

## Quarterly FunVax Review 06-01-07

Authors: [REDACTED]  
Submission Date: 06/01/07  
Review Period: 02/01/07 – 05/01/07  
Project ID: 149AZ2

### Scope:

*This report provides a summary of recommendations and conclusions based on experiments related to project ID 149AZ2 during the period of 02/01/07 and 05/01/07. This report does not contain any quantitative data and if that information is required it can be found in the original experimental reports that are listed in the section titled Summary of Experiments. In the Summary of Recommendations section, data from experiments conducted between 02/01/07 and 05/01/07 were analyzed and recommendations of future experiments are suggested. Concerns and comments from the 03/21/07 meeting at [REDACTED] [REDACTED] are also addressed in the Summary of Recommendations and Conclusion sections.*

*The objective of this phase of project ID 149AZ2 is to prepare a viral vector that will inhibit/decrease the expression of VMAT2 within a human population. Currently, tests are only scheduled for animal models. Infection of Rhesus Monkey, according to the timeline set out in Appendix 1, will begin as early as 07/02/07. A clinical team coordinated by [REDACTED] of [REDACTED] will be brought in to supervise the experiments once Rhesus Monkeys are being exposed to virus. The timeline for human trials and field tests will be determined by [REDACTED] and the role of the research group will be as support only. Upscale of the virus will be handled by [REDACTED]. The coordination between the research, clinical and manufacturing groups is outline in a report due on 06/15/07 and is in the process of being prepared by [REDACTED].*



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## Summary of Experiments

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### Summary of Experiments (02/01/07 – 05/01/07)

#### Airborne VSV Containing VMAT2 Disruption Gene

██████████, Submission Date - 02/14/07

*Abstract - Because of the vesicular stomatitis virus' ability to infect brain cells and its two step life cycle, cytolytic infections in mammals and transmission by insects, it provided a starting point to design an airborne virus that has the ability to infect the respiratory system as well as brain cells. The newly designed virus contains the typical VSV genome, a homologous region to VMAT2 and a gene from adenovirus that allows attachment to the coxsackie-adenovirus receptor (CAR) on host cells. This design allows the virus to infect the respiratory track where cytolytic infection occurs and then subsequent diffusion across the blood brain barrier to infect brain cells. 600 strains of the virus were tested in duplicate on 1,200 mice. Mice were inoculated via needle and brains tissue examined three weeks after inoculation. VSV287 had the least amount of endogenous VMAT2 protein and will be further tested to verify that it is the most efficient of the 600 strains.*

#### Dispersal Options of Vesicular Stomatitis Virus

██████████, Submission Date – 3/27/07

*Abstract - Six methods of vesicular stomatitis virus dispersal were tested - high altitude release, water supply release, insect transmission, diffusion by a ground level object such as a car, diffusion from a stationary object such as a bottle and infection of food supply such as cattle or produce. For the high altitude tests 30 liters of highly concentrated virus ( $10^{11}$  pfu/ml) had a targeted 1 sq kilometer live land rate of 150 pfu/sq meter. Stability tests in water showed that the 10% of the virus is still viable after 14 days. At 50 days, 100% of the virus is non-viable. Diffusion by a moving object showed great promise.  $10^4$  pfu/sq meter was detected at 500 sq feet from the moving object within 15 minutes. Release took place from a vehicle driving at 25 MPH, releasing approximately  $10^{11}$  pfu every 30 seconds for 5 minutes. Diffusion by stationary object is dependent on wind conditions. With a wind speed of 3 MPH, 0.1% of the starting virus could be detected 100 meters from the source after 1 hour. To examine the dispersal of VSV through the food supply, cows were injected with  $10^4$  pfu. 8 days post injection the tissue of the CNS had the greatest concentration of virus at an average of 150pfu/gram of brain tissue.*

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## Summary of Experiments

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### VMAT2 Rhesus Monkey KO

[REDACTED], Submission Date – 04/06/07

*Abstract – VMAT2 homozygous knockout monkeys die within three days while the heterozygous monkey lives what appears to be a normal live span. VMAT2 is responsible for packaging dopamine and other monoamines into vesicles that will be released at the synapse. Dopamine disruption has been shown to damage dopamine neurons. While the KO monkeys were alive, they did not feed and upon the autopsy it was concluded that they died of starvation. It appears that they had no will to live. This same conclusion was found in VMAT2 KO mice in 1997. A VMAT2 deficient monkey was developed concurrently with the KO monkey. The VMAT deficient monkey should have expression of VMAT2 80-95% lower than an average wildtype monkey. The VMAT deficient monkey should produce conclusive results by July 2007.*

### [REDACTED] Research Group Meeting 03/21/07

[REDACTED], Submission Date – 03/21/07

*Brief Summary of Minutes – Meeting commenced at 10:07am at the [REDACTED] Facility in San Diego, CA. The people present included [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED] and [REDACTED]. Topics that were discussed:*

- Current update on experiments in progress, round table
- VSV287, [REDACTED]
- Review of timeline, [REDACTED]
- Proposal for a suicide gene, [REDACTED]
- Dispersal Methods, [REDACTED]
- Testing efficiency in the field, [REDACTED]
- Inhibitors that may target a specific population, [REDACTED]
- Monkey knockout/knockdown progress, [REDACTED]
- Future experiments, [REDACTED]

*The meeting concluded at 3:35pm.*

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## Summary of Recommendation

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### Summary of Recommendation

1. *Quantitative PCR of all 600 animal subjects should be done to ensure that the data from the ELISA experiments, which showed a decrease in endogenous VMAT2 is occurring because of viral insertion and not natural variation.*

2. *Of the 600 variants of Vesicular Stomatitis Virus tested, VSV287 had the greatest decrease of endogenous VMAT2 within mice. However, this may not be the case for human subjects. All 600 strains of VSV should be retested on human subjects by the clinical group. The clinical group should be involved with this ASAP.*

3. *Bradford assays should be done on infected subjects to determine endogenous VMAT2 concentrations before and after infection, not just after infection.*

4. *Mice or other subjects should not be injected with virus since this does not test the actual dispersal method. Future experiments of VSV287 or similar strains should allow the subject to breath in the virus rather than being injected with it.*

5. *The use of FunVax could see an immediate effect within the target zones and a way to measure the rate of infection should be examined and tested before the virus is released. Two or three of the following methods should be used to approximate efficiency. The results of the mass inoculation should be proportionate to the rate of infection and could be quantitated by either behavior or biological tests.*

#### *Behavioral Indicators*

a. *Significant decrease in suicide bombings.*

b. *Decrease in armed resistance in conflict zones.*

c. *In non-conflict zones effectiveness could be measured by a decrease in people attending religious activities such as khutbahs or noon prayer.*

d. *Measureable increase in communications, telephone, email, and other forms of communication that express discontent with religion or God.*

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## Summary of Recommendation

### Biological Indicators

a. As shown by ████████ VMAT2 KO experiment on mice, in 0.25% of subjects exposed to the vaccine there is a noticeable side effect – a benign essential blepharospasm. Tests need to be done on the human population but if we assume that this side effect remains the same in humans, we can measure a sample set for blepharospasm and calculate the rate of vaccination. This would be an accurate way to estimate the rate of vaccination, but requires an examiner to be on the ground and a willing sample set that is representative of population that is being targeted. Neither of these criteria may be possible in the most contentious target zones.

b. A blood sample of militant casualties or deceased civilian would provide the most accurate estimate of the rate of vaccination. A PCR test could be used to determine if the sample contains the viral elements that are associated with FunVax. The ratio between positive and negative results would allow one to calculate the rate of vaccination.

c. Because the viral elements have been found in many cells of the body once vaccination has occurred, biological samples from living subject may be covertly taken. This may include:

1. Eating Utensils
2. Hair Follicles From Hair Brush/Razor
3. Tooth Brush
4. Q-tips
5. Under Garments
6. Cigarette Butts
7. Toilet Paper
8. Cell Phones
9. Condoms
10. Napkins
11. A Drinking Vessel
12. Clothes
13. Pillow Case or Bedding
14. Tampons
15. Dental Floss

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*Summary of Recommendation*

6. *The stability tests conducted by the group lead by ██████████ used vesicular stomatitis virus and not the virus that is currently being tested, VSV287, the airborne variant of the vesicular stomatitis virus that had the greatest decrease in endogenous VMAT2. The tests that that should be repeated using the VSV287 are:*

- a. The high atmospheric tests*
- b. Ground level diffusion test from both stationary and moving sources*

7. *Brain autopsy from monkey's in the 04/06/07 KO experiment. Knockdown experiment should include MRI, brain autopsies in addition to the test already planned by ██████████*

8. *The cell lines used in all cell culture studies should be HCN-1A line only. This will be the standards in all FunVax studies going forward by the research group. Media and growth protocols are available in Appendix 2.*

9. *The Alcohol inhibitor experiments suggested by ██████████ in the ██████████ ██████████ Research Group Meeting on 3/21/07 should be started as soon as possible. The obstacles in designing an inhibitor described at the ██████████ Meeting would be difficult to overcome. The design of a containment inhibitor is likely going to be the limiting factor in terms of having a product that is field ready.*

## Conclusions

1. *Vesicular stomatitis virus is typically transmitted by insects, however, as stated in the "Summary of FunVax Objectives" dated 5-25-05, an airborne virus would be the preferred route of infection. A strain named VSV287 has been designed to spread via air, but more studies need to be done to conclude its efficiency in both animal and human subjects.*
  - a. *Dispersal via air is possible with VSV287, however there is no accurate data that shows infection efficiency with VSV287.*
  - b. *High atmospheric tests have been done with other viruses, such as vesicular stomatitis virus and has been shown to disperse at an acceptable rate with moderate viability, however no tests have been done on VSV287.*
  - c. *The viral genome of VSV287 has been shown to integrate in various brain cells at the VMAT2 locus. Endogenous VMAT2 expression in the brain has been decreased as shown by ELISA.*
    - *52% of the mice had a decreased level of VMAT2 that was 40% below the average endogenous level of VMAT2 protein (positive). 48% of the infected mice had VMAT2 proteins at a concentration that was between 40% and 100% of the average endogenous concentration of VMAT2 (negative). It should be noted that within a single infected cell, there is most likely no endogenous VMAT2. The endogenous VMAT2 that is being measured is being expressed by non-infected cells.*
2. *Inoculation with VSV287 in mice has no serious side effects and the side effects that do occur have been shown in less than 10% of animal test subjects.*
  - a. *Side effects include benign essential blepharospasm (0.5% of cases), increase depression (6.4%) and asthma (2.5%).*
  - b. *Human subjects have not been tested, but side effects are projected to be similar.*
3. *Based on the findings by ██████████ VSV287 has been shown to be safe with minimal serious side effects and has accomplished 8 of the 9*



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## Conclusion

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bench marks laid out in the "Summary of FunVax Objectives" including the two most important objectives at this stage -

- a. An airborne virus that can insert a modified VMAT2 gene into brain cells.
- b. A significant decrease of expression of endogenous VMAT2.

Only human trials can determine VSV287's effect on religiosity and spirituality. The results obtained thus far show minimal health effects, none of which are life threatening or debilitating. Primates should be infected with VSV287 to determine the effects on systems more similar to humans.

4. Dispersal methods are still being tested. High atmospheric dispersal or dispersal by a ground level moving objects appear to be the most practical. Test will be conducted using VSV287. Once initial dispersal is accomplished infection will be transmitted person to person. The clinical group will examine the rates of person to person transmission.
5. Complete knockout of VMAT2 in mice as well as monkeys has shown to be lethal. Future experiments should examine the effects of a VMAT2 knockdown and insertion of VMAT2 mutations/alleles in monkeys.

## FunVax Research Group Timeline

- 6-01-07 – Quarterly Review Submission*
- 6-11-07 – Research Group Meeting to discuss Quarterly Review and timeline*
- 6-15-07 – Report on coordinating between research, clinical and manufacturing groups*
- 6-15-07 – Outline/proposal for field efficiency tests*
- 6-15-07 – Update on Confinement inhibitors*
- 6-15-07 – Autopsy of VMAT2 monkey brain tissue*
- 6-21-07 – Report on updated VSV287 dispersal data*
- 6-25-07 – Meeting between research, clinical and manufacturing groups to discuss report submitted on 6-15-07.*
- 7-02-07 – Provide VSV287 virus to clinical group for primate studies*
- 7-16-07 – Report on VMAT knockdown in monkeys*
- 7-16-07 – Update on confinement inhibitors*
- 7-16-07 – Report on airborne VSV strain inhalation study on mice*
- 8-01-07 – DOD meeting, transfer of responsibility to clinical group*
- 8-14-07 – Meeting with clinical group/transfer of materials*
- 9-03-07 – Quarterly Review Submission*

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Appendix 2

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## Cell Culture FunVax Research Group Standards

### *HCN-1A Cells – taken from ATTC*

Designations: HCN-1A

Depositors: Johns Hopkins University

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: neuronal  
A small square micrograph showing a single neuron with a cell body and a long, thin process extending upwards. The word "PHOTO" is printed in green at the bottom of the image.

Source: **Organ:** brain  
**Cell Type:** cortical neuron;

Cellular Products: tubulin; neurofilament protein; somatostatin; cholecystokinin-8

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

DNA Profile (STR):	Amelogenin: X CSFIPO: 10
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## Appendix 2

	D13S317: 11,12 D16S539: 12 D5S818: 11,12 D7S820: 11,12 TH01: 9.3 TPOX: 11 vWA: 17
Age:	18 months
Gender:	female
Comments:	<p>The cells stain positively for a number of neuronal markers including neurofilament protein, neuron specific enolase (NSE). [48286]</p> <p>They are also positive for tubulin, vimentin, somatostatin (SST), glutamate, gamma aminobutyric acid (GABA), cholecystokinin - 8 (CCK-8) and vasoactive intestinal peptide (VIP). [22022]</p> <p>The cells are negative for glial fibrillary acidic protein (GFAP) and myelin basis protein (MBP). [48286]</p> <p>HCN-1A cells can be induced to differentiate when cultured with a mixture of nerve growth factor (NGF), dibutyryl cyclic adenosine monophosphate (cAMP) and 1-isobutyl-3-methylxanthine (IBMX). [22022]</p> <p>Differentiation is accompanied by mature morphology and slowing of growth (doubling time greater than 120 hours). [22022]</p> <p>Unlike HCN-2 (see ATCC <a href="#">CRL-10742</a>) the growth rate of HCN-1A cells is not affected by phorbol esters. [22022]</p>
Propagation:	<p><b>ATCC complete growth medium:</b> The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.</p> <p><b>Temperature:</b> 37.0°C</p> <p><b>Growth Conditions:</b> The growth medium must be adjusted to pH 7.35 prior to filtration</p>
Subculturing:	<p><b>Protocol:</b></p> <ol style="list-style-type: none"><li>1. Remove and discard culture medium.</li><li>2. Briefly rinse the cell layer with 0.05% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.</li><li>3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).</li></ol> <p>Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.</p> <ol style="list-style-type: none"><li>4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.</li><li>5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes.</li><li>6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.</li><li>7. Place culture vessels in incubators at 37C.</li></ol>

## Appendix 2

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CRL-10442 has been shown to senesce at approximately passage 17. Current distribution stocks are prepared with a minimum of only 2 passages remaining under recommended culture conditions after cryopreservation.

**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 is recommended

**Medium Renewal:** 1 to 2 times per week

Preservation:

**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO

**Storage temperature:** liquid nitrogen vapor phase