AD_____

Award Number: W81XWH-11-1-0388

TITLE: Viral Oncolytic Therapeutics for Neoplastic Meningitis

PRINCIPAL INVESTIGATOR: Kumudu Darshini Kuruppu

CONTRACTING ORGANIZATION: The Massachusetts General Hospital
Boston, MA 02114-2621

REPORT DATE: July 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2013	Annual	1 July 2012 – 30 June 2013

4. TITLE AND SUBTITLE

Viral Oncolytic Therapeutics for Neoplastic Meningitis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0388

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Kumudu Darshini Kuruppu

E-Mail: dkuruppu@partners.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Massachusetts General Hospital
Boston, MA 02114-2621

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Neoplastic meningitis is a fatal complication of breast cancer for which there is no curative treatment at present. The project aims to develop a novel, safe and efficient therapy for neoplastic meningitis – HSV-1 oncolysis therapy. In the first year, after completing the stated tasks for that year, we developed stable breast cancer cell lines that express the renilla luciferase with the aim of using them to non-invasively image their tumors with bioluminescence. While waiting for the animal facilities at MGH to be set up in order to complete the remainder of the studies stated for the second year, we commenced developing a mouse model of meningeal metastases selecting the tripe negative human breast cancer cell line MDA-231 which we had created. During the second year we have developed a mouse model of meningeal metastases in mice and characterized the progression of meningeal metastases (Neoplastic meningitis) over time with sequential imaging with bioluminescence and MRI. The images were correlated with in vitro staining of brain sections with H&E and the development of tumors compares with the disease progression in humans.
The fully characterized model was presented at the annual meeting of the Society of nuclear medicine and molecular imaging (SNMMI) in Vancouver (June, 2013). This model is being written up for publication in Cancer Research. In addition, studies of viral and cellular kinetics with bioluminescence and PET are being written up for publication.

15. SUBJECT TERMS
Neoplastic meningitis, mouse model of meningeal metastases, breast cancer, bioluminescence, MRI, viral oncolysis, HSV-1.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER *(include area code)*

Progress Report: 01 July 2011 - 30 June 2012

Introduction:

Meningeal metastasis, also known as carcinomatous meningitis is a fatal complication of breast cancer and some solid tumors that result from the spread of cancer cells into the subarachnoid space. About 5% of breast cancer patients are diagnosed with meningeal metastases, while autopsy results suggest its presence in 20% of patients who have succumbed to systemic disease. Seeding of cancer cells in the meninges, arachnoid and the pia mater and their subsequent growth results in severe neurological complications that include abnormalities in cranial nerve functions (muscular and facial), cerebral symptoms (speech disturbances), and spinal cord symptoms (limb weakness), contributing to a life expectancy of 1 to 4 months. Treatment at present is largely palliative. Although aggressive multimodal therapies such as radiation, chemotherapy (intra-CSF and systemic) are attempted they are accompanied with toxicities and complications. Besides, chemotherapy is often cleared by the CSF. The secure guarding of the subarachnoid space by the blood-brain- barrier on one side and the blood-CSF barrier on the other offers less opportunity for chemotherapy to reach the cancer cells that reside within the meninges. Thus there is an urgent need for a new therapeutic modality to target meningeal metastases. Oncolytic, replication conditional HSV-1 is suggested as a therapeutic option for meningeal metastases.

We present here the work that has been accomplished over the second year of the grant period. We present the imaging of viral titers performed according to the SOW. In addition, while waiting for the institutional animal facilities to be reconfigured we continued on the preliminary work that was performed last year. This year we established a mouse model of meningeal metastases. This model will serve as a platform for which work proposed in the rat model in the grant can be accomplished once we commence shortly.

Body:

SOW performed:

Based on the statement of work for year two of the grant period we have studied the kinetics of viral titers with non-invasive imaging. This data will be used to determine virus titers and time of delivery in the rat model of neoplastic meningitis. In addition, using one of the cell lines that were established in year one of the grant, we developed and characterized a mouse model of meningeal metastases. This model is of great value for determining location of metastases and duration of their growth when working with the rat model.

1. Study the kinetics of virus with bioluminescence in vivo.

The kinetics of virus titers was studied in vivo with bioluminescence. Virus that expressed firefly luciferase (Fluc) was injected into flank tumors created with MDA-231 breast cancer cell line. The bioluminescence signal emitted by the virus was imaged over time with luciferin. This data (Fig-1) provides a platform to commence the virus titer and time of injection for studies performed in the rat model.

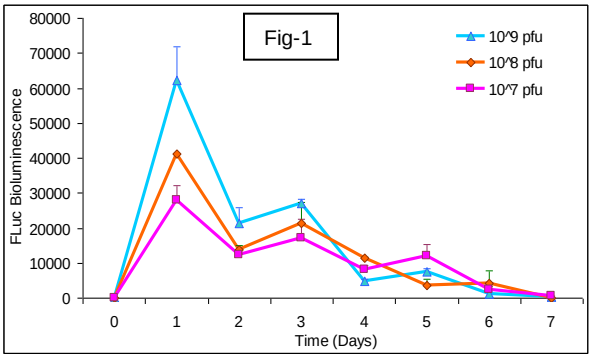


Fig-1: Waves of virus replication imaged with Fluc bioluminescence. Immediately following virus injection into the flank tumors a high bioluminescence signal is seen the following day. This signal decreases substantially by the next day and increases by day 3. Each virus titer depicts two peaks – one large peak early on followed by smaller peaks subsequently. Fluc bioluminescence is expressed as the mean ± s.d..

2. Study the kinetics of cancer cells and their response to viral oncolysis with bioluminescence in vivo.

The kinetics of tumor growth was studied using the human breast cancer cell line (MDA-231-Rluc) that expresses the bioluminescent renilla luciferase gene. Flank tumors were created and their growth was imaged with coelenterazine at several time points. Tumor volume was determined with bioluminescence

signal intensities and with external caliper measurements. Tumor growth corresponded with the intensity of the bioluminescence signal (Fig 2a, 2d) and with caliper measurements (Fig 2e). Tumor growth was inhibited following virus injection into the tumors (Fig 2b, 2d), which was also reflected in the caliper measurements (Fig 2e). The expression of virus in the tumors was imaged with luciferin over time (Fig 2c, 2f).

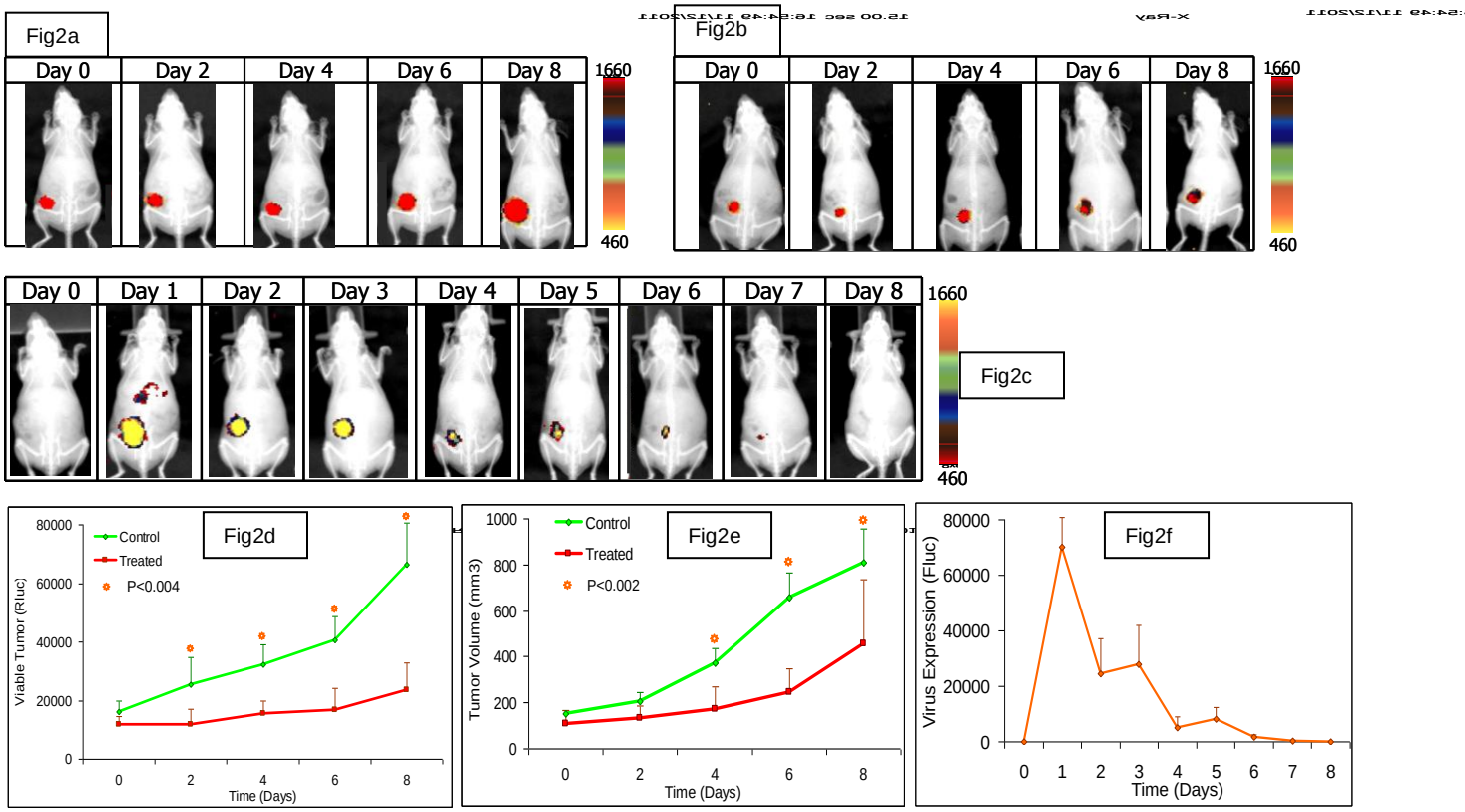


Fig 1: Tumor growth kinetics of MDA-231-Rluc and viral oncolysis (HSV-luc) kinetics imaged with dual bioluminescence.

1a: Tumor growth imaged with Rluc bioluminescence. The Rluc signal increased as the tumors grew over time. **1b:** MDA-231-Rluc tumors undergoing viral oncolysis imaged with Rluc bioluminescence. The Rluc signal decreased in tumors after injecting virus ($1x10^8$ pfu) over 8 days. **1c:** Virus expression in flank tumors imaged with Fluc bioluminescence. The Fluc signal which was high one day after virus ($1x10^8$ pfu) injection, decreased over time. **1d:** Rluc bioluminescence quantified in the control and treated groups. The signal increased over time in the control group compared to the virus treated group. Data is the mean ± s.d.. **1e:** Tumor volume in the control and virus treated groups obtained with caliper readings. The volume in the control group increased exponentially, while it was markedly inhibited in the virus treated group. Data is the mean ± s.d.. **1f:** Virus expression imaged with Fluc bioluminescence. Fluc signal showed a high peak one day after virus injection and decreases over time. Data is the mean ± s.d..

The remaining data on the kinetic of other cell lines and their response to viral oncolysis will appear in the manuscript that will be published (which is currently being written up).

3. Develop and characterize a mouse model of meningeal metastases (Neoplastic meningitis) with bioluminescence and MRI.

We have developed and characterized a mouse model of meningeal metastases where tumor growth properties have been studied with molecular imaging by Gd contrast MRI and bioluminescence over time. The model was created in nude mice using the stably transfected human MDA-MB-231-Rluc breast cancer cells that stably express renilla luciferase (Rluc). The cells ($2x10^4$ cells in 10µl) were injected into the right lateral ventricle of the mouse brain by stereotactic coordinates. The course of tumor development in the meninges and brain compartments were studied over successive time points spanning 24 days with MRI, Rluc bioluminescence and histological staining of serial brain sections.

The data was presented at the SNMMI meeting, Vacouver, 2013.

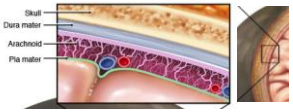
Characterization of a murine model of meningeal metastases from breast cancer

Darshini Kuruppu, Deepak Bhere, Chris Farrar, Anna-Liisa Brownell, Umar Mahmood, Misha Papisov, Kenneth Tanabe

Departments of Surgery and Radiology, Massachusetts General Hospital, Boston, MA 02114

Background

Meningeal metastasis is a fatal complication of breast cancer that affects 5-8% of patients when cancer cells seed in the meninges.



Subsequent growth of the cancer cells results in severe neurological complications involving the cranial nerves, cerebrum and spinal cord, limiting life expectancy to less than 4 months. Current treatment is largely palliative. Identifying disease progression will contribute to optimize therapeutic design and delivery.

Aim

To develop and characterize a model of meningeal metastases and characterize tumor progression with molecular MRI and bioluminescence imaging over time.

Methods

Meningeal metastases were created in nude balb/c mice using the MDA-MB-231 human breast cancer cell line that expresses Rluc. Tumor cells ($2x10^4$cells/10µl) were injected into the right lateral ventricle of the brain with stereotactic coordinates. Tumor growth in the meninges, brain and spinal cord was studied sequentially over 3 weeks with Gd-DTPA contrast T1 weighted 3D-MRI and Rluc bioluminescence imaging. Tumor volume was calculated from contrast uptake in MRI and Rluc signal intensities. The brains were studied *ex vivo* to confirm *in vivo* findings. The mice were observed for changes in body mass and external neurological symptoms.

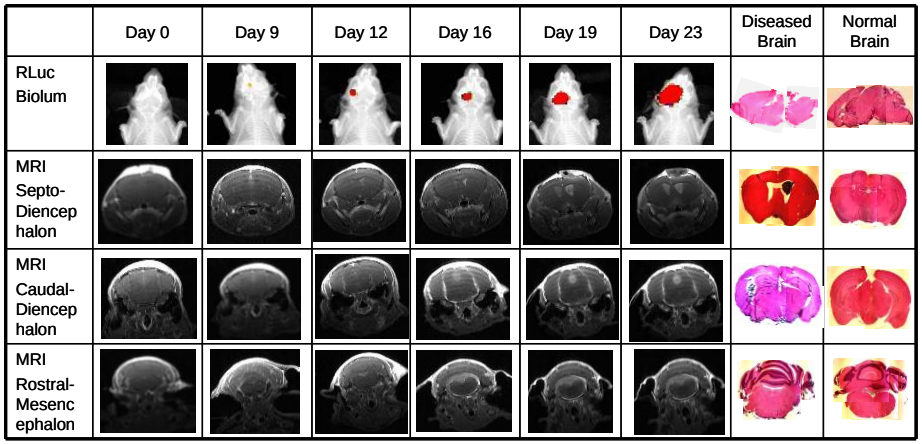


Fig 1: Serial imaging of meningeal metastases with Gd-DTPA-MRI and Rluc-bioluminescence. The Gd-DTPA contrast uptake in the brain regions are: **Septo-diencephalon:** contrast uptake seen initially in the right lateral ventricle migrates to the left lateral ventricle. **Caudal-diencephalon:** contrast visible in the 3rd ventricle and the inferior horn of the lateral ventricles. **Rostral-mesencephalon:** contrast seen in the optical track and the meningeal lining of the brain. **Rostral cerebellum:** contrast uptake in the 4th ventricle and in the base of the brain. Contrast uptake increases in intensity over time.

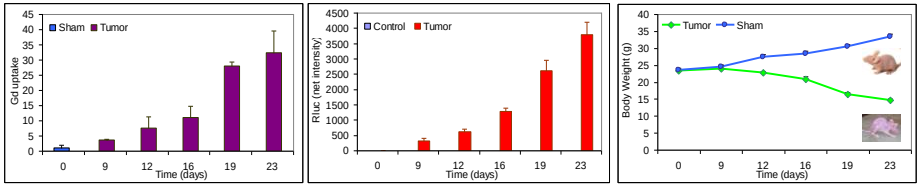


Fig 2: Defining tumor volume and body weight with meningeal metastases. 2A: The total tumor volume in the brain regions on MRI obtained with Gd uptake increases over time; 2B: Rluc signal increases over time tumor growth in the brain; 2C: As the tumors invaded the brain the diseased mice lose considerable weight compared to the controls.

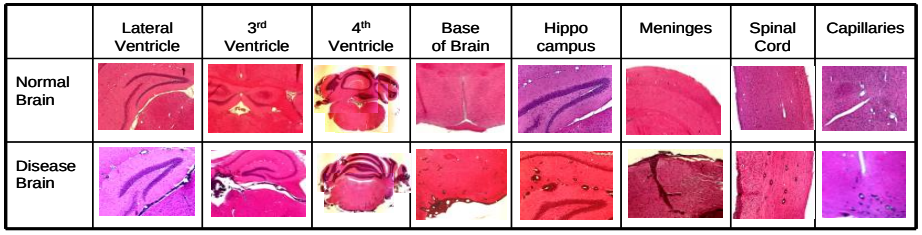


Fig 3: Identifying meningeal metastases in different regions of diseased brains compared to normal brains on histology.

Results

MRI identifies the anatomical location of the metastases. Disease progression is characterized by tumor invasion throughout the ventricular compartments and the meningeal lining of the brain. The latter stages are characterized with heavy tumor burden in the base of the brain that correlates with the severe neurological symptoms (bradykinasia, anorxia, and paralysis) in the mice. Tumor identified initially in right-lateral ventricle migrate to the left and inferior horns of the lateral ventricle, and the 3rd ventricle. Tumor is identified subsequently along the optical tracks, the 4th ventricle and the base of the brain. Histology reveals tumor aggregates in the thalamic region, hippocampus, and blood vessels of the *brain*, and along the lining of the *spinal cord* and its blood vessels. The expanding tumor volume is accompanied with a drastic reduction in body weight from emaciated body mass.

Discussion

We present a murine model of meningeal metastases with the sequential progression of disease characterized by molecular MRI and bioluminescence imaging, histology and change in body mass. The model resembles the human disease pathology and will serve as a platform to study novel treatments for meningeal metastases.

References

1. Chamberlain MC. Neoplastic meningitis. The oncologist 2008; 13; 967-977.
2. Clarke JL, Perez HR, et al. Leptomeningeal metastases in the MRI era. Neurology. 2010;74(18): 1449-54.

Funded by DOD

Kuruppu, Kumudu D.

From: Hurdle Delicia [DHurdle@snmmi.org]
Sent: Monday, June 17, 2013 3:20 PM
Subject: THANK YOU FOR YOUR POSTER PRESENTATION AT THE 60TH SNMMI ANNUAL MEETING!!!!
Importance: High

Dear Scientific Poster Presenter,

The SNMMI Scientific Program Committee appreciates your willingness to be part of the 2013 SNMMI Annual Meeting in Vancouver. Your participation and expertise greatly contributed to the overall success of the Annual Meeting educational program.

I understand the effort and time commitment involved in preparing scientific/research material. On behalf of the Scientific Program Committee, I would like to thank you for being part of the program. We appreciate and value your contribution.

Best regards,

Peter Herscovitch, M.D., FACP, FRCPC
Chair, SNMMI Scientific Program Committee

Delicia Hurdle, MBA
SOCIETY OF NUCLEAR MEDICINE AND MOLECULAR IMAGING
Sr. Program Manager, Education
office: 703.667.5121 | fax: 703.708.9015
email: dhurdle@snmmi.org
www.snmmi.org

Using this model we plan to test the efficacy of the HSV-1 virus. The mouse model will serve as a pre-study model optimization and characterization with which we can launch on our rat experimental model. The mouse model will serve as a valuable tool with which tumor cell growth can be monitored by non-invasive bioluminescence imaging and quantified. Data on the dynamics of virus spread in the meninges will serve as a platform for out studies in the rat model which will be conducted in the next year.

Key Research Accomplishments:

For the stipulated grant period we have performed the following preliminary work towards generating the proposed data and for future experiments that we plan to accomplish for the grant.

1. Determined kinetics of different titers of virus in tumors in vivo.
2. Studied with kinetics of tumor cell replication and their response to viral oncolysis in vivo.
3. Developed and characterized a mouse model of meningeal metastases.

Reportable Outcomes:

1. Abstract:
Characterization of the animal model was presented at the annual meeting of the Society of nuclear medicine and molecular imaging, Vancouver, June 2013.

2. Manuscripts –pending:
Two manuscripts are being prepared from data obtained:

- Molecular imaging with PET and bioluminescence reveals viral oncolysis kinetics and tumor viability.
- Characterization of an animal model of meningeal metastases with molecular imaging.

3. Grant submission:
An R21 grant submission was made based on the initial research data on the mouse model that was generated. Grant titled “Novel oncolytic HSV-1 targeted therapy for meningeal metastases from breast cancer” was submitted for R21 omnibus, 2013.

Conclusion

We have performed the work that was relevant for the study of viral kinetics based on the statement of work. In addition, we have developed and characterized a model of meningeal metastases in mice. This will be followed by investigating the potential therapeutic efficacy of the oncolytic HSV-1 in the mouse model. The data that will be generated will serve as a platform to complete the remainder of the statement of work in the next year in the rat model.

References:

1. Chamberlain MC. Neoplastic meningitis. J Clin Oncol. 2005 May 20;23(15):3605-13.
2. Nakamura H, Kasuya H, Mullen JT, et al. Regulation of Herpes simplex virus g134.5 expression and oncolysis of diffuse liver metastases by Myb34.5. J Clin Invest 2002;109:871–82.
3. Kuruppu D, Tanabe KK. Viral oncolysis by herpes simplex virus and other viruses. Cancer Biology & Therapy. 2005; 4(5): 524-31.
4. Kuruppu D, Brownell A, Zhu A, Yu M, Wang X, Kulu Y, Fuchs B, Kawasaki H, Tanabe KK. Positron emission tomography of herpes simplex virus 1 oncolysis. Cancer Research. 2007; 67(7): 3295.