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TITLE: Individualized Medicine in a Gyrencephalic Model of TBI Polytrauma Through the Continuum of Care

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14. ABSTRACT:
Advancements in combat casualty care for traumatic brain injury (TBI) have greatly improved mortality and morbidity rates during recent armed conflicts. Damage control resuscitation, delayed wound closure, tourniquets, and rapid evacuation for damage control surgery are examples of those observations contributing to improved care. Military casualties face numerous complications to their care that are uncommon in civilian medical practice, including the mode, multiplicity, and severity of injuries, combat- related limitations on the modalities of acute care, potentially prolonged delay to definitive care, and the possibility of prolonged aeromedical evacuation with its attendant unique environmental stressors. However, most treatment strategies are based on standard protocols which lack the subtlety to account for patient specific differences in response to therapy, thus resulting in missed opportunities for improved outcomes at the individual patient level. This study aims to identify strategies to improve outcomes associated with TBI and hemorrhagic shock (HS) by identifying biomarkers predictive of an individual's response to injury and to environmental factors (simulated prolonged en route care) with the goal of optimizing individual treatment strategies. The purpose of this study is to identify strategies to improve outcomes associated with TBI and hemorrhagic shock (HS) by identifying biomarkers predictive of an individual's response to injury and to environmental factors (simulated prolonged en route care) with the goal of optimizing individual treatment strategies. Specific aims are (1) To identify characteristics (serum and neuroimaging biomarkers, sex differences) that may predict an individual's response to injury. (2) To assess the effects of simulated aeromedical evacuation on the individual's response to injury and identify optimal evacuation strategies for the individual patient. (3) To channel knowledge gained from Phases 1 and 2 into optimized individualized therapeutic approaches.

15. SUBJECT TERMS
TBI, hemorrhagic shock, polytrauma, simulated prolonged en route care combat casualty care, damage control resuscitation, delayed wound closure, tourniquets, and rapid evacuation

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The purpose of this study is to identify strategies to improve outcomes associated with TBI and hemorrhagic shock (HS) by identifying biomarkers predictive of an individual's response to injury and to environmental factors (simulated prolonged en route care) with the goal of optimizing individual treatment strategies. Using a ferret gyrencephalic ferret model this study aims to identify strategies to improve outcomes associated with TBI and hemorrhagic shock (HS) by identifying biomarkers - including neuroimaging, physiological measurements, blood hematology and chemistries, local and systemic inflammation, as well as anatomical, cellular and molecular analysis of injured/damaged brain tissue - that are predictive of an individual's response to injury and to environmental stressors (simulated aeromedical transport) with the goal of optimizing individual treatment strategies.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Traumatic brain injury, controlled cortical impact, neuroimaging, hemorrhagic shock, aeromedical evacuation, neuroinflammation, biomarkers

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

	Timeline	***USU***	***NMRC***	***UVA***	***% complete***
Task 1. IACUC/ACURO approval for animal studies and procurement of equipment:	*Months*				
Subtask 1. Write IACUC protocol.	*1*		*Dr. Scultetus*	*Dr. Stone*	*100%*
Subtask 2. IACUC review and approval.	*1*		*Dr. Scultetus*	*Dr. Stone*	*100%*
Subtask 3. ACURO review and approval.	*1*		*Dr. Scultetus*	*Dr. Stone*	*100%*
Subtask 4. Procurement/set-up of equipment.	*1-2*		*Dr. Scultetus*	*Dr. Stone*	*100%*
Specific Aim 1: Characterizing individual responses to TBI/HS.					

Task 2. Perform animal experiments in Phase 1:					
Subtask 1. NMRC: Complete instrumented/physiological animal experiments (n= 35 ferrets). Treatment groups (1) Sham (2) controlled cortical impact (CCI) traumatic brain injury (TBI) + hemorrhagic shock (HS) and (3) fluid percussion TBI (FP) TBI + HS	*2-12*		*Dr. Scultetus*		*5%*
Subtask 2. UVA: Complete neuroimaging animal experiments (n= 45 ferrets) at 6hr and 72 hr postinjury *a. Dynamic PET imaging* *b. Magnetic resonance imaging (MRI)* *c. Diffusion tensor imaging (DTI)* *d. Magnetic resonance angiography (MRA)* *e. Arterial spin labeling (ASL)* *f. Delayed enhanced imaging (DEI)* *g. Susceptibility weighted imaging (SWI)*	*2-12*			*Dr. Stone*	*5%*
Subtask 3. USU/NMRC/UVA: Analysis of biological samples from whole blood and regional brain tissues for qRT-PCR analysis using low-density microarrays. *a. Peripheral blood-inflammatory gene mRNA transcripts* *b. Regional brain tissues- neuroinflammation, tissue damage, oxidative stress, and apoptotic gene mRNA transcripts* *c. Data to SC2i for analysis.*	*2-12*	*Dr. Bell*	*Dr. Scultetus*	*Dr. Stone*	*5%*
Subtask 4. NMRC: Histopathology samples provided to contractor for processing/analysis. UVA will participate in data analysis and interpretation.	*12*		*Dr. Scultetus*	*Dr. Stone*	
Specific Aim 2: Characterize individual responses to TBI/HS plus aeromedical transport.					
Task 3. Perform animal experiments in Phase 2:					
Subtask 1. NMRC: Complete instrumented/physiological animal	*13-23*		*Dr. Scultetus*		

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

NMRC:

- WRAIR/NMRC IACUC protocol 19-OUMD-09S approved 05MAY2019.
- ACURO approval 02JUL2019.
- Pilot study was initiated to obtain ferret blood for molecular assay development and RNA extraction.
- A dedicated post-doc was hired and trained in procedures associated with this study. This is a new model and required more intensive skill and knowledge development.

UVA:

- UVA IACUC protocol 4236 approved 26OCT2018
- ACURO approval 10APR2019
- Work commenced to create a custom MRI coil to be compatible with Siemens 3T Prisma scanner at UVA. This MRI coil is designed to a ferret specification and is capable of acquiring all of the sequences outlined for the current award.
- Acquire materials and supplies for experimentation.

- Precursor material for molecular imaging radiopharmaceuticals have been prepared for positron emission tomography (PET) procedures.
- Establish PET and MRI protocols for imaging.

USU:

- We hired a Neuroscience Post-doc, Dr. Cassie Gould, to spearhead the molecular gene expression studies in the Surgical Studies and Molecular core lab at USUHS.
- We commenced with a few pilot studies at USUHS to develop molecular methodologies to isolate mRNA and optimize yield for gene expression studies from normal ferret circulating leukocytes in presence or absence of in vitro lipopolysaccharide (LPS) stimulation.

Methodology: In a pilot study, leukocyte stimulation of whole ferret blood (Marshall Bioresources; N=6) was induced using Lipopolysaccharides (LPS; Sigma). Each blood sample was aliquoted into two, 2 mL samples; one tube served as a within-sample control (RPMI 1640 media), the other was treated with LPS (final concentration: 1 µg/mL). All tubes were then incubated for 4 hours at 37ºC. Cells were lysed with ACK lysis buffer, supernatant was removed, and the cell pellet was re-suspended in 1 mL Qiazol. Total RNA was isolated from cell pellet using RNeasy mini kit (Qiagen) and first strand cDNA was generated from 1 µg total RNA using the Reaction Ready First Strand cDNA synthesis kit (Biosciences, Frederick, MD). A set of 8 target genes and 3 housekeeping gene primer pairs were designed according to Carolan et al., 2014.

RT-PCR was conducted using the Quant Studio 7 Flex Real-Time PCR system (Rockville, MD) and the ΔCT method was used for analyses using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the housekeeping gene. A students t-test was used to assess the significance at $\alpha=0.05$.

Results. Following leukocyte stimulation with LPS, four of the eight cytokine markers exhibited significantly elevated gene expressions relative to controls (**Figure 1 and Table 1**). Ferret blood treated with LPS for 4 hours increased IL-1β fold change by 3.92 ($p=0.0261$), IL-6 by 4.49 ($p=0.0012$), IL-8 by 3.49 ($p=0.044$), and 1L-12p40 by 13.30 ($p=0.0006$). The gene expressions of IL-10, TNF-α, MCP1, or IFN-ɣ, were not elevated or significantly different relative to controls. This pilot study demonstrates that our lab is capable of measuring gene expression changes in cytokines and proteins secreted by activated leukocytes.

To further investigate potential associations between genomic changes peripherally driven by protein products secreted/produced by activated leukocytes following TBI in our ferret model, we surveyed the literature for key regulators (in addition to the markers in the pilot study) involved in mediation of acute/innate immune response, anti-inflammatory or protective mediators and apoptotic markers to find 100+ different protein products. To develop a custom PCR array, we worked with Thermofisher Scientific (TFS) to create custom primers for the activated leukocyte protein products. The bioinformatics specialists at TFS were able to design 77/100 primers (**Table 2**) and we were able to identify 16/100 primer sequences from the 30 remaining genes through published literature **Table 3**). Using the knowledge of TFS and the literature, we narrowed down the 100+ interested target genes to 41 target genes (and 3 housekeeping genes) and are currently working with TFS to create custom PCR array. An outline of the 41 target genes are shown in the custom plate layout design in **Figure 2.** Once we receive the custom PCR plates, we plan to run a second pilot study with activated leukocyte mRNA (as in the above pilot study) to ensure the sequences we and TFS designed are correctly designed against the ferret genome.

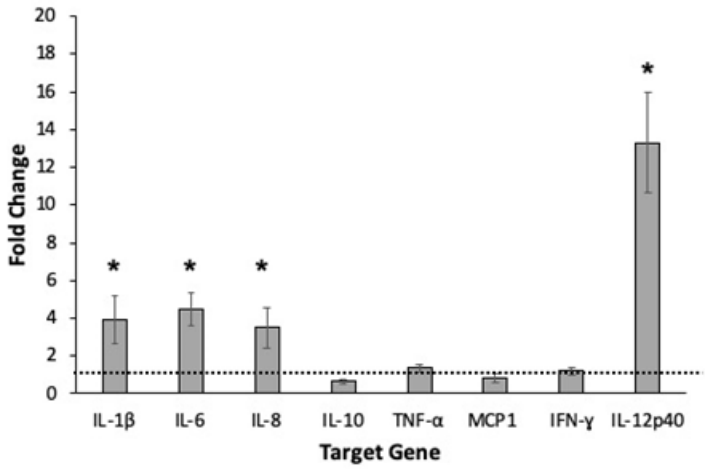


Figure 1. Levels of cytokine and leukocyte protein mRNA relative glyceraldehyde 3-phosphate dehydrogenase (GAPDH; fold change values) of activated leukocytes in ferret blood. Following LPS treatment, levels of IL-1β, IL-6, IL-8, and 1L-12p40 are significantly increased relative to untreated controls. The dashed line represents the level of each transcript normalized to "1," in RPMI 1640 treated controls, regardless of the levels across individual cytokines and leukocyte proteins. *$p<0.05$.

Target Gene	Fold Change
IL-1β	**3.92 ± 1.237**
IL-6	**4.49 ± 0.854**
IL-8	**3.49 ± 1.039**
IL-10	0.64 ± 0.147
TNF-α	1.35 ± 0.192
MCP1	0.85 ± 0.239
IFN-γ	1.2 ± 0.222
IL-12p40	**13.3 ± 2.632**

Table 1. Levels of cytokine and leukocyte protein mRNA relative to GAPDH (ΔCt values ± SEM) of activated leukocytes in ferret blood. Following LPS treatment, levels of IL-1β, IL-6, IL-8, and 1L-12p40 are significantly increased relative to untreated controls. Bolded values indicate significant difference between LPS-activated and control treated leukocytes within each target gene.

Table 2. Inflammatory mediator primer sequences developed by Thermofisher scientific (TFS). TFS was able to design 77 of the 100 requested target genes. Yellow highlighted values represent primer sequences chosen for the activated leukocyte custom PCR array.

RefSeq	Amplicon Length	Sequence Description	Gene Symbol	Assay Name	Assay ID
NM_001310164.1	80	*Mustela putorius furo* CD4 molecule (CD4), mRNA	CD4	MP-CD4	APGZJC6
NM_001310183.1	58	*Mustela putorius furo* tumor necrosis factor (TNF), mRNA	TNF	MP-TNF	APH6DW3
NM_001310187.1	69	*Mustela putorius furo* transforming growth factor beta 2 (TGFB2), mRNA	TGFB2	TGFB2	APKA7GZ
NM_001310190.1	74	*Mustela putorius furo* angiotensin I converting enzyme 2 (ACE2), mRNA	ACE2	ACE2	APMFZ2X
NM_001310191.1	69	*Mustela putorius furo* interleukin 4 (IL4), mRNA	IL4	MP-IL4	APNKVMV
NM_001310209.1	61	*Mustela putorius furo* transforming growth factor beta 1 (TGFB1), mRNA	TGFB1	TGFB1	APPRN7T
XM_004738090.2	64	*Mustela putorius furo* interleukin-3 (LOC101678790), mRNA	LOC101678790	LOC101678790	APRWHTP
XM_004739204.2	66	*Mustela putorius furo* transforming growth factor, beta 3 (TGFB3), mRNA	TGFB3	TGFB3	APT2DDM
XM_004739794.2	62	*Mustela putorius furo* heat shock protein 90kDa alpha (cytosolic), class B member 1 (HSP90AB1), mRNA	HSP90AB1	HSP90AB1	APU66XJ
XM_004741237.2	75	*Mustela putorius furo* wingless-type MMTV integration site family, member 4 (WNT4), transcript variant X1, mRNA	WNT4	WNT4	APWCZHG
XM_004742367.2	78	*Mustela putorius furo* CD8a molecule (CD8A), mRNA	CD8A	CD8A	APXGU3E
XM_004742486.2	104	*Mustela putorius furo* macrophage migration inhibitory factor (glycosylation-inhibiting factor) (MIF), mRNA	MIF	MP-MIF	APYMNNC
XM_004744199.2	68	*Mustela putorius furo* matrix metallopeptidase 2 (MMP2), mRNA	MMP2	MMP2	APZTG79
XM_004744862.2	74	*Mustela putorius furo* early growth response 1 (EGR1), mRNA	EGR1	EGR1	AP2XCT6
XM_004744982.2	89	*Mustela putorius furo* heat shock 70kDa protein 4 (HSPA4), mRNA	HSPA4	HSPA4	AP326D3
XM_004745472.2	71	*Mustela putorius furo* CD86 molecule (CD86), transcript variant X1, mRNA	CD86	CD86	AP47YXZ
XM_004746290.2	83	*Mustela putorius furo* CD40 molecule, TNF receptor superfamily member 5 (CD40), mRNA	CD40	CD40	AP7DUHX
XM_004746359.2	69	*Mustela putorius furo* secretory leukocyte peptidase inhibitor (SLPI), mRNA	SLPI	SLPI	AP9HM3V
XM_004746447.2	69	*Mustela putorius furo* lipopolysaccharide binding protein (LBP), mRNA	LBP	MP-LBP	APAAEGK
XM_004747089.2	60	*Mustela putorius furo* C-C motif chemokine 2 (LOC101686051), mRNA	LOC101686051	LOC101686051	APCE72H
XM_004747141.2	91	*Mustela putorius furo* chemokine (C-C motif) ligand 5 (CCL5), mRNA	CCL5	CCL5	APDJ2MF
XM_004747147.2	83	*Mustela putorius furo* C-C motif chemokine 3 (LOC101676470), mRNA	LOC101676470	LOC101676470	APEPV7D
XM_004747148.2	82	*Mustela putorius furo* chemokine (C-C motif) ligand 4 (CCL4), mRNA	CCL4	CCL4	APFVPTA
XM_004747973.2	91	*Mustela putorius furo* interleukin 2 (IL2), mRNA	IL2	MP-IL2	APGZJC7
XM_004748161.2	91	*Mustela putorius furo* nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1), transcript variant X1, mRNA	NFKB1	NFKB1	APH6DW4
XM_004751289.1	71	*Mustela putorius furo* beta-2-microglobulin (B2M), transcript variant X1, mRNA	B2M	MP-B2M	APKA7G2

XM_004751637.2	75	*Mustela putorius furo* jun proto-oncogene (JUN), mRNA	JUN	MP-JUN	APMFZ2Y
XM_004753059.2	74	*Mustela putorius furo* interleukin 1 receptor-like 1 (IL1RL1), transcript variant X2, mRNA	IL1RL1	IL1RL1	APNKVMW
XM_004755096.2	71	*Mustela putorius furo* forkhead box P3 (FOXP3), mRNA	FOXP3	FOXP3	APPRN7U
XM_004756714.2	81	*Mustela putorius furo* tumor necrosis factor (ligand) superfamily, member 4 (TNFSF4), transcript variant X1, mRNA	TNFSF4	TNFSF4	APRWHTR
XM_004757721.2	61	*Mustela putorius furo* caspase 3, apoptosis-related cysteine peptidase (CASP3), mRNA	CASP3	CASP3	APT2DDN
XM_004757786.2	131	*Mustela putorius furo* toll-like receptor 3 (TLR3), transcript variant X2, mRNA	TLR3	TLR3	APU66XK
XM_004758838.2	68	*Mustela putorius furo* toll-like receptor 7 (LOC101685809), transcript variant X2, mRNA	LOC101685809	LOC101685809	APWCZHH
XM_004758839.1	73	*Mustela putorius furo* toll-like receptor 8 (LOC101686197), partial mRNA	LOC101686197	LOC101686197	APXGU3F
XM_004759902.2	92	*Mustela putorius furo* bone morphogenetic protein 7 (BMP7), mRNA	BMP7	BMP7	APYMNND
XM_004760810.2	62	*Mustela putorius furo* chemokine (C-C motif) receptor 3 (CCR3), mRNA	CCR3	CCR3	APZTG9A
XM_004761627.2	73	*Mustela putorius furo* heat shock 27kDa protein 1 (HSPB1), mRNA	HSPB1	HSPB1	AP2XCT7
XM_004762732.2	97	*Mustela putorius furo* chemokine (C-X-C motif) receptor 2 (CXCR2), transcript variant X1, mRNA	CXCR2	CXCR2	AP326D4
XM_004763336.1	66	*Mustela putorius furo* bone morphogenetic protein 1 (BMP1), partial mRNA	BMP1	BMP1	AP47YX2
XM_004763991.2	102	*Mustela putorius furo* toll-like receptor 6 (TLR6), transcript variant X3, mRNA	TLR6	TLR6	AP7DUHY
XM_004764165.2	97	*Mustela putorius furo* caspase 8, apoptosis-related cysteine peptidase (CASP8), transcript variant X1, mRNA	CASP8	CASP8	AP9HM3W
XM_004764446.2	76	*Mustela putorius furo* caspase-1-like (LOC101686692), transcript variant X1, mRNA	LOC101686692	LOC101686692	APAAEGM
XM_004764452.2	61	*Mustela putorius furo* stromelysin-1 (LOC101688701), mRNA	LOC101688701	LOC101688701	APCE72J
XM_004764708.2	72	*Mustela putorius furo* T-box 21 (TBX21), transcript variant X2, mRNA	TBX21	TBX21	APDJ2MG
XM_004766247.2	104	*Mustela putorius furo* chemokine (C-X-C motif) ligand 11 (CXCL11), mRNA	CXCL11	CXCL11	APEPV7E
XM_004771000.2	59	*Mustela putorius furo* interleukin 1, alpha (IL1A), mRNA	IL1A	IL1A	APFVPTC
XM_004771315.2	106	*Mustela putorius furo* angiopoietin 1 (ANGPT1), transcript variant X1, mRNA	ANGPT1	ANGPT1	APGZJC9
XM_004771883.1	79	*Mustela putorius furo* interleukin 17A (IL17A), transcript variant X2, mRNA	IL17A	IL17A	APH6DW6
XM_004771998.2	83	*Mustela putorius furo* toll-like receptor 2 (TLR2), transcript variant X1, mRNA	TLR2	TLR2	APKA7G3
XM_004772042.2	76	*Mustela putorius furo* platelet derived growth factor C (PDGFC), transcript variant X1, mRNA	PDGFC	PDGFC	APMFZ2Z
XM_004772404.2	64	*Mustela putorius furo* bone morphogenetic protein 5 (BMP5), transcript variant X1, mRNA	BMP5	BMP5	APNKVMX
XM_004773118.2	91	*Mustela putorius furo* interleukin 23, alpha subunit p19 (IL23A), transcript variant X1, mRNA	IL23A	IL23A	APPRN7V
XM_004773176.2	100	*Mustela putorius furo* signal transducer and activator of transcription 6, interleukin-4 induced (STAT6), transcript variant X1, mRNA	STAT6	STAT6	APRWHTT
XM_004774068.2	70	*Mustela putorius furo* toll-like receptor 4 (TLR4), transcript variant X1, mRNA	TLR4	TLR4	APT2DDP
XM_004774198.2	118	*Mustela putorius furo* chemokine (C-C motif) receptor 4 (CCR4), transcript variant X1, mRNA	CCR4	CCR4	APU66XM
XM_004775013.2	99	*Mustela putorius furo* actin, beta (ACTB), mRNA	ACTB	ACTB	APWCZHJ
XM_004777248.2	66	*Mustela putorius furo* chemokine (C-X-C motif) receptor 3 (CXCR3), transcript variant X1, mRNA	CXCR3	CXCR3	APXGU3G
XM_004778431.2	61	*Mustela putorius furo* NLR family, pyrin domain containing 3 (NLRP3), transcript variant X2, mRNA	NLRP3	NLRP3	APYMNNE
XM_004780260.2	106	*Mustela putorius furo* S100 calcium binding protein B (S100B), transcript variant X1, mRNA	S100B	S100B	APZTG9C
XM_004780312.2	78	*Mustela putorius furo* interleukin 6 receptor (IL6R), transcript variant X1, mRNA	IL6R	IL6R	AP2XCT9
XM_004780396.2	61	*Mustela putorius furo* S100 calcium binding protein A8 (S100A8), mRNA	S100A8	S100A8	AP326D6
XM_004780398.2	67	*Mustela putorius furo* S100 calcium binding protein A9 (S100A9), mRNA	S100A9	S100A9	AP47YX3
XM_004781006.2	89	*Mustela putorius furo* lymphotoxin alpha (LTA), mRNA	LTA	MP-LTA	AP7DUHZ
XM_004781075.2	79	*Mustela putorius furo* Harvey rat sarcoma viral oncogene homolog (HRAS), transcript variant X1, mRNA	HRAS	HRAS	AP9HM3X
XM_004781556.2	63	*Mustela putorius furo* prostaglandin-endoperoxide synthase 2 (prostaglandin G	PTGS2	PTGS2	APAAEGN
XM_004781795.2	85	*Mustela putorius furo* toll-like receptor 5 (TLR5), transcript variant X2, mRNA	TLR5	TLR5	APCE72K

XM_013044458.1	61	*Mustela putorius furo* colony stimulating factor 1 (macrophage) (CSF1), mRNA	CSF1	CSF1	APDJ2MH
XM_013046064.1	82	*Mustela putorius furo* CD80 molecule (CD80), mRNA	CD80	CD80	APEPV7F
XM_013046568.1	61	*Mustela putorius furo* integrin, alpha M (complement component 3 receptor 3 subunit) (ITGAM), mRNA	ITGAM	ITGAM	APFVPTD
XM_013047455.1	98	*Mustela putorius furo* C-reactive protein (LOC101687507), mRNA	LOC101687507	LOC101687507	APGZJDA
XM_013048292.1	89	*Mustela putorius furo* bone morphogenetic protein 3 (BMP3), mRNA	BMP3	BMP3	APH6DW7
XM_013048913.1	121	*Mustela putorius furo* hypoxanthine phosphoribosyltransferase 1 (HPRT1), mRNA	HPRT1	HPRT1	APKA7G4
XM_013050178.1	91	*Mustela putorius furo* advanced glycosylation end product-specific receptor (AGER), transcript variant X1, mRNA	AGER	AGER	APMFZ22
XM_013051369.1	83	*Mustela putorius furo* colony stimulating factor 2 (granulocyte-macrophage) (CSF2), partial mRNA	CSF2	CSF2	APNKVMY
XM_013062218.1	98	*Mustela putorius furo* toll-like receptor 10 (TLR10), mRNA	TLR10	TLR10	APPRN7W
XM_013062395.1	67	*Mustela putorius furo* stromelysin-2 (LOC101672187), mRNA	LOC101672187	LOC101672187	APRWHTU
XM_013062542.1	63	*Mustela putorius furo* colony stimulating factor 3 (granulocyte) (CSF3), mRNA	CSF3	CSF3	APT2DDR

Table 3. Ferret specific primer sets obtained from an in-depth literature search. Several primary publications using the *Mustela putorius furo* species reported the specific inflammatory mediator primer and probe sequences.

Target Gene	Forward Sequence	Reverse Sequence	Probe	Primer Source
IL-1b	CCTGGTGCTGTATAACTCGTATGAG	TTGGTTCACACTAGTTCCGTTGA	--	Erins Test Run
ALB	TGGTTTCATCTGCCAGAGAAAG	CAAAGTCAGCTTTGGGGAATTTC	TTCAAGTGTGCCAGCCTCCAGAA	Bruder et al. 2010
ATF4	TTTACCTTCCTGCAACCACTTC	TCATGGTAATGTAAGCAGTAGAGTC	NED-CTGTCCTCCACTCCAGATCATTCCT-MGBNFQ	Bruder et al. 2010/ Carolan et al. 2014
CCL20	ATGTGCAGTAGCAAGAATTTGCTC	TTACATCTTCTTGACTCTATGGCTGAGGA	--	Qin et al. 2013
CCR6	CAGGTCACACGACAGCTAAC	TCACATGGTGAAGGACGA	--	Qin et al. 2013
CMAH	GGATTCCTGGGACTTTG	ATCCTGGATAAAAGAGTACTT	--	Erins Test Run
FOX1	GGTTATGCCGCATACCGCTAC	GGAGCAAGTGTGTGGTGGTAG	TGCCGCTGCCGCTGCCTA	Bruder et al. 2010
GAPDH	TGCGGCCAAGGCAGTAG	AGGCCATGCCAGTGAGCTT	--	Erins Test Run
GAPDH	TTGCTGACAATCTTGAGGGAGTT	CTGCTGATGCCCCCATGT	TCATACTTCTCATGGTTCACACCCATCACG	Nakata et al. 2009
Granzyme A	GGATCCTCCCTCTCCCTAAGAA	CCCAGCCTGCAACTTGACA	VIC-ATGATGTCAAACCCGAAAC-MGBNFQ	Carolan et al. 2014
HPRT	CACTGGGAAAACAATGCAGA	ACAAAGTCAGGTTTATAGCCAACA	--	Erins Test Run
IFN-g	TGGTGGGCCTCTTTTCTTAGATAT	AGAAGGAGACAATTTGGCTTTGA	TTGAAGAACTGGAGAGAGGAGAGTGACAAAAAAA	Nakata et al. 2009
IFNa	TCCATCCTGAGGAACTACTTCCAG	AGGCACAAGGGCTGTATTGC	6FAM-GAATCTCCCTCTATCTGC-MGBNFQ	Carolan et al. 2014
IFNb	ATATTTCTCCACCACGGTTCTTG	ACTCCACACTGCTGCTGCTTAG	VIC-AACTATAACTTACTTCGATTCCA-MGBNFQ	Carolan et al. 2014
IFNg	AACTGGAGAGAGGAGAGTGACAAAA	GTCTTCCTTGATGGTATCCATGC	VIC-TCTCCTTCTACTTGAAACTGT-MGBNFQ	Erins Test Run/ Carolan et al. 2014
IL-10	GCTGCGGCGCTGTCA	CTCCACCGCCTTGCTCTTAT	VIC-CGATTTCTGCCCTGTGAG-MGBNFQ	Erins Test Run/ Carolan et al. 2014
IL-10	CGAGAACCACGACCCAGAA	CCGCAGGGTCTTCAGCTTT	TCAAGGAGCACGTGAACTCGCTGG	Nakata et al. 2009
IL-12p40	GGTGCTATTCACAAGCTCAAGTATG	GGTTTGATGATGTCCCTGATGA	VIC- TACACCAGCAGCTTC-MGBNFQ	Erins Test Run / Carolan et al. 2014
IL-17	GGACGGTAAACTACCACATGAACTC	AGACTCCCTTCGCAGAACCA	VIC-TCCCCATCCAGCAAGA-MGBNFQ	Carolan et al. 2014
IL-1a	CTGAAACCTCAAAGACATCTCATCTT	GCTGGCTGCCACCATCA	FAM-CCTTCAAGGAGGATGTG- MGBNFQ	Carolan et al. 2014
IL-2	GTTAAAAATTATGAGAGCCCCAGGA	TTGAGTTCTTCTGCTAGACATTGAAGA	FAM-CTACATGCCCAAGAAG-MGBNFQ	Carolan et al. 2014
IL-4	CCAACAGATTGCTCAGAGGACTT	CACCGAACAGGTCATGTTTGC	6FAM-CAGGAACCTCAGGAACAT-MGBNFQ	Carolan et al. 2014
IL-6	GCAGAGAACAACCTAAATCTTCCAA	TGATTGAATTGAGACTGGAAGCA	6FAM-CTGGCAGAAGAGGAC-MGBNFQ	Erins Test Run/ Carolan et al. 2014
IL-6	CAAGTGGCTGAAACACGTAACAA	GGCTGAACTGCAGGAAATCC	TCACCTCATCCTACGGAGCCTTG	Nakata et al. 2009
IL-8	GGCACCTTGCATCAACATGA	AAGCAGGAAAACTGCCAAGAGA	6FAM-TTCCAAGCTGGCTGTTG-MGBNFQ	Erins Test Run/ Carolan et al. 2014
L32	TGGTTATAGGAGCAACAAGAAA	GCACATCAGCAGCACTTCA	NED-TGTTGCCCAGTGGCTTCTGG-MGBNFQ	Erins Test Run/ Carolan et al. 2014
MCP1 (CCL2)	GCAGCAAGTGTCCCAAAGAAG	GACTGGGGTCAGCGCAGAT	FAM-ATCCTCAAGACATTCCT-MGBNFQ	Erins Test Run/ Carolan et al. 2014
SFTPC	GCATCGCAGTGTATGACTATCAG	AGAGCCTCAAGACTTGGGATG	CTCCTGATTGCCTATAAGCCAGCCC	Bruder et al. 2010
TNF-a	ATGTTGTAGCAAACCCTGAAGCT	ATTGGCCAGGAGGGCATT	ACTCCAATGGCTGAGCCGACGTG	Nakata et al. 2009
TNFa	TGCCATCAGACGGGCTGTA	ACATCCTCGGCCCTTGAAG	6FAM-GAATCTCCCTCTATCTGC-MGBNFQ	Erins Test Run/ Carolan et al. 2014

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL-1a	IL-1a	IL-17	IL-17	CXCL11	CXCL11	HSPa4	HSPa4	Granenzyme A	Granenzyme A	CRP	CRP
B	IL-1β	IL-1β	TNF	TNF	CCL20	CCL20	HSP90AB1	HSP90AB1	CSF1	CSF1	CASP3	CASP3
C	IL-2	IL-2	TNF-α	TNF-α	CXCR5	CXCR5	HSPB1	HSPB1	CSF2	CSF2	CASP8	CASP8
D	IL-4	IL-4	IFN-α	IFN-α	CCR6	CCR6	TLR2	TLR2	CSF3	CSF3	L32	L32
E	IL-6	IL-6	IFN-β	IFN-β	CD80	CD80	TLR3	TLR3	PTGS2	PTGS2	HPRT	HPRT
F	IL-8	IL-8	IFN-γ	IFN-γ	CD86	CD86	NFKB1	NFKB1	SLP1	SLP1	GAPDH	GAPDH
G	IL-10	IL-10	MCP1 (CCL2)	MCP1 (CCL2)	TGFβ1	TGFβ1	FOXp3	FOXp3	gDNA	gDNA	RQ1	RQ2
H	IL-12p40	IL-12p40	CCL5	CCL5	TGFβ2	TGFβ2	MIF	MIF	RT	PCR	PCR	PCR

= Primer we designed for pilot study

= Primer from the literature

= Primer designed by TFS

= Housekeeping genes (also from literature

= required RT-PCR controls

Figure 3. Custom RT-PCR plate layout to assess mRNA transcripts for 41 target inflammatory mediator genes in circulating leukocytes. This assay will assess circulating leukocyte mRNA from both sham uninjured controls and injured ferrets. Peach boxes represent custom primers designed and already tested by our laboratory. Blue highlighted boxes represent primers reported in primary research articles. Yellow highlighted boxes indicate primers designed by Thermofisher Scientific (TFS; see **Table 2** for more information). Pink boxes represent housekeeping genes previously used in the literature. Green highlighted boxes represent other required RT-PCR controls.

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

NMRC:
Post-doc, research assistant and veterinary personnel involved with this project received training to learn and optimize all surgical and animal handling techniques, which led to acquisition of new skills and knowledge.

UVA:
All research personnel participating on this program have received training on working with ferret as a laboratory animal.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of

these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are planning on initiating the study experiments and generate data that will be presented at conferences and published in peer review journals.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*

- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

There are no changes in approach.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The timeline for execution of animal experiments was delayed due to the introduction of a new species and new skill sets that required longer than anticipated preparation time and training. We plan to catch up on the timeline by increase the monthly experiment throughput and do not foreseen any delays of the overall schedule at this point.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

There are no impacts on expenditures.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use of biohazards and/or select agents

Nothing to report

there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation);*

status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*

- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

NMRC:
Name: Anke Scultetus, MD
Project Role: NMRC Site PI
Nearest person month worked: 1
Contribution to Project: Oversight of NMRC physiology studies

Name: Stephen Ahlers, PhD
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Scientific expertise on TBI

Name: Francoise Arnaud, PhD
Project Role: Senior Scientist
Nearest person month worked: 2
Contribution to Project: Oversight of laboratory analyses

Kapinga Ngalula, PhD
Project Role: Post-doc
Nearest person month worked: 2
Contribution to Project: animal experiments

Name: Melissa Mehalick, PhD
Project Role: Scientist
Nearest person month worked: 2
Contribution to Project: statistical analysis

Name: Noemy Carballo
Project Role: Senior Research Assistant
Nearest person month worked: 2

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

USU:
Name: Randy Bell, MD
Project Role: PI
Nearest person month worked: 1
Contribution to Project: Principal Investigator

Name: Thomas Davis, PhD
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Oversight of molecular analyses studies

Name: Cassie Gould, PhD
Project Role: Post doc
Nearest person month worked: 1
Contribution to Project: Develops, implements and conducts molecular investigations to assess the effects of traumatic brain injury on systemic and neuroinflammation investigating teams at the other two partnering sites.

UVA:

Name: James R. Stone, MD, PhD
Project Role: UVA Site PI
Nearest person month worked: 1
Contribution to Project: Oversight and support of experimental studies

Name: Miles Lankford

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Individualized Medicine in a Gyrencephalic Model of TBI Polytrauma Through the Continuum of Care.

DM180181 JPC-6 Precision Trauma Care Research Award

PI: CDR Randy S. Bell, MD, MC, USN **Org:** Dept. of Surgery, USU & WRNMMC **Award Amount:** $4.1M

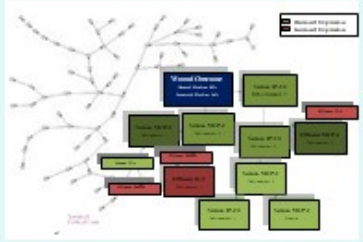
Study/Product Aim(s)

In a gyrencephalic ferret model of combat trauma (traumatic brain injury [TBI] + hemorrhagic shock [HS]), we will assess individual response(s) to trauma, aeromedical evacuation (AE), and selected promising therapies.

Approach

Ferrets will be instrumented and undergo closed head TBI + hemorrhagic shock. Outcome measures will include hemodynamics, blood chemistry, trauma biomarkers, coagulation profiles, etc. Natural history will be assessed with parallel longitudinal imaging studies of un-instrumented animals. Three phases will respectively characterize individual response(s) to:

- Trauma - TBI + HS (Phase 1).
- Simulated AE (hypobaria) after trauma (Phase 2).
- Selected promising therapeutic intervention(s) (Phase 3).



Bayesian Belief Network Analysis of Wound Outcome

Multiplex assays, neuroimaging & data analysis

Change in clinical practice

En Route Care

Timeline and Cost

Activities CY	1	2	3
IACUC Approvals, Purchasing, Set-up			
Phase 1: Characterize individual response(s) to TBI/Polytrauma			
Phase 2: Characterize individual response(s) to aeromedical evacuation			
Phase 3: Characterize individual response(s) to selected therapies			
Prepare/submit Completion Reports			
Estimated Budget ($K)	**1.247 M**	**1.337 M**	**1.5M**

Updated: November 20, 2019

Goals/Milestones

CY1 Goal – Regulatory approvals and setup; Phase 1

√ IACUC approvals, purchasing supplies/equipment

☐ Complete Phase 1 experiments

☐ Prepare/submit Completion Report & manuscript for publication

CY2 Goal – Phase 2

☐ Complete Phase 2 experiments

☐ Prepare/submit Completion Report & manuscript for publication

CY20 Goal – Phase 3; Complete study

☐ Complete Phase 3 experiments

☐ Prepare/submit Final Report and manuscript for publication

Comments/Challenges/Issues/Concerns

Budget Expenditure to Date

Actual Expenditure:
$852,444.90