DEPARTMENT OF THE AIR FORCE
59TH MEDICAL WING (AETC)
JOINT BASE SAN ANTONIO - LACKLAND TEXAS

8 DEC 2016

MEMORANDUM FOR SGVT
ATTN: CAPT BRITTANY LENZ

FROM: 59 MDW/SGVU

SUBJECT: Professional Presentation Approval

1. Your paper, entitled **HLA-B Sequencing in Patients with Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis** presented at/published to **AAD Annual Meeting, Orlando, FL, 3-7 March 2017** in accordance with MDWI 41-108, has been approved and assigned local file #**16399.**

2. Pertinent biographic information (name of author(s), title, etc.) has been entered into our computer file. Please advise us (by phone or mail) that your presentation was given. At that time, we will need the date (month, day and year) along with the location of your presentation. It is important to update this information so that we can provide quality support for you, your department, and the Medical Center commander. This information is used to document the scholarly activities of our professional staff and students, which is an essential component of Wilford Hall Ambulatory Surgical Center (WHASC) internship and residency programs.

3. Please know that if you are a Graduate Health Sciences Education student and your department has told you they cannot fund your publication, the 59th Clinical Research Division may pay for your basic journal publishing charges (to include costs for tables and black and white photos). We cannot pay for reprints. If you are 59 MDW staff member, we can forward your request for funds to the designated wing POC.

4. Congratulations, and thank you for your efforts and time. Your contributions are vital to the medical mission. We look forward to assisting you in your future publication/presentation efforts.

PAUL T. BARNICOTT, GS-15-DAF
Deputy Director, Clinical Research Division

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### INSTRUCTIONS

1. The author must complete page two of the 59 MDW Form 3039 (this form):

   a) In Section 2, add the funding source for your study [e.g., 59 MDW CRD Graduate Health Sciences Education (GHSC) [SG5 O&M]; SG5 R&D; Tri-Service Nursing Research Program (TSNRP); Defense Medical Research & Development Program (DMRDP); NIH; Congressionally Directed Medical Research Program (CDMRP); Grants; etc.]

2. Print your name, rank/grade, sign and date the form in the author's signature block or use electronic signature.

3. Attach a copy of the 59th MDW IRB or IACUC approval letter for the research related study. If this is a technical publication/ presentation, state the type (e.g., case report, QA/QI study, program evaluation study, informational report/briefing, etc.) in the "Protocol Title" box of the 59 MDW Form 3039.

4. Attach a copy of your abstract, paper, poster and other supporting documentation.

5. Save and forward, via email, the processing form and all supporting documentation to your Unit Commander, Program Director or immediate supervisor for review/approval.

6. On page 2, have either your Unit Commander, Program Director or immediate supervisor:

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7. Contact the 59th CRD/Publications and Presentations Section at (292-7141) for instructions for submitting the request form.

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**NOTE:** All abstracts, papers, posters, etc., should contain the following disclaimer statement:

***"The views expressed are those of the [author(s)] [presenter(s)] and do not reflect the official views or policy of the Department of Defense or its Components."***

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***"The voluntary, fully informed consent of the subjects used in this research was obtained as required by 32 CFR 219 and DoDI 3216.02_AFI 40-402, Protection of Human Subjects in Biomedical and Behavioral Research ."***

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***"The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication No. 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966, as amended."***

## PROCESSING OF PROFESSIONAL MEDICAL RESEARCH PUBLICATIONS/PRESENTATIONS

| TO: | FROM: Author's Name, Rank, Grade, Office Symbol | PROTOCOL NUMBER: |
|---|---|---|
| Clinical Research Division/SGVU<br>(59 MDW/SGVU) | BRITTANY LENZ, CAPT, O-3, 59 TRS/SGVT | |

**PROTOCOL TITLE** - ***[NOTE: For each new release of medical research or technical information as a publication/presentation, a new 59 MDW Form 3039 must be submitted for review and approval.]***

**1. TITLE OF MATERIAL TO BE PUBLISHED OR PRESENTED**
HLA-B SEQUENCING IN PATIENTS WITH STEVENS-JOHNSON SYNDROME AND TOXIC EPIDERMAL NECROLYSIS

**2. FUNDING RECEIVED FOR THIS STUDY?** ☐ YES ☒ NO **FUNDING SOURCE:**

**3. IS THIS MATERIAL CLASSIFIED?** ☐ YES ☒ NO

**4. IS THIS MATERIAL SUBJECT TO ANY LEGAL RESTRICTIONS FOR PUBLICATION OR PRESENTATION THROUGH A COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA), MATERIAL TRANSFER AGREEMENT (MTA), INTELLECTUAL PROPERTY RIGHTS AGREEMENT ETC.?** ☐ YES ☒ NO
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| ☒ | **POSTER (To be demonstrated at meeting/Name of Meeting, City, State, and Date of Meeting)**<br>AAD ANNUAL MEETING, ORLANDO, FL, 03-07 MARCH 2017 |
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**6. WHAT IS THE EXPECTED DATE YOUR PRESENTATION/PUBLICATION WILL BE SUBMITTED TO THE DEFENSE TECHNICAL INFORMATION CENTER (DTIC)?**

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| 7. WHO IS THE PRIMARY 59 MDW POINT OF CONTACT? (Last, First, MI.) (Include email) | DUTY PHONE/PAGER No. |
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| LENZ, BRITTANY L.<br>brittany.lenz@us.af.mil | 210-594-1733 |

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| d. Thomas Beachkofsky | Maj/O-4 | 59 MDSP/SGMD | |
| e. | | | |
| f. | | | |
| g. | | | |

**I CERTIFY ANY HUMAN OR ANIMAL RESEARCH RELATED STUDIES WERE APPROVED AND PERFORMED IN STRICT ACCORDANCE WITH 32 CFR 219, AFMAN 40-401_IP AND 59 MDWI 41-108. I HAVE READ THE FINAL VERSION OF THE ATTACHED MATERIAL AND CERTIFY THAT IT IS AN ACCURATE MANUSCRIPT FOR PUBLICATION AND/OR PRESENTATION.**

| AUTHOR'S PRINTED NAME/RANK/GRADE | AUTHOR'S SIGNATURE | DATE |
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| **APPROVING AUTHORITY'S PRINTED NAME, RANK, TITLE** | **APPROVING AUTHORITY'S SIGNATURE** | **DATE** |
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**1st INDORSEMENT (SGVU Use Only)**

| TO: Clinical Research Division (59 MDW/SGVU) (Contact 292-7141 for email instructions) | 1. DATE RECEIVED: Nov 17, 2016 | 2. ASSIGNED PROCESSING REQUEST FILE NUMBER: 16399 |
|---|---|---|
| 3. DATE REVIEWED: Dec 6, 2016 | | 4. DATE FORWARDED TO PA: |

**5. AUTHOR CONTACTED FOR RECOMMENDED OR NECESSARY CHANGES**

☐ NO ☒ YES If yes give date: Nov 17, 2016 ☐ N/A

**6. COMMENTS**

☒ APPROVED ☐ DISAPPROVED

IRB approved retrospective poster with appropriate disclaimers

| PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER | DATE | SIGNATURE OF REVIEWER |
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|---|---|---|

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|---|---|---|
| Kevin Iinuma, SSgt/E-5, 59 MDW Public Affairs | Dec 8, 2016 | IINUMA.KEVIN.MITSUGU.1296227613 Digitally signed by IINUMA.KEVIN.MITSUGU.1296227613 DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USAF, cn=IINUMA.KEVIN.MITSUGU.1296227613 Date: 2016.12.08 07:37:03 -06'00' |

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|---|---|---|
| | | |

# HLA-B Sequencing in Patients with Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

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**Patrick J. Brown, MD, and Thomas M. Beachkofsky, MD**
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## Introduction

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse drug reactions (ADRs) associated with significant morbidity and mortality (Figures 1-4). Though the exact pathogenesis remains unclear, targeted genomic analysis has identified relationships with certain immunologic markers and enzymes associated with drug metabolism. HLA-B types in particular have been associated with ADRs; however to date these associations exist for only a few specific drugs and patient populations.

Given the unique presentation and ability to obtain target tissue for analysis, severe cutaneous ADRs provide an ideal target for research using precision medicine techniques. We initially sought to examine whether certain HLA-B alleles were present at an increased frequency in patients with SJS/TEN. Now through whole genome sequencing and epigenetic analysis, we aim to identify novel biomarkers for cutaneous adverse drug reactions.

## Methods

We conducted a retrospective study of SJS/TEN patients admitted to the San Antonio Military Medical Center (SAMMC) Burn Unit between 2001 and 2015. Targeted sequencing of the HLA-B gene was performed on 28 formalin-fixed paraffin-embedded (FFPE) skin biopsy samples from cases with a known offending drug. Typically, HLA-B gene alleles are determined by sequencing Exons 2 and 3 of the gene, using primers that anneal in a non-variable region of the introns. This was not possible with FFPE samples due to fragmentation of DNA; therefore we used commercial primers designed from the least variable areas of the exons possible.

Using the Sanger sequencing method, we identified the potential HLA-B alleles present in the FFPE samples and evaluated for an association with the offending drug (Table 1).

We would like to thank the San Antonio Military Medical Center Burn Unit, Wilford Hall Ambulatory Surgical Center, and the 59th MDW Clinical Research Division for their contributions to this project.



## Clinical Presentation

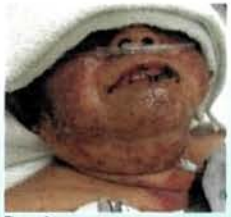
Figure 1

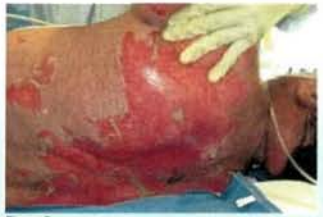
Figure 2

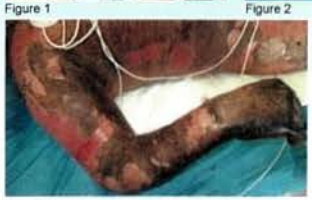
Figure 3


Figure 4

## HLA-B Sequencing

| Specimen # | Causative agent | Ethnicity | ALLELE 1 | ALLELE 2 |
|---|---|---|---|---|
| 1 | Bactrim | White | B*07 or B*44 | B*13 or B*44 |
| 2 | Bactrim | White | B*07, B*15, B*27, B*44, B*51, or B*53 | |
| 3 | Bactrim | Mexican | B*07 | B*08, B*38,B*42, B*44, or B*55 B*41, |
| 4 | Bactrim | Black | B*53 | B*07, B*15, or B*44 |
| 5 | Bactrim | Other | Unable to determine alleles | |
| 6 | Bactrim | Other | B*40:249 | B*37:11 |
| 7 | Bactrim | White | 51:197 | B*08:129 |
| 8 | Bactrim | Other | B*07, B*15, B*27, B*44, B*51, or B*53 | |
| 9 | Bactrim | Black | Unable to determine alleles | |
| 10 | Bactrim | White | Unable to determine alleles | |
| | | | | |
| 11 | Allopurinol | Other | B*15:10 | B*13 or B*44 |
| 12 | Allopurinol | Hispanic | B*51 | B*58:08 |
| 13 | Allopurinol | White | B*13 or B*42 | B*7 or B*44 |
| | | | | |
| 14 | Amikacin | White | Unable to determine alleles | |
| | | | | |
| 15 | Amoxicillin | Black | B*51 | |
| | | | | |
| 16 | Azithromycin | Unlisted | B*08, B*35, B*41, B*42, B*44 | |

| Specimen # | Causative agent | Ethnicity | ALLELE 1 | ALLELE 2 |
|---|---|---|---|---|
| 17 | Ceftriaxone | Unlisted | B*07 or B*81 | B*58 |
| 18 | Ceftriaxone | Hispanic | B*35 | B*51 or B*53 |
| | | | | |
| 19 | Ciprofloxacin | Other | B*53 | B*41 or B*51 |
| | | | | |
| 20 | Cotrimoxazole | Black | B*07, B*35, or B*58 | B*07, B*42, B*58, B*81 or B*83 |
| | | | | |
| 21 | Diclofenac | White | B*27, B*35, B*40, B*48, B*51, or B*58 | |
| | | | | |
| 22 | Phenytoin | White | B*7 | B*44, B*41, or B*53 |
| | | | | |
| 23 | Dapsone | White | B*35, 39 | B*35 |
| | | | | |
| 24 | Ibuprofen | White | B*52 | |
| | | | | |
| 25 | Lamotrigine | White | Unable to determine alleles | |
| 26 | Lamotrigine | White | B*35 | |
| | | | | |
| 27 | Levofloxacin | White | B*40:06 | |
| | | | | |
| 28 | Voriconazole | White | B*08 or B*35 | |

Table 1

## Results

Multiple potential HLA-B alleles were identified in most of our specimens. This highlights the limitations of DNA sequencing using FFPE samples, as the fragments of DNA and non-overlapping sequences limited our ability to determine an exact HLA type for every specimen. Based on these results, we were unable to determine statistically significant associations regarding frequency of HLA type or inciting drugs. However, our data show HLA-B*44 as a potential allele in 9 of the 28 samples, including 5 of 10 cases associated with Bactrim. This is consistent with prior reports of HLA-B*44 associated with SJS/TEN due to sulfonamides and SJS/TEN with severe ocular complications.

## Discussion & Prospective Study

Severe cutaneous ADRs remain a significant cause of morbidity and mortality in health care and are often unpredictable. To further investigate potential genetic risk factors for SJS/TEN, we designed a prospective study using whole genome sequencing and transcriptome studies to examine epigenetic changes during ADRs (see below).

| Time since enrollment | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 17 (Continued once weekly while inpatient) | Day 30-60 (Following resolution of initial skin lesion or hospital discharge) |
|---|---|---|---|---|---|---|---|---|
| Peripheral blood for transcriptome analysis | X | X | X | X | X | X | X | X |
| Skin biopsy for transcriptome analysis | X | | | | | | | X |
| Peripheral blood for whole genome sequencing | X | | | | | | | |

We are currently enrolling patients at SAMMC through 2018. A secondary goal of this study is the establishment of a tissue repository at the Collaborative Health Initiative Research Program at the Uniformed Services University to aid in future studies examining adverse drug reactions.

**References**

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