Report of the Investigation into the Allegation of Research Misconduct by Dr. Georgiy Aslanidi

Submitted to Dr. David Norton, Vice President for Research

October 24, 2016

DIO 6242

Executive Summary

The Investigation addressed an allegation brought against Dr. Georgiy Aslanidi (Respondent) by Dr. Arun Srivastava, (Complainant), Professor and Division Chief, and Dr. Roland Herzog, (Complainant), Professor, both from the Division of Cellular & Molecular Therapy, Department of Pediatrics, College of Medicine, University of Florida (UF). According to Drs. Srivastava and Herzog, there were falsified and/or fabricated figures in 3 journal articles from 2011 through 2016 (namely Figure 3 in Article 1, Figure 4 in Article 2 and Figure 6 in Article 3) (Page 3745 in Attachment 1a, Page 297 in Attachment 1b, Page 22 in Attachment 1c).

According to the National Institutes of Health (NIH) regulation, 42 Code of Federal Regulations (CFR), Parts 50 and 93 and the UF policy for Dealing with Conduct in Research, "fabrication is making up data or results and recording or reporting them" and "falsification is manipulating research materials, equipment or processes, or changing or omitting data or results such that the research is not accurately represented in the research record". Further, a finding of research misconduct, according to federal policy and the UF policy requires that: 1) there is a significant departure from accepted practices of the relevant research community; and 2) the misconduct be committed intentionally, knowingly or recklessly; and 3) the allegation be proven by a preponderance of evidence.

Preliminary information-gathering and preliminary fact-finding from the inquiry indicated that:

- Allegations 1, 2 and 3 for Figure 3A in Article 1, Figure 4 in Article 2 and Figure 6 in Article 3 may have substance. Thus, it was determined that these allegations warranted further investigation.
- Allegation 1 for Figure 3B in Article 1 did not have substance. Thus, it was determined that this allegation did not warrant further investigation.

An Investigation Committee was formed to review the allegations. The Investigation Committee concluded that Figure 3A in Article 1 (Page 3745 in Attachment 1a) represented a clear incident of research misconduct. However, the Investigation Committee was unable to conclude whether the respondent Dr. Aslanidi or a post-doc who previously worked in the laboratory, Dr. Giridhara Jayandharan (Dr. Rao), fabricated or manipulated Figure 3A in Article 1 (Page 3745 in Attachment 1a). The Investigation Committee indicated there was not enough direct evidence to either implicate or exonerate either of these individuals. The Investigation Committee also concluded that for Figure 4 in Article 2 (Page 297 in Attachment 1b) and Figure 6 in Article 3 (Page 22 in Attachment 1c), Dr. Aslanidi's actions were sloppy and constituted errors in accepted scientific practice rather than research misconduct).

Description of the Allegation:

1. Allegation 1

Specifically Figure 3A (Page 3745 in Attachment 1a), "*Adeno-associated virus (AAV)-enhanced green fluorescent protein (EGFP) vector-mediated transduction of primary human monocyte-derived dendritic cells (DCs) in the presence of nuclear factor kappa-light-chain enhancer of activated B cell (NF-KB) modulators*", in the article, "*Activation of the NF-kB pathway by Adeno-associated virus (AAV) vectors and its Implications in Immune Response and Gene Therapy",* in the journal "*Proceedings of the National Academy of Sciences, 108: 3743-3748, 2011*" had the following falsifications or fabrications:

a. The second panel (scAAV-EGFP) was labeled as "Mock" in the original data.
b. The first panel (Mock) appears to have some cells deleted in the lower left of the upper right quadrant.
c. There is no original data to support the fourth (+Cytokines) and fifth (+Cytokines +Bay 11) panels.
    a. The fifth panel (+Cytokines) was labeled as "scAAV2-CB-EGFP" in the original data.
    b. The fourth panel (+Cytokines +Bay 11) was labeled as "scAAV2-CB-EGFP+ IOμM VP 16" in the original data.
d. The threshold lines in the five panels appear to have been lowered from the original data.
e. The origin of the third panel (+ VP 16) is unknown.
f. The % values in the five panels differ from the original data.

2 Allegation 2

Specifically, Figure 4 (Page 297 in Attachment 1b), "*In vivo imaging of tumor growth progression in C57BL/6 mice, injected with adeno-associated virus serotype 6 (AAV6) vectors carrying the prostatic acid phosphatase (PAP) gene, evaluated by activity of luciferase stably expressed in murine prostate cancer cells (RMI)*" in the article "*Reprogramming Immune Response With Capsid-Optimized AAV6 Vectors for Immunotherapy of Cancer*", in the journal, "*Journal of Immunotherapy, 38:292-298, 2015*", had the following falsifications or fabrications:

a. Tumor sizes were potentially misrepresented since the minimum and maximum bioluminescence intensities could be affected by background, binning, focal stop and exposure time settings. However, information about these parameters was not provided in the figure captions or the methods section. Further, information about the signals and details about how these settings were controlled across images taken weeks apart was also not presented in the methods section of the article.

3 Allegation 3

Specifically, Figure 6, (Page 22 in Attachment 1c) "*In vivo imaging of tumor growth progression in mice, engrafted with human liver cancer cells, and injected with AAV6 vectors, evaluated by luciferase activity*", in the article "*Development of a Novel AAV Serotype 6 based Vectors Selective Tropism for Human Cancer Cells*", in the journal, "*Gene Therapy, 23, 18–25, 2016*", had the following falsifications or fabrications:

a. Tumor sizes and vector efficiency potentially are misrepresented since the minimum and maximum bioluminescence intensities can be affected by background, binning, focal stop and exposure time settings, yet information about these parameters was not provided in the figure captions or the methods section. Further, information about the signals and

details about how these settings were controlled across images taken weeks apart was also not presented in the methods section of the article.

b. The AAV6-RGD and AAV6-RGD-Y705-731F+T492V+K531E mice image panels were acquired 8 days apart, potentially misrepresenting vector efficiency.

Name and Position of the Respondent:

Dr. Georgiy Aslanidi, Research Assistant Professor, Department of Pediatrics, College of Medicine, UF.

PHS/NIH Support Information:

Awards referenced in the "Acknowledgements" section of published Articles.

Allegation 1: This research was supported in part by:

1. The Wistar Institute Consortium Agreement award to UF with prime award P01 HL-078810 from NIH/NHLBI; R. Herzog (PI); Immune Responses to AAV-Mediated Fix Gene Transfer: PROJ 3 Strategies to Prevent Cytotoxic T Lymphocyte Responses to the Transgene Product in Viral Gene Transfer
2. R01 AI051390, NIH/NIAID; R. Herzog (PI) ; Immunology of Factor IX Gene Transfer to Liver
3. R01 HL076901, NIH/NHLBI; A. Srivastava (PI); Human Parvovirus B19 Vectors: Mechanism of Transduction
4. P01 DK058327, NIH/NIDDK; T. Flotte/B. Byrne (PI); Recombinant AAV for Correction of Genetic Abnormalities
5. R01 HL097088, NIH/NHLBI; A. Srivastava (Contact PI), R. Herzog, Guangping Gao, Sergei Zolotukhin; Next Generation of Recombinant AAV Serotype Vectors for Gene Therapy

Allegations 2 and 3: This research was supported in part by:

1. Children's Miracle Network; G. Aslanidi (PI) – Gift awards
2. Children's Miracle Network; Chen Ling (PI) – Gift awards

Applicable Regulations:

1. National Institutes of Health (NIH) regulation, 42 Code of Federal Regulations (CFR), Parts 50 and 93 found at http://www.admin.ufl.edu/DDD/attach06-07/R10101-0704.pdf and https://ori.hhs.gov/sites/default/files/42_cfr_parts_50_and_93_2005.pdf.
2. UF Regulation 6C1-1.0101; Policy for Dealing with Conduct in Research found at http://www.admin.ufl.edu/DDD/attach06-07/R10101-0704.pdf.

Inquiry Process:

The inquiry process was conducted by Dr. Brandi Ormerod, Associate Professor and Director of Graduate Student Diversity and Professional Development, Department of Biomedical Engineering, College of Engineering, and Dr. Irene Cooke and Mr. Michael Scian, Director and Assistant Director, respectively, of the Division of Research Compliance, Office of Research.

During the inquiry, the sequestered information was reviewed, there were discussions with Drs. Srivastava and Herzog, Dr. Aslanidi was interviewed and there was email correspondence with Dr. Rao in India.

The results of the inquiry found the following:

Allegation 1: Drs. Rao, Srivastava, Herzog and Aslanidi all agreed that Figure 3A in Article 1 (Page 3745 in Attachment 1a) was fabricated. Thus, the allegation had substance and warranted further investigation. However, it was not clear who was responsible for this fabrication. This warranted additional investigation. However, for Figure 3B in Article 1 (Page 3745 in Attachment 1a), it was determined that the while the western blot presented in Dr. Rao's November 2, 2009, laboratory presentation (Attachment 6c in Attachment 11) was not shown in its entirety, the western blot bands that were presented were the same as that in the laboratory presentation. Thus, the allegation did not have substance and did not warrant further investigation.

Allegations 2 and 3: Minimum and maximum bioluminescence intensities could be affected by background, binning, focal stop and exposure time settings. Varying these parameters could influence the appearance of size of a tumor, yet information about these parameters was not provided in the figure captions or the methods section of Articles 2 or 3 (Attachments 1b and 1c). Further, information about the signals and details about how these settings were controlled across images taken weeks apart was also not presented in the methods section of either article. Additionally, for Figure 6 in Article 3 (Page 22 in Attachment 1c), discrepancies in the manuscript confuse which cancer cell type the mice were injected with, the long-time intervals between when groups imaging sessions require a careful description of imaging parameters given that bioluminescent intensities are compared and the lack of labeling on the graphs in Figure 6B (Page 22 in Attachment 1c) bring into question whether separate mice or regions within the same mice were imaged. Although variable imaging settings may be standardized in the photons emitted per second per cm^2 settings used in the articles, all of these reasons warranted additional investigation.

Charges to Consider for the Investigation:

1. Determine who fabricated Figure 3A in Article 1.
2. Determine whether Figure 4 in Article 2 and Figure 6 in Article 3 were falsified or fabricated.
3. Determine whether the allegations meet the definition of research misconduct; in this case, falsification or fabrication.
4. Determine whether there was a significant departure from the accepted practices of the relevant research community.
5. Determine whether the falsification or fabrication (if present) was unintentional or intentional, knowing or reckless based on the facts of the case.
6. Determine whether the allegation can be proven by a preponderance of evidence.

Investigation Process:

Investigation Committee

The Investigation Committee consisted of:

- Dr. Grant McFadden, Professor, Department of Molecular Genetics and Microbiology, College of Medicine.
- Dr. Brandi K. Ormerod, Associate Professor and Director of Graduate Student Diversity and Professional Development, Department of Biomedical Engineering, College of Engineering.
- Dr. Edward W. Scott, Professor and Director, Department of Molecular Genetics and Microbiology, College of Medicine.

Dr. Irene Cooke and Mr. Michael Scian, Director and Assistant Director, respectively, of the Division of Research Compliance, Office of Research, supported the Investigation Committee throughout the process.

Meetings and Interviews:

The Investigation Committee met formally on August 5, August 30 and September 12, 2016. During the meetings, the inquiry report and supporting documentation (Attachment 11) was reviewed, questions for the interviews and format for the interviews were finalized, and responses provided by the interviewees were discussed. The Investigation Committee also reviewed information and corresponded via telephone calls and emails, as needed.

The Investigation Committee interviewed Drs. Aslanidi, Srivastava and Herzog on September 30, 2016. Drs. Srivastava and Herzog were interviewed together. Dr. Rao was only willing to correspond with the Investigation Committee via email (Attachment 2). However, the Investigation Committee determined that they already had the information they needed (Attachment 3) from previous email correspondence with Dr. Rao in the Inquiry Report (Attachments 14 and 19 in Attachment 11). The interview recordings were provided to each interviewee for their review and comment (Attachments 8 and 9).

Information Reviewed by the Investigation Committee:

1. Journal Articles Referenced in Investigation Report
    a. G.R. Jayandharan, G.V. Aslanidi, A.T. Martino, S.C. Jahn, G.Q. Perrin, R.W. Herzog, and A. Srivastava (2011) Activation of the NF-kB Pathway by Adeno-associated virus (AAV) Vectors and its Implications in Immune Response and Gene Therapy. Proc. Natl. Acad. Sci., USA, 108: 3743-3748
    b. Munjal Pandya, Kellee Britt, Brad Hoffman, Chen Ling, and George V. Aslanidi (2015) Reprogramming Immune Response With Capsid-Optimized AAV6 Vectors for Immunotherapy of Cancer. J. Immunotherapy 38:292-298
    c. R Sayroo, D Nolasco, Z. Yin, Y. Colon-Cortes, M. Pandya, C. Ling and G. Aslanidi (2016) Development of a Novel AAV Serotype 6 based Vectors Selective Tropism for Human Cancer Cells. Gene Therapy 23, 18–25

    d. George V. Aslanidi, Angela E. Rivers, Luis Ortiz, Lakshamanan Govindasamy, Chen Ling, Giridhara R. Jayandharan, Sergei Zolotukhin, Mavid Agbandje-McKenna, and Arun Srivastava (2012) High-efficiency Transduction of Human Monocyte-derived Dendritic Cells by Capsid-modified Recombinanat AAV2 Vectors. Vaccine 30(26):3908-3917
2. Emails to and from Drs. Rao and Cooke around August 31, 2016, regarding a request for a Skype interview
3. Emails dated September 7, 2016, from the Investigation Committee regarding the Skype interview with Dr. Rao
4. Emails dated April 19, 2012, to and from Drs. Srivastava and Wilson, University of Pennsylvania
5. Dr. Rao's laboratory notebook titled D Sequence
6. Page dated 25/5/9, with Post-it note from Dr. Rao's laboratory notebook titled D Sequence
7. Aslanidi_Poster ASCGT and 'Aslanidi G' PowerPoint_5-10-10
8. Interview recording of Dr. Aslanidi
    a. Interview recording comments from Dr. Aslanidi
9. Interview recording of Drs. Srivastava and Herzog
    a. Interview recording comments from Drs. Srivastava and Herzog
    b. PowerPoint presented by Drs. Srivastava and Herzog at the interview
10. Comments from Drs. Aslanidi and Rao to the draft report. A draft report of the findings of the Investigation Committee was submitted to Drs. Aslanidi and Rao for comment on October 4, 2016. Dr. Aslanidi replied on October 11, 2016, and Dr. Rao replied on October 10, 2016. The final report considered the comments provided by Drs. Aslanidi and Rao to the draft report.
11. Inquiry Report with attachments

Investigation Analysis:

Allegation 1: Based on a review of the information and the interviews, the Investigation Committee determined that Figure 3A in Article 1 (Page 3745 in Attachment 1a) contains duplicated, altered and mislabeled flow cytometry plots that represent clear fabrications and falsifications of scientific data. The details are listed in Allegation 1 above. Figure 6A of Article 1 (Page 3747 in Attachment 1a), done by Dr. Rao, also contains a duplicated and cropped pair of control fluorescent micrograph images that are fabrications and falsifications in the published research record. This duplication/falsification was not part of the original Allegation 1, but surfaced during Dr. Aslanidi's interview (Attachment 8). Drs. Srivastava and Herzog stated in their interview that Dr. Aslanidi had dendritic cell expertise and Dr. Rao had AAV expertise (Attachment 9). Thus, they were tasked to perform the experiments for Figure 3 (Page 3745 in Attachment 1a). No primary data from any of the flow cytometric or fluorescent micrographs were found for evaluation by the committee. Further, no laboratory notebooks were found that contained evidence that the experiments described in Figure 3A (Page 3745 in Attachment 1a) were performed. Only one large removable Post-It note (Attachment 6) affixed to one of Dr. Rao's notebook pages (Attachment 5) provided any evidence that the experiments described by Figure 3A (Page 3745 in Attachment 1a) were performed. The Post-It note (Attachment 6) was sparsely annotated without a clear outline of what experiment was performed, however, it did indicate that the cells types discussed in Figure 3A in

Article 1 (Page 3745 in Attachment 1a) were employed in the study. The date on the Post-It note (Attachment 6) was recorded with ink that differed in color from ink used to record information in the main body of text on the Post-It note and the date was not faded like the text in the body of the note, suggesting post-dating.

The precise method and timing of the data manipulation in Figure 3A in Article 1 (Page 3745 in Attachment 1a) could not be determined due to the very sparse and poor records kept by both Drs. Aslanidi and Rao regarding the study. The first known presentation of the altered Figure 3A was by Dr. Aslanidi in a poster and PowerPoint, both dated May 10, 2010 (Attachment 7). Dr. Aslanidi in his interview (Attachment 8) stated that he neither made the part of the poster nor the PowerPoint slide of the figure that showed the 5-panels. Further, he stated that he did not perform the experiments for the figure that showed the 5-panels. He only presented the poster because Dr. Rao had left UF to begin his new position in India. Dr. Rao indicated via email that he did not create the altered 5-panel version of Figure 3A (Attachments 14 and 19 in Attachment 11). Dr. Rao also pointed out that the first draft of the PNAS paper that he created and sent back to UF on May 12, 2010, after the poster presentation, still contained the original 3-panel version of Figure 3A (Attachments 14 and 19 in Attachment 11). Dr. Rao declined to videoconference with the committee to present his version of the incidents in question and stated that he would only respond to written questions via email (Attachment 2). The type of data manipulations used to create Figures 3A (Page 3745 in Attachment 1a) and 6A (Page 3747 in Attachment 1a) could have easily been performed via simple post-experiment manipulations using an image processing program such as Photoshop. They did not require functional knowledge of how to perform flow cytometry or fluorescent microscopy, just how to execute simple photo manipulations on a computer.

Allegations 2 and 3: The figures in question in Articles 2 (Page 297 in Attachment 1b) and 3 (Page 22 in Attachment 1c) were examined. The data as presented did not meet the standards required for rigorous scientific proof. Insufficient experimental details concerning machine settings and experimental design were presented to support the conclusions stated in the articles regarding these live animal images. The Investigation Committee concluded that these figures represented examples of poor scientific article review and further established the pattern of a less rigorous approach to data collection on the part of Dr. Aslanidi. However, the Investigation Committee concluded that the lack of scientific rigor in these articles did not rise to the level of intentional scientific research misconduct or data falsification.

It should be noted that during the course of the interview with Drs. Srivastava and Herzog (Attachment 9), another incident of improper figure construction by Dr. Aslanidi came to light (Page 4, Slide 7 in Attachment 9b). In the initial submission phase of an article (when published as “epub ahead of print”), a colleague noted improper duplication and labeling of a series of fluorescent micrographs, and brought it to the attention of Dr. Srivastava (Attachment 4). The errors were in a figure created by Dr. Aslanidi. During the interview, Dr. Aslanidi agreed that he had mixed up panels in assembling the figure and the errors/duplications were corrected prior to the final publication of the paper (Figure 4, Page 3913 in Attachment 1d). This once again illustrates a general lack of rigor in the handling of scientific data for publication by Dr. Aslanidi. In his interview Dr. Aslanidi freely admitted making these errors of placing incorrect micrographs within the figure. He pointed out that he accepted full responsibility at the time and corrected the errors prior to publication.

Investigation Findings:

A finding of research misconduct, according to the Department of Health and Human Services Public Health Service Policy on Research Misconduct (42 CFR Part 93) and the UF Policy for Dealing with Conduct in Research requires that: 1) the allegation meets the definition of research misconduct (fabrication, falsification or plagiarism), and 2) there is a significant departure from accepted practices in the relevant research community, and 3) the misconduct be committed intentionally, knowingly or recklessly.

According to both federal and the UF Policy, "falsification is manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record."

Based on their investigation, the Investigation Committee concluded that Figure 3A in Article 1 (Page 3745 in Attachment 1a) represented a clear incident of research misconduct. The data in Figure 3A in Article 1 (Page 3745 in Attachment 1a) was falsified with the intent to deceive by duplication, fabrication and mislabeling of data presented in the figure. No honest mistake could account for the errors in Figure 3A in Article 1 (Page 3745 in Attachment 1a). The control duplication in Figure 6A in Article 1 (Page 3747 in Attachment 1a) is similar in nature to the falsification in Figure 3A in Article 1 (Page 3745 in Attachment 1a), but could have arisen by honest duplication error of the control panels or by using the wrong picture file for the second control panel.

The record keeping for the primary data presented in Figures 3A (Page 3745 in Attachment 1a) and 6A (Page 3747 in Attachment 1a) in Article 1 did not meet the accepted minimum standards for scientific research. No primary data files, experimental details, or records for the flow cytometry experiments of Figure 3A in Article 1 (Page 3745 in Attachment 1a) can be produced. Only one detachable Post-It note (Attachment 6) even indicates any experiments were carried out. No laboratory notebooks provided evidence that the experiments in Figure 3A (Page 3745 in Attachment 1a) were performed as described in the published article. However, based on their roles and responsibilities in the laboratory and in the research, Drs. Aslanidi or Rao were the most likely to have falsified Figure 3A (Page 3745 in Attachment 1a). In addition, the lack of rigorous record keeping prevented Drs. Aslanidi and Rao from absolving themselves from blame for this research misconduct. There was no evidence that anyone else Falsified Figure 3A (Page 3745 in Attachment 1a). However, because the falsification could have been made with simple photo manipulations on a computer it is possible that anyone else with access to the filed could have falsified Figure 3A. Thus, no clear determination of guilt can be made against anyone in this incident.

Lastly, the Investigation Committee also concluded that for Figure 4 in Article 2 (Page 297 in Attachment 1b) and Figure 6 in Article 3 (Page 22 in Attachment 1c), Dr. Aslanidi's actions were sloppy and constituted errors in accepted scientific practice rather than research misconduct.

Additional Comments and Recommended Institutional Actions:

It was noted during the inquiry that Dr. Srivastava was already collaborating with the PNAS journal to retract either Figure 3A or the entire article, depending on whatever was feasible. It was confirmed during the investigation that this is still in progress. The Investigation Committee recommends that the entire article be retracted. In addition, the Investigation Committee recommends that Figures 3A and 6A be corrected wherever they have been published (e.g. videos/ PubMed, etc.). The Investigation Committee also recommends that appropriate funding agencies be notified of the scientific misconduct.

Further, Drs. Srivastava and Herzog should be encouraged to ensure that the trainees that they supervise keep better records to reduce the likelihood that their trainees commit similar scientific misconduct in the future. While preventing determined data fraud is difficult, requiring better record keeping reduces the opportunity for fraud.

Also, the Investigation Committee recommends that for two years, the Department Chair or designee, review and verify proposed publications/proposals/presentations from Dr. Aslanidi's work (raw data, analysis etc.) for scientific validity before they are submitted.

Submitted on October 24, 2016

Investigation Committee Members (alphabetical order)

Grant McFadden, Ph.D.
Professor
Department of Molecular Genetics and Microbiology
College of Medicine

Brandi Ormerod, Ph.D.
Associate Professor and Director
Department of Biomedical Engineering
College of Engineering

Edward Scott, Ph.D.
Investigation Committee Chair
Professor and Director
Department of Molecular Genetics & Microbiology
College of Medicine