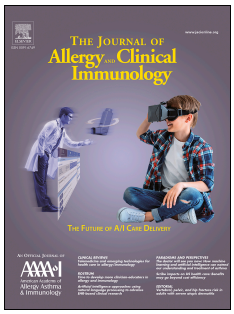
Journal Pre-proof



Cross-reactivity between vancomycin, teicoplanin and telavancin in HLA-A*32:01 positive vancomycin DRESS patients sharing an HLA-Class II haplotype

Nontaya Nakkam, PhD, Andrew Gibson, PhD, Effie Mouhtouris, BSc, Katherine Konvinse, PhD, Natasha Holmes, MBBS, PhD, Kyra Y. Chua, MBBS, PhD, Pooja Deshpande, PhD, Danmeng Li, MD, David A. Ostrov, PhD, Jason Trubiano, MBBS, PhD, Elizabeth J. Phillips, MD

PII: S0091-6749(20)30687-4

DOI: https://doi.org/10.1016/j.jaci.2020.04.056

Reference: YMAI 14571

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 9 March 2020

Revised Date: 30 April 2020

Accepted Date: 30 April 2020

Please cite this article as: Nakkam N, Gibson A, Mouhtouris E, Konvinse K, Holmes N, Chua KY, Deshpande P, Li D, Ostrov DA, Trubiano J, Phillips EJ, Cross-reactivity between vancomycin, teicoplanin and telavancin in HLA-A*32:01 positive vancomycin DRESS patients sharing an HLA-Class II haplotype, *Journal of Allergy and Clinical Immunology* (2020), doi: https://doi.org/10.1016/j.jaci.2020.04.056.

Title: Cross-reactivity between vancomycin, teicoplanin and telavancin in HLA-A*32:01 positive vancomycin DRESS patients sharing an HLA-Class II haplotype

Authors:

Nontaya Nakkam, PhD[1,2], Andrew Gibson, PhD[3], Effie Mouhtouris, BSc[4], Katherine Konvinse, PhD[2], Natasha Holmes, MBBS, PhD[4], Kyra Y. Chua, MBBS, PhD[4], Pooja Deshpande, PhD[3,5], Danmeng Li, MD[6], David A. Ostrov, PhD[6], Jason Trubiano, MBBS, PhD[4,7], Elizabeth J. Phillips, MD[2,3]

[1]Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; [2]Vanderbilt University Medical Center, Nashville, USA; [3]Institute for Immunology and Infectious Diseases, Murdoch University, Perth, Australia; [4]Department of Infectious Diseases, Austin Health, University of Melbourne, Melbourne, Australia; [5]School of Human Sciences, University of Western Australia, Western Australia, Australia; [6]Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, USA; [7]Peter McCallum Cancer Centre, Melbourne, Australia

Corresponding author:

Dr. Elizabeth J. Phillips

1161 21st Avenue S, A2200 MCN

Nashville, TN 37232

Email: elizabeth.j.phillips@vumc.org

Phone: +1 (615) 322-9174; Fax: (615) 343-6160

Journal: *The Journal of Allergy and Clinical Immunology* (JACI)

Article Type: A Letter to the Editor

Current Word Count: 1356

Sources of Funding Related to this Project: EJP receives funding from related to this National Institutes of Health (1P50GM115305-01, R21AI139021 and R34AI136815 and 1 R01 HG010863-01) and the National Health and Medical Research Council of Australia). JAT is supported by a National Health and Medical Research Council (NHMRC) postgraduate scholarship (GNT 1139902) and post graduate scholarship from the National Centre for Infections in Cancer (NCIC). KCK is supported by the National Institutes of Health (F30AI131780 and T32GM7347).

IRB: Approvals were in place at all participating sites

Conflicts of Interest: EJP and KCK hold a provisional patent for the detecton of HLA-A*32:01 in connection with determining drug reaction with eosinophilia and systemic symptoms (DRESS). The authors have no other conflicts relevant to the content of this publication.

Capsule Summary:

All fifteen patients with HLA-A*32:01 restricted vancomycin-induced DRESS, showed negative *ex vivo* responses to dalbavancin however two showed cross-reactivity to teicoplanin and telavancin. Adjunctive diagnostic testing should be considered to detect potential cross-reactivity amongst glycopeptides.

Key words:

Vancomycin-induced DRESS, HLA-A*32:01, cross-reactivity, teicoplanin, telavancin

To the Editor:

Vancomycin is a glycopeptide antibiotic used to treat resistant gram-positive infections. It is associated with a life-threatening, delayed T-cell–mediated reaction, drug reaction with eosinophilia and systemic symptoms (DRESS) presenting with fever, rash, hematological abnormalities, lymphadenopathy and organ involvement that occurs 2-6 weeks after vancomycin initiation.[1] We demonstrated that HLA-A*32:01 is strongly associated with vancomycin-induced DRESS in European populations.[2] All glycopeptide antibiotics contain a heptapeptide core structure and cross-reactivity should be considered when treating patients who have had a previous hypersensitivity reaction to vancomycin (Supplemental Figure E1).[3] Cross-reactivity remains controversial as some patients presenting with teicoplanin-induced DRESS showed subsequent tolerability to vancomycin[4-7] and patients with teicoplanin-induced DRESS confirmed by positive intradermal skin test had a negative skin test to vancomycin.[8]

To examine the immunological cross-reactivity amongst four glycopeptide antibiotics including vancomycin, teicoplanin, dalbavancin and telavancin, adults $\geq$18 years with a probable diagnosis of vancomycin DRESS defined by a corresponding Naranjo adverse drug reaction (ADR) score of $\geq$5 (probable ADR), a RegiSCAR score of $\geq$4 (probable DRESS) and who carried HLA-A*32:01, the recently described risk allele for vancomycin-induced DRESS, were recruited between January 2010 and September 2019 through drug allergy clinics and inpatient facilities at participating institutions (Vanderbilt University Medical Center in Nashville, Tennessee; Austin Health, Peter MacCallum Cancer Centre, Fiona Stanley Hospital and Royal Perth Hospital in Perth, Western Australia, Australia). All

patients provided informed consent for collection of saliva and blood to be stored as DNA and peripheral blood mononuclear cells (PBMCs).

Interferon-gamma (IFN-γ) release in response to overnight incubation with implicated drugs was performed by ELISpot assay (3420-2H; Mabtech, Stockholm, Sweden) in triplicate from thawed PBMCs (rested overnight) and included negative (unstimulated) and positive (anti-CD3 Mabtech antibody, staphylococcal enterotoxin B, and/or cytomegalovirus pp65) controls. Control PBMCs from glycopeptide unexposed HLA-A*32:01 positive and negative individuals were also used. PBMCs plated at 200,000 cells/well were incubated with vancomycin, teicoplanin, dalbavancin, telavancin and other implicated drugs at concentrations representative of maximum serum concentrations, as well as those 10-fold higher and 10-fold lower (Figure 1). A positive response was defined as more than 50 spot-forming units (SFU)/million cells after background removal as per previous definitions.[9] High-resolution 4-digit HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DR, and HLA-DQ typing was performed by using sequence-based typing with previously published protocols.[2]

Fifteen patients who met the clinical inclusion criteria for vancomycin-induced DRESS syndrome were enrolled into this study. The demographics, clinical characteristics, DRESS history are described in Supplemental Table E1 and full HLA typing of all in Supplemental Table E2.

All vancomycin DRESS cases exhibited a dose-dependent positive IFN-γ ELISpot response to vancomycin (Figure 1, Supplemental Table E3) and all had a clear negative response to both concentrations of dalbavancin (Figure 1). Three cases overall showed cross-reactivity with 3 showing a positive response to telavancin and 2 of these also demonstrating a positive IFN-γ ELISpot response to teicoplanin. One of the two patients who had positive IFN-γ ELISpot responses to vancomycin, teicoplanin and telavancin (Patient ID 15, Supplemental Table E3) was intradermally skin tested to both vancomycin and teicoplanin

and showed positive responses to both (Supplemental Figure E2), which was not seen in glycopeptide unexposed controls (n=5) and 3 patients (Patient ID 9, 10, and 11) with HLA-A*32:01 positive vancomycin DRESS who showed positive delayed intradermal testing and IFN-γ ELISpot to vancomycin but negative IFN-γ ELISpot and intradermal testing to teicoplanin. Patients ID 1,3 and 5 also tolerated ingestion challenges with medications concurrently administered at the time of vancomycin DRESS.

In samples with sufficient cell numbers, PBMCs were tested against other concurrently administered medications potentially implicated in DRESS development (Supplemental Figure E3). IFN-γ ELISpot was also performed on PBMCs from non-HLA-matched healthy donors (n=5) and a HLA-A*32:01 positive vancomycin naïve control (n=1); all exhibiting a negative response to all four drugs (data not shown).

Vancomycin is implicated in up to 40% of antibiotic-related DRESS cases.[1] The prevalence of vancomycin DRESS appears to be increasing and it is the second most common cause of DRESS overall reported to the FDA Adverse Event Reporting System (FAERS) between 1999 and 2019, https://open.fda.gov/data/faers/ (accessed March 2, 2020). HLA-A*32:01 has recently been reported as a genetic risk factor for vancomycin-induced DRESS in the European population, within which the allelic prevalence is approximately 6.8%.[2] Due to the high prevalence of the risk allele, the high incidence of vancomycin-induced DRESS and the potential cross-reactive risk with dalbavancin given its extremely long half-life of 14 days, the detection of cross-reactivity to alternative glycopeptide antibiotics is very important for reducing the risk of DRESS and providing patients with future therapeutic options. Our study is reassuring in demonstrating that 100% of vancomycin DRESS cases with IFN-γ ELISpot responses showed a negative IFN-γ ELISpot response to dalbavancin, suggesting no or very low cross-reactivity between vancomycin and dalbavancin. Dalbavancin differs from vancomycin through a structural modification of the

lipophilic side chain which enhances its binding affinity to the cell membrane and prolongs its half-life.[3] Approximately 87 % (13/15) and 73% (8/11) of vancomycin DRESS cases showed no cross-reactivity to teicoplanin and telavancin, respectively. Two vancomycin DRESS cases demonstrated immunological cross-reactivity to teicoplanin, and telavancin using ELISpot, supported by a positive intradermal skin test to teicoplanin in the one patient where this was performed. Of note, one of these two patients had a short interval between original reaction and IFN-γ ELISpot assay (Patient ID 8, Supplemental Table E1). Telavancin is a semi-synthetic derivative of vancomycin and that dalbavancin is a semi-synthetic lipoglycopeptide derived from a glycopeptide structure more similar to teicoplanin. In addition to the long lipophilic side chain of dalbavancin that extends its half-life and improves affinity for the D-Ala-D-Ala target in the bacterial cell wall, dalbavancin lacks the acetylglucosamine group of teicoplanin. Intriguingly the two patients (Patient ID 8, 15, Supplemental Table E2 & E3) with shared *ex vivo* cross-reactivity amongst vancomycin, teicoplanin and telavancin shared the same class II HLA haplotype.

To determine if vancomycin, teicoplanin and telavancin have the potential to bind class II HLA molecules in particular shared by the patients that exhibited cross-reactive specificities, we used molecular docking (AutoDock Vina). Vancomycin, teicoplanin and telavancin were predicted to bind HLA-DQ (DQA1*01:01, DQB1*05:03) with estimated ΔG values of -7.7, -7.4 and -7.2 kcal/mol, respectively whereas dalbavancin was predicted to bind only weakly (Figure 2). Teicoplanin and telavancin may hence bind class II HLA as the molecular basis for cross-reactive T cell responses in HLA-A*32:01 positive patients who have experienced vancomycin DRESS. This could suggest a new model for cross-reactivity including recognition of drug/class II HLA complexes by CD8+ T cells matured by positive selection by HLA-A*32:01 or alternatively, CD4+ T cells may recognize teicoplanin and telavancin in the context of class II HLA molecules such as DQ.

A limitation of our study is the lack of in vivo and rechallenge cross-reactivity data. We cannot be certain that the clinical phenotype of patients with prior vancomycin DRESS and *ex vivo* cross-reactivity to teicoplanin and telavancin and positive delayed intradermal skin testing in the one patient performed would also be DRESS, however, given the structural similarity of these drugs and the half-life of dalbavancin of greater than 1 week, rechallenge of these individuals would not be ethical unless the clinical need outweighed any risk.

This study is the first evidence that elucidates a risk for potential immunological cross-reactivity pattern between vancomycin, teicoplanin and newer glycopeptide antibiotics in patients with previous DRESS induced by vancomycin. The lack of apparent cross-reactivity is reassuring for dalbavancin, particularly given the long half-life of this lipoglycopeptide, however, clinicians should be aware of the low but detectable risk of cross-reactivity in particular amongst teicoplanin, telavancin and vancomycin in the HLA-A*32:01 restricted vancomycin DRESS. Our study suggests that *ex vivo* IFN-γ ELISpot assay or skin tests in combination with HLA typing could be performed to risk-stratify patients with a history of previous vancomycin DRESS for potential risk of cross-reactivity between vancomycin, teicoplanin, and telavancin to aid in making decisions for future treatment. The shared class II HLA haplotype amongst two patients with cross-reactivity between vancomycin, teicoplanin and telavancin and virtual docking of these drugs to HLA class II suggest a potential novel mechanism for cross-reactivity following sensitization that deserves further exploration.

Nontaya Nakkam, PhD[1,2], Andrew Gibson, PhD[3], Effie Mouhtouris, BSc[4], Katherine Konvinse, PhD[2], Natasha Holmes, MBBS, PhD[4], Kyra Y. Chua, MBBS, PhD[4], Pooja Deshpande, PhD[3,5], Danmeng Li, MD[6], David A. Ostrov, PhD[6], Jason Trubiano, MBBS, PhD[4,7], Elizabeth Phillips, MD[2,3]

[1]Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; [2]Vanderbilt University Medical Center, Nashville, USA; [3]Institute for Immunology and Infectious Diseases, Murdoch University, Perth, Australia; [4]Department of Infectious Diseases, Austin Health, University of Melbourne, Melbourne, Australia; [5]School of Human Sciences, University of Western Australia, Western Australia, Australia; [6]Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, USA; [7]Peter McCallum Cancer Centre, Melbourne, Australia

References

1. Blumenthal KG, Peter JG, Trubiano JA, Phillips EJ. Antibiotic allergy. Lancet. 2019;393(10167):183-98.

2. Konvinse KC, Trubiano JA, Pavlos R, James I, Shaffer CM, Bejan CA, et al. HLA-A*32:01 is strongly associated with vancomycin-induced drug reaction with eosinophilia and systemic symptoms. J Allergy Clin Immunol. 2019;144(1):183-92.

3. Van Bambeke F. Lipoglycopeptide Antibacterial Agents in Gram-Positive Infections: A Comparative Review. Drugs. 2015;75(18):2073-95.

4. Kwon HS, Chang YS, Jeong YY, Lee SM, Song WJ, Kim HB, et al. A case of hypersensitivity syndrome to both vancomycin and teicoplanin. J Korean Med Sci. 2006;21(6):1108-10.

5. Lye D, Athan E, O'Brien D. Teicoplanin hypersensitivity syndrome. Int J Antimicrob Agents. 2007;29(4):476-8.

6. Hsiao SH, Chen HH, Chou CH, Lin WL, Liu Yeh PY, Wu TJ. Teicoplanin-induced hypersensitivity syndrome with a preceding vancomycin-induced neutropenia: a case report and literature review. J Clin Pharm Ther. 2010;35(6):729-32.

7. Miyazu D, Kodama N, Yamashita D, Tanaka H, Inoue S, Imakyure O, et al. DRESS Syndrome Caused by Cross-reactivity Between Vancomycin and Subsequent Teicoplanin Administration: A Case Report. Am J Case Rep. 2016;17:625-31.

8. Ben Romdhane H, Chadli Z, Ben Fredj N, Chaabane A, Boughattas NA, Aouam K. Teicoplanin-induced DRESS syndrome: The importance of skin tests. Med Mal Infect. 2018;48(4):291-3.

9. Trubiano JA, Strautins K, Redwood AJ, Pavlos R, Konvinse KC, Aung AK, et al. The Combined Utility of Ex Vivo IFN-gamma Release Enzyme-Linked ImmunoSpot Assay and In Vivo Skin Testing in Patients with Antibiotic-Associated Severe Cutaneous Adverse Reactions. J Allergy Clin Immunol Pract. 2018;6(4):1287-96 e1.

Figure legends

Figure 1. IFN-γ release ELISpot results using PBMCs from 15 potential vancomycin DRESS patients after 20 hours of incubation with vancomycin, teicoplanin, dalbavancin, and telavancin (Telavancin stimulation was tested on 11 cases). Means of the triplicates are plotted. Error bars indicate interquartile range of the median ELISpot results from all cases after background subtraction. A positive response was defined as >50 SFU/million cells after background removal. Two patients (Patient ID 8 and 15) showed cross-reactivity between vancomycin, teicoplanin and telavancin and one patient (Patient ID 7) showed potential cross-reactivity between vancomycin and telavancin at 500 μg/mL (see Supplemental Table E3 for details).

Figure 2. Prediction of binding interactions between vancomycin, teicoplanin, telavancin and HLA-DQ. A model of HLA-DQ (DQA1*01:01 in green, DQB1*05:03 in beige) is shown. AutoDock Vina was used for molecular docking with vancomycin (red), teicoplanin (cyan), telavancin (yellow). Molecular docking of vancomycin, teicoplanin and telavancin with class II HLA molecules, HLA-DR, -DQ and -DP sequences were obtained from the HLA/IMGT database (http://www.ebi.ac.uk/ipd/imgt/hla/allele.html). Atomic homology models were generated with SWISS-MODELLER based on the most closely related crystal structures. The class II HLA complex models were then geometry minimized by using PHENIX. Vancomycin, teicoplanin and telavancin were docked into the HLA-A*32:01 model with AutoDock Vina. The scoring grid dimensions were 40 × 40 × 40 Å centered on a site corresponding to the Cα of the fifth peptide amino acid position (P5). Vancomycin was docked, with exhaustiveness set to 40. The top 9 scoring orientations were determined and compared. PyMOL was used to generate molecular graphics (PyMOL Molecular Graphics System, version 1.8; Schrödinger, Cambridge, Mass).