnel lists using the IRB modification form. In the circumstance where the PI of that study believes that your expertise is deemed essential for the safety and welfare of research participants, the PI may request an exception from the IRB's prohibition of your involvement in the research by providing a justification as to why your continued participation in these studies is necessary and appropriate.

3. An ad-hoc subcommittee, consisting of the Chairs and Vice-Chairs of the Clinical IRB Committees, has been convened to review and evaluate exception requests by PIs who believe you should continue participating in their research. The subcommittee will also work to ensure study personnel transitions occur without negatively impacting the safety and welfare of research participants. The subcommittee may also perform other functions related to this matter as deemed appropriate by the IRB.

If you have any questions about this process, you may contact us at david.asmuth@ucdmc.ucdavis.edu and john.anderson@ucdmc.ucdavis.edu. Please copy both of us on all correspondence.

Sincerely,

David Asmuth, MD
Chair
Clinical Committee A

John Anderson, MD
Chair
Clinical Committee B

/sh

cc: Ralph Hexter, Provost & Executive Vice Chancellor
Harris Lewin, Vice Chancellor for Research
Claire Pomeroy, Vice Chancellor for Health Sciences and Dean, School of Medicine
John Meyer, Vice Chancellor for Administrative and Resource Management
Sidney Scudder, Vice Chair, IRB-A
Deborah Diercks, Vice Chair, IRB-B

From: Elodia Tarango [elodia.tarango@ucdmc.ucdavis.edu]
Sent: Friday, September 16, 2011 11:44 AM
To: Rudolph Schrot; J.paul Muizelaar
Cc: hexter@ucdavis.edu; lewin@ucdavis.edu; Claire Pomeroy; jameyer@ucdavis.edu; john.anderson@ucdmc.ucdavis.edu; david.asmuth@ucdmc.ucdavis.edu
Subject: RESENDING - IRB COMMUNICATION
Attachments: 2153_0001.pdf

Importance: High

This communication is being resent to you. There was a scanning error in the prior communication. Thank you

Elodia Tarango
Interim IRB Director
IRB Administration
University of California, Davis
Phone: (916) 703-9154 Fax: (530) 752-3472
E-Mail: elodia.tarango@ucdmc.ucdavis.edu
CTSC Bldg., 2921 Stockton Blvd.
Suite 1400, Rm. 1429, Sacto, CA 95817
www.research.ucdavis.edu/IRBAdmin

----- Forwarded by Elodia Tarango/SOM/HS/UCD on 09/16/2011 11:40 AM -----

From: irb@reprographics.ucdavis.edu
To: "Lody" <elodia.tarango@ucdmc.ucdavis.edu>
Date: 09/16/2011 11:40 AM
Subject: Attached Image

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SACRAMENTO, CALIFORNIA 95817

CONFIDENTIAL

September 16, 2011

Via electronic and campus mail

J. PAUL MUIZELAAR, MD
NEUROLOGICAL SURGERY

RUDOLPH SCHROT, MD
NEUROLOGICAL SURGERY

Re: "Probiotic Intracranial Therapy for Malignant Glioma"

Dear Drs. Muizelaar and Schrot:

The Institutional Review Board (IRB) reviewed the August 31, 2011 report on Investigation Findings related to the three surgical procedures performed under the activity "Probiotic Intracranial Therapy for Malignant Glioma" from October 2010 through March 2011. In addition, the IRB reviewed your undated written response to the Investigation Findings and your Prospectus dated March 9, 2011.

In accordance with University Policy 240, the IRB is responsible for reviewing all research conducted at the institution involving human subjects and as required by state and federal regulations. The main issue before the IRB is whether IRB review and approval should have been obtained by you prior to performing the procedures.

The IRB understands that for Patient #1, you indicated to then-IRB Director Eric Mah that the procedure was only performed in the course of the practice of medicine as an innovative/unconventional treatment for a patient facing certain death and in urgent need. In addition, it was a one-time event using bacteria which was locally obtained.

While the treatment of Patient #1 with the bacteria in the course of the practice of medicine alone may not have constituted human subjects research, the IRB investigation revealed that the bacteria was not locally derived but originated from ATCC of Manassas, Virginia. In addition, ATCC had specifically instructed that it was not intended for human use. The IRB has determined that providing inaccurate information to IRB staff led to an incorrect determination that no IRB review was needed for this one patient.

Furthermore, the IRB determined that administering the bacteria to Patient #2 and Patient #3 constituted human research without IRB review and approval in violation of Policy 240 and other established procedures and institutional requirements. The IRB believes the activity performed possibly increased the risk to patient-subjects but the IRB cannot fully evaluate the specific risks because the IRB was never presented with a protocol for these three patients treated prior to the surgeries.

While the IRB recognizes that you believed you were acting in the best interests of your patients, the IRB has concluded that serious and continuing noncompliance occurred and the procedures you performed required IRB review and approval.

The IRB requires the following:

1) You not use this bacteria or perform this procedure with similar aims using any other unapproved substance without IRB and FDA review and authorization.

2) On all human research for which you serve as Principal Investigators (PIs) or co-Principal Investigators, you must immediately halt all enrollment of new subjects.

    - Please report within 10 days of the date of this letter in a tabular format, the project title, IRB protocol number (as applicable), the current subject enrollment, whether the research is a treatment or non-treatment protocol, and whether ongoing participation by subjects is in the clinical best interests of the subjects and why.

3) Within 10 days of the date of this letter, please provide a report to the IRB on all human research for which you serve as study personnel and/or co-investigators, as applicable.

    - In this report, referencing the project title and IRB protocol number (as applicable), please clarify your specific roles in the research, the current subject enrollments, whether the research is a treatment or non-treatment protocol, and whether ongoing participation by subjects is in the clinical best interests of the subjects and why.

Based on your report and review of the individual protocols, the IRB will make study-by-study determinations on the appropriateness of your continued involvement in these research projects and whether your involvement minimizes risks to subjects in accordance with University policy and federal regulations.

In addition, the use of an unapproved agent may also have violated other University policies, which fall outside of the purview of the IRB. Consequently, this case will also be referred to:

- The Committee on Biosafety regarding the use of the bacteria in humans
- The UC Davis Health System for allowing non-approved products in a surgical setting

- Campus Academic Personnel as a potential violation of Academic Personnel Manual 015 (Faculty Code of Conduct) for conducting human research without prior IRB review and approval, in violation of Policy 240.

We are also required to report IRB findings to certain external entities, including the Food & Drug Administration. You will be copied on any such correspondence.

Finally, because you did not obtain IRB approval, the IRB reminds you that most medical journals require IRB approval when reviewing manuscripts involving human participants; your activity required IRB approval, and use of identifiable data requires current IRB approval. From the IRB's perspective, this unsanctioned research activity should not be submitted for dissemination or publication.

If there is additional information you wish to share with the IRB, which you would like considered before the above actions are taken, please submit that to us within seven (7) business days.

If you have any questions about this process, you may contact us at david.asmuth@ucdmc.ucdavis.edu and john.anderson@ucdmc.ucdavis.edu. Please copy both of us on all correspondence.

Sincerely,

David Asmuth, MD
Chair
Clinical Committee-A

John Anderson, MD
Chair
Clinical Committee-B

cc: Ralph Hexter, Provost & Executive Vice Chancellor
Harris Lewin, Vice Chancellor for Research
Claire Pomeroy, Vice Chancellor for Health Sciences and Dean, School of Medicine
John Meyer, Vice Chancellor for Administrative & Resource Management

DRAFT Health System P&P
Innovative Use Policy

I. Purpose

To provide guidance and support physicians for the innovative use and novel application of medical therapies, devices and/or medications in the treatment of patients. This policy is restricted to innovative use in the clinical setting.

II. Setting

UC Davis Medical Center

III. Definitions

a. ***Innovative Use*** is the application of a therapy, device, or medication in a manner that departs in a significant way from standard or accepted practice. Innovative use includes any use of an unapproved drug, biologic, or device that has IRB approval in clinical practice. Innovative Use is not common "off label" use of FDA-approved drugs or devices.

b. An ***Unapproved Drug, Biologic, or Device*** is any product intended for use in the diagnosis, cure, mitigation, treatment, or prevention of human disease but that has not been approved or cleared for marketing by FDA.

III. Policy

A. Institutional Objective

UCDMC recognizes that medical circumstances arise in which non-standard therapies for treatment or novel uses for drugs or devices may be warranted.

B. Physician Responsibility

1. Any physician who wishes to utilize an Unapproved Drug, Biologic, or Device shall first obtain approval from the IRB (or, in an emergency, from the FDA with notification to the IRB consistent with IRB-approved emergency use/expanded access policies and guidelines).

2. The ordering and/or prescribing physician shall request a consultation regarding an Innovative Use by directly contacting the Chief Medical Officer.

3. The physician shall document in the medical record the informed consent of the patient/patient's representative reflecting that person's understanding that the treatment represents a novel approach and has not been fully tested or approved prior to using or administering the innovative use.

C. Chief Medical Officer

1. The Chief Medical Officer shall review requests, monitor outcomes to minimize continued use of ineffective or unsafe practices, and assure that the physician and institution meet ethical and legal obligations.

OFFICE OF THE GENERAL COUNSEL

Health Sciences Research Advisory: *Emergency or "Compassionate" Use of Unapproved Drugs*

October 17, 2011

Q. What options are available for a patient with a life-threatening illness when no approved drug is available to treat the illness and the patient is not eligible for participation in a clinical trial investigating a new drug with the potential to help?

A. Options vary depending on the circumstances. The most promising may be treatment under FDA's expanded access program.

Background/Executive Summary

A drug is a medicine, biologic, compound, or other substance ("article") intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease; or to affect the structure or function of the body; or otherwise recognized in the official United States Pharmacopoeia. Foods, dietary ingredients, and dietary supplements may be considered drugs in some circumstances.

The Federal Food, Drug & Cosmetic Act ("FDCA") prohibits the shipment in interstate commerce of "adulterated" and "misbranded" drugs. Unapproved drugs and approved drugs utilized "off-label" (that is, inconsistent with FDA-approved labeling), may be adulterated or misbranded. FDCA and regulations FDA has promulgated under the law permit use of these drugs only under limited circumstances, for example in connection with a clinical investigation performed pursuant to an investigational new drug application ("IND") or an IND exemption. FDA does not, however, regulate the practice of medicine. Thus, duly licensed physicians may prescribe approved drugs off-label without triggering FDA oversight, provided that state laws and institutional policies permit such use, and the patient is appropriately informed.[*]

Pharmacy compounding may also be an option in some circumstances, though these are limited.

FDA has developed procedures that may be utilized to facilitate treatment with an unapproved (that is, "investigational") product when a physician determines that no approved therapies are viable. *Expanded access* or *compassionate use* is "the use of an investigational drug outside of a clinical trial, for the sole purpose of treating a patient or patients with a serious or life-threatening disease who have no acceptable medical options."[†] Serious diseases or conditions are those associated with morbidity that has a substantial impact on day-to-day functioning. Whether a disease is serious is a matter of clinical judgment. Examples cited by FDA include epilepsy and *in situ* breast cancer. The remainder of this advisory describes the procedures to follow to take advantage of FDA's expanded access program.

[*] Off-label prescribing may, however, subject physicians or their employers to professional liability if it is inconsistent with the standard of care and results in patient injury; and off-label promotion by drug manufacturers has been the subject of intensive federal enforcement initiatives in recent years.

[†] The expanded access rules are not entirely consistent in scope or in approach with rules found at 21 C.F.R. part 312 subpart E governing drugs intended to treat only life-threatening or severely debilitating illnesses. The rules in subpart E are directed primarily at product manufacturers to facilitate development, evaluation and marketing of new therapies, and are not easily utilized by academic medical centers and their physicians to facilitate clinical treatment of individual patients.

Physician/Investigator Responsibilities Under the Expanded Access Program

A physician who wishes to administer an investigational drug under the expanded access program is responsible for procuring the drug from the sponsor, securing IRB approval and providing informed consent consistent with standard FDA rules (subject to emergency use exceptions provided in those rules), reporting adverse drug events to the sponsor, and maintaining accurate drug accountability and patient case history records. Depending on the circumstances, additional requirements may apply. A physician who is unable or unwilling to meet these demands should not proceed.

Eligibility and Procedures

Note: This document describes minimal regulatory requirements. Local IRBs and medical centers may have adopted additional requirements and specific procedures for addressing expanded access. For example, medical staff policies may require consultation with the Chief Medical Officer or an innovative use, patient safety, or ethics committee, in addition to IRB approval. Please consult with your local IRB and medical center policies for additional information.

1. Procurement of Drug

Before pursuing an emergency or single use IND, the physician should confirm that the drug's manufacturer is willing and able to provide the drug. If there is no commercial manufacturer and the drug is to be manufactured locally, FDA will require detailed manufacturing information. The agency has published guidance on current good manufacturing practices ("cGMP") for Phase I studies and for later stage investigations. Additional information is available online.

2. FDA Evaluation

FDA also has issued detailed guidance describing the steps a physician must take to secure FDA permission to treat a patient under the expanded access program. Briefly, the physician must specify whether he or she is seeking an emergency IND or a single-patient IND; provide information about the patient's history and prognosis, treatment plan, and proposed intervention, and attach a completed IND application. It is unnecessary to prepare a formal research protocol designed to obtain meaningful scientific data – FDA fully recognizes that the purpose of the expanded access program is primarily to diagnose, monitor, or treat a patient's condition; and expects very limited data collection (described in further detail below). A sample cover sheet that may be used to facilitate development of a single IND application is provided below.

3. IRB Approval or Notification

Advance IRB approval is generally required for single-patient INDs even though the purpose of the use of the test article is for clinical care and not research. In the event of an emergency (a life-threatening or severely debilitating situation where no standard acceptable treatment is available and there is not sufficient time to obtain IRB approval), the investigator (the physician responsible for administering the investigational product, or "test article") may rely on an emergency use exemption under 21 C.F.R. § 56.104(c). "Life-threatening" includes diseases or conditions where the likelihood of death or major irreversible morbidity such as blindness, loss of limb, loss of hearing, paralysis, or stroke is high if promising treatment is delayed until the IRB can convene. In this case, the investigator must report the emergency use to the IRB within five (5) days of administration. This exemption to the IRB approval requirement applies only to a single use of the test article; any subsequent use requires prospective IRB review and approval. However, FDA has acknowledged that it would be inappropriate to deny emergency treatment to a second individual if the only obstacle was that the IRB had not had sufficient time to convene a meeting to review the issue.

Once IRB approval has been secured, standard rules for conducting clinical trials generally apply. For example, the investigator must promptly report any changes in activity under the IND, as well as unanticipated problems

involving risks to patients or others. In addition, the investigator may not make any changes to the activities proposed under the emergency or single patient IND without IRB approval, except where necessary to eliminate apparent immediate hazards to patients.

4. Informed Consent

The informed consent requirements found at 21 C.F.R. part 50 apply to emergency and single-patient INDs. Accordingly, informed consent for administration of such drugs may not include exculpatory language (*e.g.*, assumption of risk or waiver of a patient's right to sue). The only exception to the informed consent requirement is for emergency use where both the investigator and an uninvolved physician certify in writing to FDA and the IRB, under 21 C.F.R. § 50.23(a) that:

- The patient is confronted by a life-threatening situation necessitating the use of the test article.
- Informed consent cannot be obtained because of an inability to communicate with, or obtain legally effective informed consent from, the subject.
- Time is not sufficient to obtain consent from the subject's legal representative.
- No alternative method of approved or generally recognized therapy is available that provides an equal or greater likelihood of saving the subject's life.

If, in the investigator's opinion, immediate use is required to preserve the patient's life, and time is not sufficient to obtain an independent physician's determination that the above conditions apply, the investigator may make the determination and, within five (5) working days after use of the product, have the determination reviewed and evaluated in writing by an uninvolved physician.

A sample consent form that may be submitted to FDA and the IRB with the IND and IRB approval applications is provided below (though individual IRBs may require different forms depending on their local standard operating procedures). Note that the form references the fact that patients may be responsible for the costs of an investigational drug administered through the expanded access program. While FDA does permit sponsors to charge for investigational drugs under certain conditions, that fact does not mean that Medicare or other payors will reimburse those costs, and in fact many will not.

5. Application of Standard IND Rules

The investigator is responsible for ensuring that the drug is administered in accordance with the information provided in the emergency or single patient IND, as well as other documentation provided to the sponsor; for protecting the rights, safety, and welfare of patients to whom the drug is administered, and for controlling the investigational drug supply. Specifically, the investigator must: (1) administer the investigational drug only to subjects under his or her personal supervision or the supervision of a subinvestigator (listed in a Form 1572 submitted with the emergency or single patient IND application) responsible to the investigator; (2) produce and maintain adequate and accurate records; (3) maintain control of the investigational agent; and (4) submit progress and safety reports to the sponsor (or, if there is no sponsor, then directly to FDA).

Additional Information

- National Library of Medicine (Clinical Trials Registry), http://clinicaltrials.gov
  To locate listed expanded access programs in ClinicalTrials.gov, select "Search for Clinical Trials" from the http://clinicaltrials.gov website, select "Advanced Search" on the Search Page, find the field titled "Study Type" and select "Expanded Access Studies" from the pull-down menu. Additional search terms (e.g., condition) may be added to the Advanced Search. Select "Search" to see a list of expanded access programs. Alternatively, you may view a list of all currently available Expanded Access studies. If a drug is not listed, a request can still be made of the sponsor.[‡]

- California Department of Public Health, http://www.cdph.ca.gov
  The site includes information on CDPH regulation of drugs and devices, including information on the Department's Drug Safety Program and the text of the Sherman Food, Drug & Cosmetic Law.

FDA Links

- Investigational New Drug Regulations
- Expanded (Drug) Access Website
- Final Rule (2009) on Expanded Access to Investigational Drugs
- Final Rule (2009) on Charging for Investigational Drugs

Attachments

- Current Expanded Access Regulatory Text
- Sample Informed Consent Document
- Sample Cover Sheet for IND Submission (to be Attached to FDA-1571)

More Information

Contact: Rachel Nosowsky, OGC (510) 987-9407

[‡] Adapted from http://www.fda.gov/ForConsumers/ByAudience/ForPatientAdvocates/SpeedingAccesstoImportantNewTherapies/ucm177138.htm.

PHARMACY COMPOUNDING

FDA recognizes that licensed pharmacists routinely compound and manipulate reasonable quantities of human drugs in response to valid prescriptions written by licensed health care providers for individual patients, and generally defers to state authorities for oversight and enforcement of these activities. However, FDA is more likely to identify FDCA violations and initiate federal enforcement in response to the following activities:

1. Compounding drugs in anticipation of receiving prescriptions, except in very limited quantities in relation to the amounts of drugs compounded after receiving valid prescriptions.
2. Compounding drugs that were withdrawn or removed from the market for safety reasons. [A list of those drugs is available online.]
3. Compounding finished drugs from bulk active ingredients that are not components of FDA approved drugs without an [IND].
4. Receiving, storing, or using drug substances without first obtaining written assurance from the supplier that each lot of the drug substance has been made in an FDA-registered facility.
5. Receiving, storing, or using drug components not guaranteed or otherwise determined to meet official compendia requirements.
6. Using commercial scale manufacturing or testing equipment for compounding drug products.
7. Compounding drugs for third parties who resell to individual patients or offering compounded drug products at wholesale to other state licensed persons or commercial entities for resale.
8. Compounding drug products that are commercially available in the marketplace or that are essentially copies of commercially available FDA-approved drug products [except in special situations, for example where medical need can be established].
9. Failing to operate in conformance with applicable state law regulating the practice of pharmacy.

U.S. Food and Drug Administration, CPG 460.200: Pharmacy Compounding Compliance Policy Guides Manual (Tab M). Additional guidance on pharmacy compounding and information on FDA's related enforcement initiatives is posted online.

REGULATORY TEXT FOR EXPANDED ACCESS
(21 C.F.R. §§ 312.300, 312.305, 312.310)

Sec. 312.300 General

(a) *Scope*. This subpart contains the requirements for the use of investigational new drugs and approved drugs where availability is limited by a risk evaluation and mitigation strategy (REMS) when the primary purpose is to diagnose, monitor, or treat a patient's disease or condition. The aim of this subpart is to facilitate the availability of such drugs to patients with serious diseases or conditions when there is no comparable or satisfactory alternative therapy to diagnose, monitor, or treat the patient's disease or condition.

(b) *Definitions*. The following definitions of terms apply to this subpart:
Immediately life-threatening disease or condition means a stage of disease in which there is reasonable likelihood that death will occur within a matter of months or in which premature death is likely without early treatment.
Serious disease or condition means a disease or condition associated with morbidity that has substantial impact on day-to-day functioning. Short-lived and self-limiting morbidity will usually not be sufficient, but the morbidity need not be irreversible, provided it is persistent or recurrent. Whether a disease or condition is serious is a matter of clinical judgment, based on its impact on such factors as survival, day-to-day functioning, or the likelihood that the disease, if left untreated, will progress from a less severe condition to a more serious one.

Sec. 312.305 Requirements for all expanded access uses.

The criteria, submission requirements, safeguards, and beginning treatment information set out in this section apply to all expanded access uses described in this subpart. Additional criteria, submission requirements, and safeguards that apply to specific types of expanded access are described in 312.310 through 312.320.

(a) *Criteria*. FDA must determine that:
(1) The patient or patients to be treated have a serious or immediately life-threatening disease or condition, and there is no comparable or satisfactory alternative therapy to diagnose, monitor, or treat the disease or condition;
(2) The potential patient benefit justifies the potential risks of the treatment use and those potential risks are not unreasonable in the context of the disease or condition to be treated; and
(3) Providing the investigational drug for the requested use will not interfere with the initiation, conduct, or completion of clinical investigations that could support marketing approval of the expanded access use or otherwise compromise the potential development of the expanded access use.

(b) *Submission*. (1) An expanded access submission is required for each type of expanded access described in this subpart. The submission may be a new IND or a protocol amendment to an existing IND. Information required for a submission may be supplied by referring to pertinent information contained in an existing IND if the sponsor of the existing IND grants a right of reference to the IND.
(2) The expanded access submission must include:
(i) A cover sheet (Form FDA 1571) meeting the requirements of 312.23(a);
(ii) The rationale for the intended use of the drug, including a list of available therapeutic options that would ordinarily be tried before resorting to the investigational drug or an explanation of why the use of the investigational drug is preferable to the use of available therapeutic options;
(iii) The criteria for patient selection or, for an individual patient, a description of the patient's disease or condition, including recent medical history and previous treatments of the disease or condition;
(iv) The method of administration of the drug, dose, and duration of therapy;
(v) A description of the facility where the drug will be manufactured;
(vi) Chemistry, manufacturing, and controls information adequate to ensure the proper identification, quality, purity, and strength of the investigational drug;

(vii) Pharmacology and toxicology information adequate to conclude that the drug is reasonably safe at the dose and duration proposed for expanded access use (ordinarily, information that would be adequate to permit clinical testing of the drug in a population of the size expected to be treated); and
(viii) A description of clinical procedures, laboratory tests, or other monitoring necessary to evaluate the effects of the drug and minimize its risks.
(3) The expanded access submission and its mailing cover must be plainly marked "EXPANDED ACCESS SUBMISSION." If the expanded access submission is for a treatment IND or treatment protocol, the applicable box on Form FDA 1571 must be checked.

(c) *Safeguards*. The responsibilities of sponsors and investigators set forth in subpart D of this part are applicable to expanded access use under this subpart as described in this paragraph.
(1) A licensed physician under whose immediate direction an investigational drug is administered or dispensed for an expanded access use under this subpart is considered an*investigator* , for purposes of this part, and must comply with the responsibilities for investigators set forth in subpart D of this part to the extent they are applicable to the expanded access use.
(2) An individual or entity that submits an expanded access IND or protocol under this subpart is considered a*sponsor* , for purposes of this part, and must comply with the responsibilities for sponsors set forth in subpart D of this part to the extent they are applicable to the expanded access use.
(3) A licensed physician under whose immediate direction an investigational drug is administered or dispensed, and who submits an IND for expanded access use under this subpart is considered a*sponsor-investigator* , for purposes of this part, and must comply with the responsibilities for sponsors and investigators set forth in subpart D of this part to the extent they are applicable to the expanded access use.
(4) *Investigators*. In all cases of expanded access, investigators are responsible for reporting adverse drug events to the sponsor, ensuring that the informed consent requirements of part 50 of this chapter are met, ensuring that IRB review of the expanded access use is obtained in a manner consistent with the requirements of part 56 of this chapter, and maintaining accurate case histories and drug disposition records and retaining records in a manner consistent with the requirements of 312.62. Depending on the type of expanded access, other investigator responsibilities under subpart D may also apply.
(5) *Sponsors*. In all cases of expanded access, sponsors are responsible for submitting IND safety reports and annual reports (when the IND or protocol continues for 1 year or longer) to FDA as required by 312.32 and 312.33, ensuring that licensed physicians are qualified to administer the investigational drug for the expanded access use, providing licensed physicians with the information needed to minimize the risk and maximize the potential benefits of the investigational drug (the investigator's brochure must be provided if one exists for the drug), maintaining an effective IND for the expanded access use, and maintaining adequate drug disposition records and retaining records in a manner consistent with the requirements of 312.57. Depending on the type of expanded access, other sponsor responsibilities under subpart D may also apply.

(d) *Beginning treatment* – (1) *INDs*. An expanded access IND goes into effect 30 days after FDA receives the IND or on earlier notification by FDA that the expanded access use may begin.
(2) *Protocols*. With the following exceptions, expanded access use under a protocol submitted under an existing IND may begin as described in 312.30(a).
(i) Expanded access use under the emergency procedures described in 312.310(d) may begin when the use is authorized by the FDA reviewing official.
(ii) Expanded access use under 312.320 may begin 30 days after FDA receives the protocol or upon earlier notification by FDA that use may begin.
(3) *Clinical holds*. FDA may place any expanded access IND or protocol on clinical hold as described in 312.42.

§ 312.310: Under this section, FDA may permit an investigational drug to be used for the treatment of an individual patient by a licensed physician.

(a) *Criteria*. The criteria in 312.305(a) must be met; and the following determinations must be made:
(1) The physician must determine that the probable risk to the person from the investigational drug is not greater than the probable risk from the disease or condition; and

(2) FDA must determine that the patient cannot obtain the drug under another IND or protocol.

(b) *Submission*. The expanded access submission must include information adequate to demonstrate that the criteria in 312.305(a) and paragraph (a) of this section have been met. The expanded access submission must meet the requirements of 312.305(b).
(1) If the drug is the subject of an existing IND, the expanded access submission may be made by the sponsor or by a licensed physician.
(2) A sponsor may satisfy the submission requirements by amending its existing IND to include a protocol for individual patient expanded access.
(3) A licensed physician may satisfy the submission requirements by obtaining from the sponsor permission for FDA to refer to any information in the IND that would be needed to support the expanded access request (right of reference) and by providing any other required information not contained in the IND (usually only the information specific to the individual patient).

(c) *Safeguards*. (1) Treatment is generally limited to a single course of therapy for a specified duration unless FDA expressly authorizes multiple courses or chronic therapy.
(2) At the conclusion of treatment, the licensed physician or sponsor must provide FDA with a written summary of the results of the expanded access use, including adverse effects.
(3) FDA may require sponsors to monitor an individual patient expanded access use if the use is for an extended duration.
(4) When a significant number of similar individual patient expanded access requests have been submitted, FDA may ask the sponsor to submit an IND or protocol for the use under 312.315 or 312.320.

(d) *Emergency procedures*. If there is an emergency that requires the patient to be treated before a written submission can be made, FDA may authorize the expanded access use to begin without a written submission. The FDA reviewing official may authorize the emergency use by telephone.
(1) Emergency expanded access use may be requested by telephone, facsimile, or other means of electronic communications. For investigational biological drug products regulated by the Center for Biologics Evaluation and Research, the request should be directed to the Office of Communication, Outreach and Development, Center for Biologics Evaluation and Research, 301-827-1800 or 1-800-835-4709, e-mail:*ocod@fda.hhs.gov* . For all other investigational drugs, the request for authorization should be directed to the Division of Drug Information, Center for Drug Evaluation and Research, 301-796-3400, e-mail:*druginfo@fda.hhs.gov* . After normal working hours (8 a.m. to 4:30 p.m.), the request should be directed to the FDA Emergency Call Center, 866-300-4374, e-mail:*emergency.operations@fda.hhs.gov* .
(2) The licensed physician or sponsor must explain how the expanded access use will meet the requirements of 312.305 and 312.310 and must agree to submit an expanded access submission within 15 working days of FDA's authorization of the use.

INFORMED CONSENT FOR TREATMENT WITH AN UNAPPROVED DRUG

You have been diagnosed with: _____.
This is a serious or life-threatening disease. You and your doctor have discussed your options. Your doctor believes that:

- √ There is no FDA-approved drug available to treat your condition because:
  - o No such drug exists; or
  - o Approved drugs have been tried but not worked; or
  - o Approved drugs cause side-effects that you cannot tolerate.
- √ You cannot find or get into a clinical trial of an experimental drug that might help.
- √ There are no other acceptable medical options.

Your doctor has told you that an unapproved drug, _____, might help. This drug has not been proven to be safe or effective for your treatment. The Food and Drug Administration (FDA), however, has given your doctor permission to treat you with this unapproved drug under its "expanded access" program. More information about the program is online at: http://tinyurl.com/UCM176098 and http://tinyurl.com/UCM177138. If you cannot access these articles on a computer, please ask your doctor to print them out for you.

The known risks of the unapproved drug include: _____
_____.

Your doctor has a financial interest in the drug or its manufacturer as: ☐ an inventor (may receive royalties if the drug is approved and marketed); ☐ a consultant/advisor/spokesperson (receives or in the last 12 months has received professional service fees); ☐ an officer or director or employee; ☐ other: _____; ☐ none (your doctor has no financial interest in the drug or its manufacturer).

Your doctor has told you that treatment with this unapproved drug is not the same as regular drug treatment:

- √ **While the drug may help you, it may not. There is no guarantee.**
- √ **The drug has not been approved by FDA. Treatment may cause unknown side effects. These may include serious injury or pain, disability, or death. No compensation is available for these side effects.**
- √ **Your insurance may not cover the cost of the drug or treatment for its side effects. These are costs you may need to pay. To find out more about possible costs, contact your health plan.**

You may want to discuss your options further with your doctors, your family, your friends, or others before you decide what to do. You may also contact the [IRB NAME/NUMBER] with questions about your rights. If you choose to receive the unapproved drug, please sign below.

I understand my diagnosis and my options. I know that information about my treatment and response will be kept confidential, but may be given to the drug's manufacturer and/or FDA as required by law. My questions have been answered. I would like to receive the unapproved drug. This decision is voluntary and I understand that I can discontinue treatment at any time without penalty or loss of benefits to which I may otherwise be entitled.

Patient Name: _____ MRN: _____

Signature: _____ Date: _____

If the patient is unable to consent (a minor, incompetent, or incapacitated), please add the following information and signature:

Name of Legally Authorized Representative ("LAR"): _____

LAR's Authority to Sign: ☐ Parent (of Minor) ☐ Legal Guardian ☐ Other: _____

Signature of LAR: _____ Date: _____

I have explained the proposed treatment to the above patient/LAR, including risks, potential benefits, and alternatives, as well as any financial interest I may have in the treatment.

Physician Name: _____ Tel.: _____

Signature: _____ Date: _____

REQUEST FOR EMERGENCY OR INDIVIDUAL PATIENT IND (COVER SHEET)

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/ucm107434.htm

Request Type *(check one)*: ☐ Emergency IND ☐ Individual Patient IND

Drug Name:

Brief Clinical History
Diagnosis
Disease Status
Prior Therapy
Response to Prior Therapy
Rationale[*]

[*] *Include a list of available therapeutic options that would ordinarily be tried before the investigational drug or an explanation of why use of the investigational drug is preferable.*

Treatment Plan
Drug
Dose
Route of Administration
Planned Duration
Monitoring Procedures
Modifications for Toxicity[†]

[†] *Describe dose reduction, treatment delay, or other modifications that may be made.*

Narrative:

To the extent not already addressed above, describe the justification for the proposed treatment plan, explain the basis of the determination that there are no comparable or satisfactory therapeutic alternatives to the unapproved drug or biologic, and confirm that you have determined that the probable risk to the patient from the investigational drug is not greater than the probable risk from the patient s disease or condition.

Chemistry, Manufacturing, and Controls Information and Pharmacology and Toxicology Information:

Include a description of the manufacturing facility. The requirement for this information may be met by providing a Letter of Authorization (LOA) to refer to this information if it has been previously submitted to FDA (for example, to an existing IND or NDA). The treating physician should contact the sponsor of the previously submitted information for such authorization and letter. The letter of authorization should include relevant identifying information, such as the sponsor s relevant application (e.g., IND) number.

References:

Reference a published protocol or journal article (or articles) if appropriate.

Informed Consent and IRB Approval:

☐ Informed consent and approval of the use by an appropriate Institutional Review Board (IRB) will be obtained prior to initiating treatment.
☐ This is an emergency use request. An appropriate IRB will be notified of the emergency treatment within 5 working days of the treatment. Informed consent will be sought unless the requirements of 21 C.F.R. § 50.23 are met and appropriately documented.

Physician Information
Name
E-Mail
Phone
Fax
Qualifications[§]
Signature

[§] *Describe training, experience, and licensure or attach relevant portions of the physician's curriculum vitae.*