# COVER PAGE

1. Program Director: **Peter Dowling, M.D., East Orange VA Medical Center, Neurology Service (127C), 385 Tremont Avenue, East Orange, NJ 07018. Tel: (973) 676-1000 Ext. 3616**

2. Name of Organization/Institution: **New Jersey Health Care System, East Orange VA Medical Center**

3. Grant Title: **Erythropoietin-derived peptides for treatment of acute brain injury**

4. Grant Number: **08-3208-BIR-E-1**

5. Grant Period Covered by the Report: **06/01/08 – 5/31/15**

6. Date of Submission of the Report: **12/16/15**

## BODY OF REPORT

1. Original aims of the project.

   **S.A. 1: Compare the effects of our small EPO peptides to full-length erythropoietin therapy on modifying the clinical and histopathologic outcome in wild type C57BL/6 mice after traumatic brain injury** (histologically quantifying dying and proliferating neural cells, MHC II alterations and mononuclear/T cell infiltrate in treated animals versus sham treated traumatic brain injured controls at 1, 3, 7, 14, 42 and 90 days post-injury, composite neuro score, hanging wire task, radial and water maze, clinical correlations).

   **S.A. 2: Determine the therapeutic window for achieving a beneficial effect with our new peptides** (neuropathology and clinical correlations).

   **S.A. 3: As a further test of our hypothesis, determine if EPO therapy can favorably modify the clinical outcome in RAG1$^{-/-}$ immunodeficient mice that have received labeled wild type T cells and/or other immune functional cells by adoptive transfer** (T and B cell purification, flow cytometry, in vivo bioluminescent imaging to track labeled cell migration and pathology time course by immunohistochemistry).

2. Project successes.

   The enclosed manuscript entitled "Beneficial effect of erythropoietin short peptide on acute traumatic brain injury" (in Neurotherapeutics) was accepted Dec 15, 2015 and it contains a listing of all the project successes: we established that short erythropoietin peptide JM4 was capable of substantial side effect free neuroprotection following brain trauma and that a therapeutic window of more than 9 hours exists for our compound. We developed a new set of criteria for the physical exam in neurologically impaired brain injured animals and presented data showing that our short EPO peptide readily crosses the blood brain barrier. This information and other background was submitted to the NIH-RAID Pilot Program in January 2011 and our proposal to develop our lead compound for human use was approved (Feb 13, 2012). The NIH has committed to developing JM4 complete to an IND filing and this funded project is ongoing (1 X01 NS073526-01A1).

3. Project challenges.
   None.

4. Implication for future research and/or clinical treatment.

   <u>Clinical treatment</u>: Our success in obtaining NIH support for the development of JM4 through to and IND filing indicates that we will likely have the opportunity of testing neuroprotective JM4 in human subjects in about 1 year, assuming final bioassays for toxicity etc. remain favorable.

Future research: We chose to further define the active inflammatory cells in passive transfer experiments using normal animals with intact immune systems rather than the Rag1 -/- model (S.A. 3). We have now found strong evidence that the injurious cell belongs to elements contained within the CD11b+ cell population and that CD11b negative cells are incapable of generating pathology. These robust findings form the basis of our next NJCBIR grant proposal now under consideration entitled "Important role of the spleen in acute TBI" (PI: Y Maeda, MD). Importantly, we have developed new highly effective cell sorting procedures utilizing the magnetic activated cell-sorting (MACS) system (Miltenyi Biotec, Auburn, CA).

5. Plans to continue the research, including applications submitted to other sources for ongoing support.

   As indicated above, we currently have our next NJCBIR grant under active consideration at this time. We have greatly enlarged our studies to investigate the surprisingly beneficial effect of JM4 therapy on animal models of neurodegeneration. We have received a new four-year RR&D VA Merit Award for these studies (RR&D VA Merit Award: Novel EPO peptide therapy for a new mouse model of dementia, Grant# 1 I01 RX001305-01A2) and our compound is remarkably effective at blocking chronic neuroinflammation, slowing clinical progression and lowering levels of hyperphosphorylated tau to barely detective levels (The 140th Annual meeting of American Neurological Association, Sep 27-29, 2015, Neuroinflammation as a therapeutic target in neurodegenerative disorders). This observation has JM4 therapeutic implications for yet another major brain trauma entity – acute/chronic traumatic encephalopathy where neuroinflammation and aggregated tau are the most prominent neuropathologic characteristics. We plan to investigate the neuroprotective role of JM4 on the clinical response and behavior of hyperphosphorylated tau in animal models of repetitive mild brain injury.

   RR&D VA Merit Award
   Title: Novel EPO peptide therapy for a new mouse model of dementia
   Grant #: 1 I01 RX 001305-01A2

   The 140th annual meeting of American Neurological Association (Sep 27-29, 2015)
   Authors: Wei Lu, Yasuhiro Maeda, Michelle Marchese, Esther Rodriguez, Bo Wang and Peter Dowling
   Tile: Neuroinflammation as a therapeutic target in neurodegenerative disorders (vol. 78, supplement page S85, 2015).

6. Explain how you have leveraged NJCBIR funding to obtain additional federal or other support for brain injury research and list the appropriate funding organizations.

   We obtained additional funding from the NIH for JM4 drug development and we obtained additional support from the VA for studying neuroinflammation as a therapeutic target in neurodegenerative disorders (again JM4 therapeutic studies).

Compound Summary
BrIDGs Program

12/9/15

**PI Name:** **Dr. Peter Dowling, M.D.**

Affiliation: University of Medicine and Dentistry of New Jersey (UMDNJ)

Project Title: Short Stabilized EPO-Peptide as Side Effect Free Therapeutic Agent for Multiple Sclerosis and Acute Brain Trauma

Agent Category: Peptide

Molecular Target: Glial MHC II

Level of Development: Complete to IND

NSC:

Molecular Weight: 2030 MSDS:

Common Name: JM4

Chemical Name:

Other: EPO is active in animal models of multiple sclerosis (MS) and traumatic brain injury (TBI) but beneficial properties cannot be separated from its hematopoetic effects. A series of peptides were prepared to isolate the positive neurologic effects. The lead sequence for development is JM4, amino acids 28-46 of the A-B chain, (Gly-Cys-Ala-Glu-His-Cys-Ser-Leu-Asn-Glu-Asn-Ile-Thr-Val-Pro-Asp-Thr-Lys-Val).

Monthly Summary: 11.18.15 Received internal standard, the RF studies tentatively scheduled for mid December. PJ/JC

**Background**

Application Receipt Date: 01/11/2011 Review Date: Apr 14, 2011 Approval Date: February 13, 2012

7. List and include a copy of all publications emerging from this research, including those used in preparation.

   1. Wang B, Kang M, Marchese M, Rodriguez E, Lu W, Maeda Y, Dowling P. Beneficial effect of erythropoietin short peptide on acute traumatic brain injury. Neurotherapeutics, 2015 (final manuscript accepted for publication on Dec 15, 2015).

   2. Wei Lu, Yasuhiro Maeda, Michelle Marchese, Esther Rodriguez, Bo Wang and Peter Dowling. Neuroinflammation as a therapeutic target in neurodegenerative disorders. Annals of Neurology, vol. 78, supplement page S85, 2015.

   3. Yuan R, Wang Bo, Lu W, Maeda Y and Dowling P. A distinct region in erythropoietin that induces immuno/inflammatory modulation and tissue protection. Neurotherapeutics, 2015: DOI10. 1007/s13311-015-0379-1.

8. Financial summary.

   Final financial summary has already been directly sent to the NICBIR through the VBRI. Please contact Ms. Kristen Bourgerie at 973-676-1000 Ext. 3875 or kristen.bourgerie@va.gov for further inquiries.