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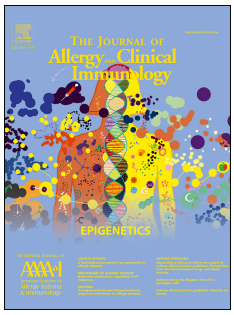

HLA-A*32:01 is strongly associated with vancomycin-induced drug reaction with eosinophilia and systemic symptoms

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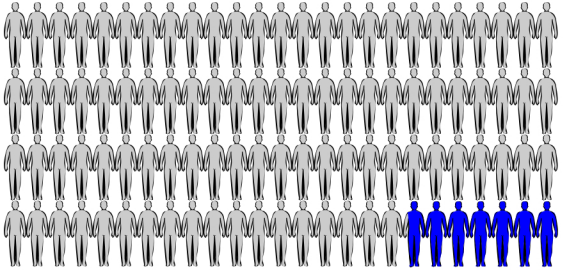
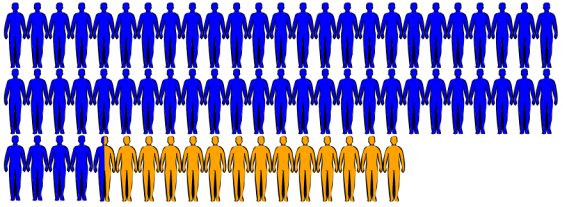
HLA-A*32:01 is strongly associated with vancomycin-induced drug reaction with eosinophilia and systemic symptoms

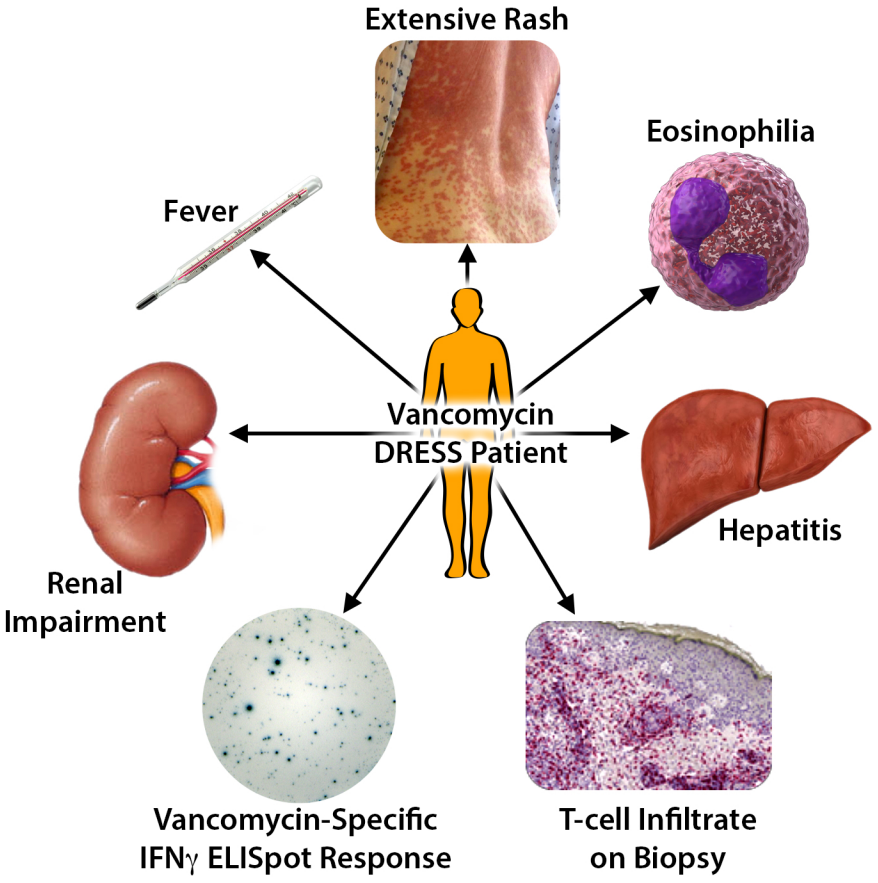
~6.8% of individuals with European ancestry carry HLA-A*32:01



~20% of HLA-A*32:01+ will develop DRESS



Therefore, ~75 would need to be tested to prevent one case



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ABSTRACT

Background

Vancomycin is a prevalent cause of the severe hypersensitivity syndrome drug reaction with eosinophilia and systemic symptoms (DRESS) which leads to significant morbidity and mortality and commonly occurs in the setting of combination antibiotic therapy which impacts future treatment choices. Variations in human leukocyte antigen (HLA) class I in particular have been associated with serious T-cell mediated adverse drug reactions which has led to preventive screening strategies for some drugs.

Objective

To determine if variation in the HLA region is associated with vancomycin-induced DRESS.

Methods

Probable vancomycin DRESS cases were matched 1:2 with tolerant controls based on sex, race, and age using BioVU, Vanderbilt's deidentified electronic health record database. Associations between DRESS and carriage of HLA class I and II alleles were assessed by conditional logistic regression. An extended sample set from BioVU was utilized to conduct a time-to-event analysis of those exposed to vancomycin with and without the identified HLA risk allele.

Results

Twenty-three individuals met inclusion criteria for vancomycin-associated DRESS. 19/23 (82.6%) cases carried HLA-A*32:01 compared to 0/46 (0%) of the matched vancomycin tolerant controls ($p=1x10^{-8}$) and 6.3% of the BioVU population (n=54,249) ($p=2x10^{-16}$). Time-to-event analysis of DRESS development during vancomycin treatment among the HLA-A*32:01 positive group indicated that 19.2% developed DRESS and did so within four weeks.

Conclusions

HLA-A*32:01 is strongly associated with vancomycin DRESS in a population of predominantly European ancestry. HLA-A*32:01 testing could improve antibiotic safety, help implicate vancomycin as the causal drug and preserve future treatment options with co-administered antibiotics.

Clinical Implications

HLA-A*32:01 testing to help preempt and implicate vancomycin as the causal drug for DRESS could improve the safety and efficacy of antibiotic treatment.

Capsule Summary

In a population of predominantly European ancestry, we show that carriage of the HLA-A*32:01 allele is strongly associated with the development of vancomycin DRESS. HLA-A*32:01 testing could increase the safety of vancomycin treatment.

Keywords: vancomycin, drug reaction with eosinophilia and systemic symptoms, human leukocyte antigen, antibiotic allergy, delayed hypersensitivity, T-cell hypersensitivity

Abbreviations: DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms), HLA (Human Leukocyte Antigen), EHR (Electronic Health Record), IRB (Institutional Review Board), PBMC (Peripheral Blood Mononuclear Cell), ELISpot (Enzyme-Linked ImmunoSpot), SEB (Staphylococcal Enterotoxin B), SFU (Spot Forming Units), Cmax (maximum serum concentration), ADR (Adverse Drug Reaction), IDT (Intradermal Testing), H&E (Hematoxylin and Eosin), IHC (Immunohistochemistry), *MRSA* (Methicillin-resistant *Staphylococcus aureus*),

MRSE (Methicillin-resistant *Staphylococcus epidermidis*), AML (Acute Myeloid Leukemia), *E. coli (Escherichia coli*), ALL (Acute Lymphoblastic Leukemia), CLL (Chronic Lymphoblastic Leukemia), *MSSA* (Methicillin-sensitive *Staphylococcus aureus*), HIV/AIDS (Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome), IM-ADR (Immune-Mediated Adverse Drug Reaction).

INTRODUCTION

Vancomycin is a widely used antibiotic of global importance for the treatment of serious, deep-seated, antibiotic-resistant Gram-positive infections which frequently require prolonged treatment courses. Worldwide the use of vancomycin is increasing because of the increasing incidence of methicillin-resistant *Staphylococcus aureus* infections. Vancomycin is associated with infusional pruritus and rash ("red man syndrome") which is managed by slow infusion and anti-histamines. However, vancomycin is also a very common cause of a life-threatening delayed T-cell mediated reaction known as drug reaction with eosinophilia and systemic symptoms (DRESS) and has been implicated in up to 40% of antibiotic-related cases[1,2]. DRESS, otherwise known as drug-induced hypersensitivity syndrome, typically develops 2-8 weeks after drug initiation and presents with features including fever, a widespread rash, facial edema, white cell abnormalities, and involvement of internal organs such as the liver, kidneys, heart and lungs[3]. The mortality of DRESS is 1-10% and long-term morbidity such as autoimmune disease has been described up to 4 years following acute disease[4,5]. When DRESS develops in the setting of combination antibiotics and other co-administered drugs, all treatment is stopped and future exposure to all concurrently-dosed drugs is contraindicated due to the associated risks of morbidity and mortality if DRESS reoccurs and the inability to implicate any one drug on clinical grounds alone. The ability to more definitively diagnose DRESS associated with vancomycin may allow patients not only to avoid the current and future risk of vancomycin exposure but also to continue or resume therapy, particularly with other falsely implicated antibiotics.

METHODS

Vancomycin DRESS cases

Retrospective patients were detected using Vanderbilt's BioVU repository, a deidentified electronic health record (EHR) database linked to a DNA biobank in operation since February 7, 2007. Prospective patients with potential vancomycin DRESS were enrolled to confirm genetic findings from the BioVU analysis using vancomycin-specific immunological studies to support clinical diagnoses. Patients were prospectively recruited between 2010 and 2018 through drug allergy clinics and inpatient facilities at participating institutions (Vanderbilt University Medical Center in Nashville (Tennessee, USA), Austin Health, Peter MacCallum Cancer Centre, and Alfred Health in Melbourne (Victoria, Australia), Fiona Stanley Hospital and Royal Perth Hospital in Perth (Western Australia, Australia)). Institutional review board (IRB) approvals were in place for the BioVU study and for all sites contributing to the prospective study. All aspects of the study including the collection and storage of DNA, plasma, peripheral blood mononuclear cells (PBMCs) and skin were IRB-approved and all patients provided written or electronic informed consent. Saliva and blood were routinely collected from prospective patients, processed and stored as repositories of DNA, PBMCs and plasma. Patients >17 years of age who were diagnosed with DRESS with vancomycin identified as a primary implicated drug, a corresponding Naranjo adverse drug reaction score of ≥5 (probable adverse drug reaction), a RegiSCAR score of ≥4 (probable DRESS) and available DNA or genotyping were included in the study[6,7]. Any potential duplicate cases between the prospective patients and the BioVU cohort were eliminated by an observer blinded to identifiable patient information.

Vancomycin tolerant controls

Controls from the BioVU genotyped cohort (n=54,249) were defined as individuals who tolerated intravenous vancomycin for greater than 5 weeks and had at least five vancomycin therapeutic trough levels over the treatment period recorded in the Vanderbilt EHR. 297/54,249 individuals were prescribed at least 5 weeks of vancomycin treatment. Using this subset, controls were matched 2:1 with cases on sex, race and age within five years. Vancomycin tolerance and length of treatment was verified by manual review of the EHR by a reviewer blinded to the HLA results. Additional controls for vancomycin Enzyme-Linked ImmunoSpot (ELISpot) assays were recruited from our Vanderbilt IRB-approved studies to investigate drug responses in individuals with a broad range of immune-mediated adverse drug reactions and healthy volunteers.

Human leukocyte antigen (HLA) typing

High resolution four-digit HLA A B C DP DR DQ typing was performed using either sequence-based typing on 454FLX or Illumina Miseq[8,9] or imputed from SNP data from HumanExome BeadChip and GWAS platforms by Expanded Multi-Ethnic Genotyping Array (MEGA^{EX}, Illumina), HumanOmni-Quad, HumanOmni5-Quad and Human660W-Quad using SNP2HLA as previously described[10]. Imputation for HLA-A*32:01 using SNP2HLA has a reported accuracy of 99.46%[10]. Associations between DRESS and carriage of HLA-A/B/C/DRB1/DQA1/DQB1/DPB1 alleles at the 4-digit level were assessed by conditional logistic regression to accommodate the matching. Analyses were carried out in R version 3.4.3. (R Core Team (2017)). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

Enzyme-Linked ImmunoSpot (ELISpot) assays

Overnight IFN-γ ELISpot assays were performed in triplicate (Mabtech Kit 3420-2H) as previously described[11-13] using negative (unstimulated) and positive (anti-CD3 Mabtech antibody and/or Staphylococcal enterotoxin B (SEB)) controls. PBMCs plated at 200,000 cells per well were incubated with pharmacy stock vancomycin and other implicated drugs at concentrations representative of peak serum concentrations (Cmax) as well as 10-fold higher and 10-fold lower than Cmax. As supported by consensus in the literature, a positive response was defined as >50 spot forming units (SFU)/million cells after background removal[11-13]. Figures representing ELISpot results were generated using GraphPad Prism 7.0a Macintosh Version, GraphPad Software, La Jolla California USA, www.graphpad.com.

Time-to-Event Analysis of Vancomycin-Exposed BioVU Cohort

In the BioVU cohort of 54,249 patients with available genotyping, we identified 137 patients that were HLA-A*32:01 positive and 1,672 who did not carry HLA-A*32:01 and for whom at least two weeks of intravenous vancomycin treatment was intended. 137 of the 1,672 HLA-A*32:01 negative individuals were randomly selected to serve as an equal-sized control group. The deidentified EHRs of the 274 patients in both sub-cohorts were reviewed during the period of vancomycin exposure to determine patient sex, race, age, longest treatment period, development of an adverse drug reaction (ADR) and specifically, the development of possible DRESS. Since DRESS is an immune-mediated reaction and vancomycin is renally cleared, patient immunosuppression, chronic renal failure and end stage renal failure with dialysis were documented as potential covariates. ADR latency, defined as the length of time from initiation of vancomycin to symptom onset, as well as any concurrent antimicrobials were documented.

Analyses were carried out by Fisher's exact tests, logistic and Cox regression as appropriate in R version 3.4.3.

Skin Testing and Histopathology

Intradermal skin testing (IDT) with 0.05, 0.5, 5 and 50 mg/mL of sterile pharmacy grade vancomycin was performed with readings at 20 minutes, 24 and 48 hours on two subjects: patient 18, a prospectively enrolled patient who had experienced probable vancomycin DRESS 6.5 months earlier and C50, an HLA-A*32:01 positive, vancomycin-naïve healthy control. For patient 18, histopathology was examined from the acute DRESS reaction and from a biopsy of the positive 5 mg/ml vancomycin delayed IDT. Formalin-fixed, paraffin-embedded skin biopsies were sectioned at 5 μm intervals. Slides were deparaffinized and stained with hematoxylin and eosin (H&E). For the immunohistochemistry (IHC), slides were placed on the Leica Bond Max IHC stainer and deparaffinized. Slides were incubated with anti-FOXP3 (Cat.14-4777-82, eBioscience, Inc.) for one hour at a 1:100 dilution, Ready-To-Use anti-CD4 (PA0427, Leica) for one hour, or Ready-To-Use anti-CD8 (MM39-10, StatLab) for 15 mins. The Bond Polymer Refine detection system was used for visualization. A dermatopathologist scored all slides.

*Molecular Docking of Vancomycin with HLA-A*32:01*

Sequences of HLA-A*32:01 and HLA-A*29:02 were obtained from the HLA/IGMT database (http://www.ebi.ac.uk/ipd/imgt/hla/allele.html). An atomic homology model for HLA-A*32:01 was generated with SWISS-MODELLER[14] based on the most closely related crystal structure, PDB 6EI2, 92 % identical. To generate a peptide/HLA-A*32:01 complex model, the peptide from the crystal structure of 6EI2 was positioned into the antigen binding cleft of the HLA-

A*32:01 model using SSM in the COOT program package, then mutated to RLYGKSLYSF, a peptide eluted from HLA-A*32:01[15]. The peptide/HLA-A*32:01 complex model was then geometry minimized using PHENIX[16].

Vancomycin was docked into the HLA-A*32:01 model with AutoDock Vina[17]. The scoring grid dimensions were 40 × 40 × 40 Å, centered on a site corresponding to the Cα of the fifth peptide amino acid (P5). Vancomycin was docked with exhaustiveness set to 40. The top nine scoring orientations were output and compared. PyMol was used to generate molecular graphics (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.).

RESULTS

Baseline demographics

Twenty-three individuals were identified that met inclusion criteria for clinically diagnosed vancomycin-associated DRESS in our study including 15 prospectively recruited patients (7 from Vanderbilt University Medical Center in Nashville, 5 from Melbourne, and 3 from Perth) and 8 retrospectively ascertained individuals from Vanderbilt's BioVU repository (Tables 1 and S1). The patient cohort was primarily of European ancestry and included 9 women and 14 men from 17 to 76 years of age who developed DRESS between 2004 and 2018. Only one patient was identified by a blinded observer that overlapped between the Vanderbilt retrospectively identified BioVU cohort and the prospectively collected patients and the duplicate was eliminated. All patients had Naranjo adverse drug reaction scores of 8 to 10 (probable or definite adverse drug reaction) and RegiSCAR scores of 4 to 7 (probable or definite DRESS). 21/23 (91%) patients were being treated with other antibiotics concurrently with vancomycin. The median latency period from vancomycin initiation to the first symptoms of DRESS was 21 days (mean, 22.9 days; range, 14 – 50 days) (Table 1). Age, race and sex matching was successful (Table S2) and indications for vancomycin treatment were similar between cases and controls (Tables 1 and S3). Similar to the DRESS cases who had a median vancomycin trough of 16 μg/mL (mean, 17.2 μg/mL; range, 3 – 44 μg/mL; n = 116), the tolerant controls had a median vancomycin trough of 18 μg/mL (mean, 19.6 μg/mL; range, 2 – 86 μg/mL; n = 644).

HLA Associations with DRESS

341 The HLA-A*32:01 allele was carried by 19/23 (86%) DRESS cases compared with 0/46 (0%) of
342 the matched vancomycin tolerant controls ($p=1x10^{-8}$, conditional logistic with Bonferroni
343 multiple

comparisons correction) (Tables 1, S3, S4 and S5 and Figure 1A). After adjusting for HLA-A*32:01 carriage, no other alleles were significant (p=0.13). In a larger BioVU cohort of DNA samples from 54,249 Vanderbilt patients, the HLA-A*32:01 allele carriage rate was 6.30% which matches the carriage rate in other cohorts of predominant European ancestry[18,19]. Carriage of HLA-A*32:01 in our BioVU cohort (n= 54,249) was more prevalent in European Americans (6.78%) than African American populations (2.78%).

IFN-γ ELISpot Responses in DRESS

IFN-γ ELISpot assays were performed on all prospectively enrolled cases for which cryopreserved PBMCs were available (14/15). PBMCs from 12/14 (86%) DRESS cases had a positive IFN-γ ELISpot response to vancomycin (Figure 1B). Analyses restricted to immunologically confirmed cases and matched controls, revealed that 11/12 (92%) IFN-γ ELISpot positive patients carried HLA-A*32:01 compared with 0/24 (0%) of the matched controls ($p=9x10^{-7}$, conditional logistic) (Figure 1A). Three IFN-γ ELISpot positive patients had multiple blood draws at time points distant from the initial reaction with repeat positive results (Figure S1). In Patient 4, a positive IFN-γ ELISpot to vancomycin was demonstrated 9 years after the initial DRESS reaction. In samples with sufficient cell numbers, PBMCs were routinely tested against all other concurrently administered medications potentially implicated in DRESS development (Figure S2). Notably, patient 21, one of the two patients with a negative vancomycin IFN-γ ELISpot, is HLA-A*32:01 negative and demonstrated a reproducible positive IFN-γ ELISpot to rifampin stimulation leading us to conclude that her DRESS syndrome was associated with rifampin. Thirteen controls were tested concurrently with cases and none demonstrated a positive vancomycin IFN-γ ELISpot (Figure 1B and Table S6).

Time to DRESS Analysis of the Vancomycin-Exposed BioVU Cohort

While at least two weeks of vancomycin was intended in all patients, 22/137 (16%) HLA-A*32:01 positive and 18/137 (13%) HLA-A*32:01 negative patients completed <1 week of vancomycin

therapy. Possible and definitive DRESS cases in the HLA-A*32:01 carriers occurred after one week to four weeks of vancomycin therapy and the estimated probability of developing DRESS was 19.2% at four weeks (Figures 2 and S3). The median time to DRESS symptoms after vancomycin initiation was 18 days in this cohort. In comparison, none of the 119 HLA-A*32:01 negative individuals who were exposed to at least one week of uninterrupted vancomycin treatment developed DRESS or symptoms suggestive of DRESS ($p=6x10^{-5}$). Development of non-DRESS ADRs did not differ between risk allele positive and negative groups (p=0.35). Within the HLA-A*32:01 positive group, when considered jointly by logistic regression with DRESS as outcome, hemodialysis (p=0.03) and immunosuppression (p=0.04) were both protective factors against DRESS development. Among the DRESS cases, 2/13 (15%) had either hemodialysis or immunosuppression compared with 64/124 (52%) carrying HLA-A*32:01 who tolerated vancomycin (p=0.02). Notably, 18 HLA-A*32:01 positive individuals tolerated vancomycin for $\geq$5 weeks. This demonstrates that not all HLA-A*32:01 positive individuals will develop DRESS after prolonged vancomycin treatment.

Skin testing, oral rechallenge, and skin histology results

Vancomycin intradermal testing (IDT) produced strong immediate histamine responses at 20 minutes in both HLA-A*32:01 positive individuals who were tested, but only the patient with a history of DRESS developed a delayed positive IDT with dermal induration and erythema at

vancomycin concentrations of 0.5, 5 and 50 mg/mL recorded 24 and 48 hours after drug placement

(Figure 3A and 3B). In addition, DRESS patient 18 had negative immediate testing, delayed IDT and oral challenge to levofloxacin which had been co-administered with vancomycin. H&E staining from skin biopsies obtained from patient 18 from the acute DRESS reaction and the 5 mg/ml vancomycin positive IDT skin test showed the papillary dermal edema, epidermal spongiosis and dense lymphocytic infiltrate classically seen in DRESS histology (Figure 3C and 3D). Immunohistochemistry of these same biopsies showed no appreciable difference in the distribution of CD4 and CD8 positive cells in the dermal infiltrate between the acute and skin test biopsies. The acute biopsy did, however, demonstrate a substantially higher number of intraepidermal CD8+ T cells when compared to the skin test biopsy. Conversely, dermal FOXP3+ T regulatory cells were present in the skin test biopsy but absent in the acute biopsy (Figure 3E).

*Molecular Docking of Vancomycin with HLA-A*32:01*

We used molecular docking to estimate potential interactions between vancomycin and HLA-A*32:01. We generated a homology model of peptide/HLA-A*32:01 complex based on the most similar solved structure (PDB 6EI2, HLA-A68, 92 % identical) and used AutoDock Vina to predict binding orientations and scores. Vancomycin was not predicted to bind HLA-A*32:01 with high affinity when peptide occupied the antigen binding cleft (ΔG=-7.3 kcal/mole) (RLYGKSLYSF, corresponding to a peptide eluted from HLA-A*32:01). However, vancomycin was predicted to

bind the antigen binding cleft of HLA-A*32:01 with higher affinity in the absence of peptide, ΔG=-7.7 kcal/mole (Figure 4). These data suggest that vancomycin has the potential to bind within the antigen binding cleft of HLA-A*32:01 in the absence of peptides that conform to the canonical HLA-A*32:01 binding motif (9mer P1 K or R, PΩ F, I or L)[20]. Vancomycin was predicted to bind antigen binding cleft residues in HLA-A*32:01 that differ between closely related alleles not associated with vancomycin induced DRESS, such as HLA-A*29:02 (polymorphic differences shown in magenta in Figure 5.9). Since the on-target mechanism of action for vancomycin is binding D-Ala-D-Ala in the bacterial cell wall, we asked if vancomycin has the potential to bind consecutive alanine residues in HLA-A*32:01. Molecular docking suggests that vancomycin is not likely to bind consecutive alanine residues (L isomers) in HLA-A*32:01. The top scoring molecular docking orientation shows that the vancomycin atoms contacting D-Ala-D-Ala were predicted to bind HLA-A*32:01 in the central region of the cleft normally occupied by the central positions in peptide backbone (shown in cyan in Figure 4).

DISCUSSION

The international implementation of routine pre-prescription screening for HLA-B*57:01 has eliminated abacavir hypersensitivity as a clinical entity and has paved the way for the translation of other HLA screening strategies for the prevention of drug hypersensitivity reactions into clinical practice[8,9,21,22]. Similar to the discovery of abacavir and HLA-B*57:01, our study highlights the utility of using large clinical databases and prospectively defined cases in combination with adjunctive immunological information to define genetic associations with a specific clinical phenotype[8,9,23]. Since vancomycin is frequently prescribed empirically in an urgent manner for acute life-threatening infections, and since DRESS typically takes 2 weeks to occur, unlike previous models that suggest HLA screening prior to intended prescription of a drug, use of HLA-A*32:01 typing may be more appropriate following initiation of vancomycin, when bacterial culture information is available, and in patients destined to receive longer or multiple treatment courses to identify those that could be at risk for vancomycin DRESS. This would be facilitated through the development of a single allele assay for HLA-A*32:01, similar to approaches developed for HLA-B*57:01 and HLA-B*15:02, which are now widely available through commercial laboratories with short turnaround times. Since certain HLA alleles are known to influence the natural history of some infections, one potential limitation of this study is that we were not able to match controls based on indication for vancomycin treatment. However,

given that HLA-A*32:01 was not represented at all in our matched tolerant controls despite the good distribution of almost identical Gram positive and mixed infections and similar host risk factors and co-morbidities that led to the intent for prolonged vancomycin treatment in both vancomycin DRESS cases and tolerant controls, we feel confident that this finding is not a disease association.

Our time-to-event analysis suggests that the risk of DRESS approaches 20% at four weeks of therapy in those carrying HLA-A*32:01 (Figures 2 and S3). Based on this analysis and the prevalence of HLA-A*32:01 of approximately 6.8% in individuals of European ancestry, we can estimate that approximately 70 patients started on vancomycin would need to undergo HLA-A*32:01 testing to prevent or preempt one case of vancomycin DRESS, which is a favorable ratio compared with other well-defined HLA-drug associations where HLA testing is used in clinical practice[3].

Ex vivo and *in vivo* diagnostic approaches such as IFNγ ELISpot assays and IDT warrant further study for their sensitivity, specificity and safety for vancomycin and concurrently administered medications[24]. Additionally, if these techniques were to become routine in the diagnosis of vancomycin DRESS, standardized IFNγ ELISpot assays would need to be commercially available for clinical laboratories. In patient 18, evidence of a localized DRESS reaction on histopathology from a positive IDT biopsy demonstrates that the immunopathology of the acute reaction can be recapitulated in the skin following disease recovery. Consistent with previous studies showing that the ratio of FOXP3+ T cells to overall CD3+ T cells in acute DRESS skin positively correlates with longer times from start of symptoms to skin biopsy, we observed an

increase is FOXP3+ regulatory T cells in the dermis of recovery phase skin following intradermal vancomycin administration (Figure 3E)[25]. This also suggests that regulatory T cells may reside in the skin weeks to months following acute DRESS. While these immunohistopathologic results are compelling, they are from a single patient and require further study in additional patients with vancomycin DRESS.

Vancomycin has been associated with other ADRs including linear IgA bullous dermatosis, fixed drug eruption, acute generalized exanthematous pustulosis, and Stevens-Johnson syndrome/toxic epidermal necrolysis[1,26]. We have enrolled 10 individuals with non-DRESS vancomycin immune-mediated adverse drug reactions in our broader drug hypersensitivity studies. While the heterogeneity and small number of patients limits our ability to rule out other HLA associations with non-DRESS vancomycin-induced reactions, only 1/10 is HLA-A*32:01 positive. These HLA typing results suggest that this association is specific for vancomycin DRESS and that HLA screening would not prevent other vancomycin-induced delayed hypersensitivity reactions. Our study was powered to identify a strong association between HLA-A*32:01 and vancomycin DRESS in a population of primary European ancestry and we cannot generalize at this point to non-European ancestries where HLA associations with vancomycin DRESS will need to be independently studied.

Although the specific mechanism of vancomycin DRESS is unknown, our data may provide clues to the immunopathogenesis of this syndrome. The strong association with HLA-A*32:01 supports that vancomycin DRESS is an HLA Class I-restricted, CD8+ T-cell mediated process. For the HLA-B*57:01-restricted abacavir hypersensitivity reaction, immunologically-confirmed

hypersensitivity can occur as early as 1.5 days of first dosing suggesting that a pre-existing memory T-cell response may be mechanistic[9]. In contrast, vancomycin DRESS in ours and other studies is characterized by a long latency period (median 21 days)[27]. Further, HLA-A*32:01 positive individuals who have not been exposed to vancomycin were observed to have negative responses to vancomycin by both *in vivo* (intradermal challenge) (n=1) and *ex vivo* (IFNγ ELISpot) assessments (n=4). These data might suggest that vancomycin DRESS pathogenesis is dependent upon a naïve T-cell response requiring CD4+ T-cell help. Vancomycin is a large glycopeptide and is excreted unchanged in the urine. Unlike abacavir which has been shown to alter the repertoire of self-peptides presented to T cells in HLA-B*57:01 positive individuals[28,29], our model suggests that vancomycin may bind within the antigen binding cleft of HLA-A*32:01 in the absence of peptides that conform to the canonical HLA-A*32:01 binding motif (Figure 4).

Currently, the use of HLA testing in clinical practice has been limited to pre-prescription screening strategies. This discovery of a strong association between HLA-A*32:01 and one of the most serious immunologically-mediated reactions associated with a commonly used antibiotic, vancomycin, raises the possibility that HLA testing could be used as a diagnostic risk stratification tool after initiation of vancomycin treatment but prior to development of vancomycin DRESS. Patients with complex and life-threatening infections commonly receive vancomycin dosed concurrently with beta-lactams or fluoroquinolone antibiotics as was noted in 21/23 (91%) of our cases. This often leads to patients with DRESS being labeled as allergic to all of these antibiotic classes, which significantly restricts current and future treatment options. In those found to be HLA-A*32:01 positive, vancomycin treatment could either be rationalized where therapeutically appropriate or continued under close clinical observation and laboratory

monitoring with discontinuation of vancomycin at the first sign of early DRESS symptoms. Alternatively, for those who develop possible vancomycin-induced DRESS or who have a known history suggestive of vancomycin DRESS, HLA-A*32:01 testing could be combined with adjunctive testing such as IFNγ ELISpot to vancomycin and other co-administered drugs to improve drug causality assessment. These strategies have the immediate potential to improve patient care by improving drug safety, increasing short-term drug efficacy and reducing future constriction of antibiotic choices.

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TABLES

ID	Age	Sex	Race	Latency	RegiSCAR	Naranjo	HLA-A*32:01	Trough	Indication for vancomycin	Other implicated medications
1	56	F	W	18	5	8	Positive	27	*MRSA* cervical spine post-operative wound infection	rifampin
2	52	M	W	26	6	8	Positive	9	Culture negative post-operative soft tissue infection of right foot	ceftriaxone, ciprofloxacin
3	59	M	W	19	4	8	Positive	14	Gram-positive cocci right chronic calcaneal osteomyelitis	ciprofloxacin
4	66	M	W	21	4	8	Positive	3	*MRSA* osteomyelitis	rifampin
5	27	M	W/H	29	5	8	Positive	21	Lumbar spine osteomyelitis	isoniazid, rifampin, ethambutol, pyrazinamide
6	33	M	W	28	5	8	Positive	14	*MRSE*-infected mesh post bariatric surgery abdominal repair	None
7	52	M	W	17	6	8	Positive	16	Epidural abscess and osteomyelitis with Gram-positive bacteremia	ceftriaxone
8	48	M	B	21	5	8	Negative	12	Cellulitis post exploratory laparotomy and inguinal hernia repair	piperacillin-tazobactam
9	53	F	W	23	7	8	Positive	7	Culture negative soft tissue infection with underlying rib osteomyelitis	levofloxacin, fluconazole
10	29	M	W	26	5	8	Positive	19	Traumatic arm injury and possible osteomyelitis	trimethoprim/sulfamethoxazole, piperacillin-tazobactam, ciprofloxacin
11	58	M	W	36	6	8	Positive	22	Culture negative osteomyelitis	ciprofloxacin
12	62	M	W	21	4	8	Negative	21	Implantable cardioverter defibrillator pocket infection	None
13	17	M	W	29	4	8	Positive	10	*MRSA* bacteremia secondary to right pelvic myositis and phlebitis	ibuprofen, hydroxyzine
14	51	F	W	14	6	8	Positive	23	*Enterococcus faecium* bacteremia	ceftazidime
15	57	M	W	15	5	8	Negative	24	Culture negative febrile neutropenia and neutropenic colitis during AML treatment	meropenem
16	24	F	W	14	6	10	Positive	16	*MRSE* and *Bacteroides fragilis* post-operative wound infection following caesarean section and supracervical hysterectomy for uterine necrosis	azithromycin, clindamycin, gentamicin, piperacillin-tazobactam, amoxicillin,

										meropenem, metronidazole
17	38	M	W	18	5	8	Positive	14	*MRSA* right chest phlegmon, deep soft tissue infection, underlying osteomyelitis of 2nd rib with fracture	rifampin
18	47	F	W	17	6	8	Positive	13	*MRSA* and *E. coli* bacteremia w/ chest infiltrate	levofloxacin
19	76	M	W	27	6	8	Positive	12	*Enterococcus* bacteremia and endocarditis	gentamicin, benzylpenicillin
20	61	F	W	50	5	8	Positive	N/A	*MRSA* wound infection leading to hip prosthesis removal and placement of vancomycin spacer	ciprofloxacin
21	71	F	W	28	5	8	Negative	16	*MRSA* and *E. coli* hardware infection post knee replacement	ceftriaxone, rifampin
22	40	F	W	15	4	8	Positive	17	*E. coli* and *Enterococcus faecalis* urosepsis during pregnancy	ceftriaxone
23	47	F	W	15	5	8	Positive	22	*Staphylococcus epidermis* ventriculitis	ceftriaxone

Table 1. Summary of case basic demographics, clinical characteristics, HLA risk allele carriage and DRESS history. Legend: ID, patient identification; Age, age at time of vancomycin treatment; Latency, days between vancomycin initiation and symptoms development; HLA, human leukocyte antigen; Trough, last vancomycin trough level before development of hypersensitivity symptoms in μg/mL. F, female; M, male; W, Caucasian; W/H, Caucasian/Hispanic; B, African American; N/A, not available; *MRSA*, Methicillin-resistant *Staphylococcus aureus*; *MRSE*, Methicillin-resistant *Staphylococcus epidermidis*; AML, acute myeloid leukemia; *E. coli, Escherichia coli.*

FIGURE LEGENDS

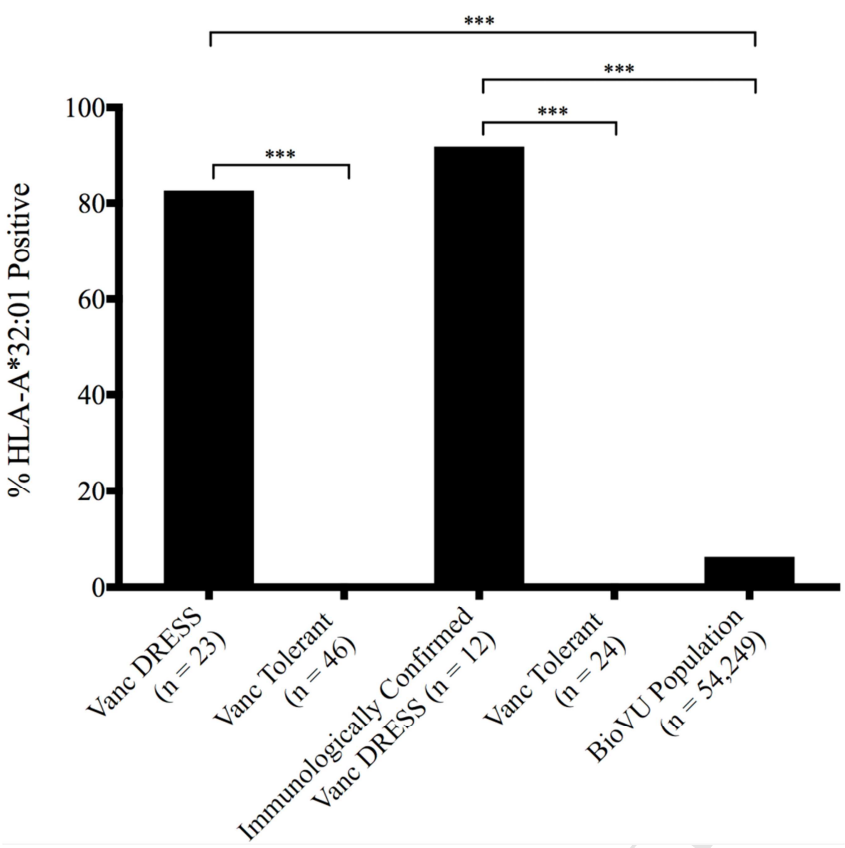
Figure 1. HLA-A*32:01 is strongly associated with vancomycin DRESS. A. 19/23 (83%) DRESS cases carried HLA-A*32:01 compared with 0/46 (0%) of the matched vancomycin tolerant controls (p=1x10-8, conditional logistic). If analyses are restricted to immunologically confirmed cases, then 11/12 (92%) vancomycin ELISpot positive patients carried HLA-A*32:01 compared with 0/24 (0%) of the BioVU matched controls (p=9x10-7, conditional logistic). HLA-A*32:01 carriage in all identified vancomycin DRESS cases and immunologically confirmed cases was also very significantly overrepresented compared to HLA-A*32:01 carriage in the entire BioVU cohort (6.3%) (p=2x10-16 and p=2=7x10-13 respectively, exact binomial tests). There was no significant difference in HLA-A*32:01 carriage between the vancomycin tolerant populations and the BioVU cohort (p=0.12 for all controls, p=0.40 for controls matched to immunologically confirmed cases, exact binomial tests). Additionally, there was no significant difference in HLA-A*32:01 carriage between the immunologically confirmed vancomycin DRESS cases and those that were not immunologically confirmed (p=0.32, Fisher's exact test). All analyses shown included Bonferroni correction for multiple comparisons. **B.** IFN-γ release ELISpot results after 18-hour incubation with vancomycin at concentrations of 250 μg/mL (grey) or 500 μg/mL (black) using peripheral blood mononuclear cells from vancomycin DRESS patients. Controls included cells from vancomycin-naïve, HLA-A*32:01 positive healthy donors (n = 3) including the son of case patient 18 and the vancomycin skin test negative control C50, an HLA-A*32:01 positive individual tolerant of 4 weeks of vancomycin (n = 1), patients who had developed a non-DRESS immune-mediated adverse reaction to vancomycin (n = 5) and non-HLA matched healthy donors (n = 4). Means of the replicates are plotted. In patients with multiple blood draws at time points distant from the reaction, ELISpot results from the first blood draw are plotted. 12/14 (85.7%) DRESS cases had a positive vancomycin ELISpot compared to none of the controls (p=0.005 (DRESS vs. HLA-A-32:01 positive controls), p=0.002 (DRESS vs. non-DRESS ADRs), p=0.005 (DRESS vs. non-HLA matched healthy donors)). Positive results are those above the dotted line intersecting the y-axis at 50 SFU/million cells. Differences in proportion of positive responses between groups were assessed using Fisher's exact tests. Patient and control PBMCs were also stimulated with vancomycin at concentrations of 5 μg/mL and 50 μg/mL and exhibited a dose-dependent response (data not shown). Legend: HLA, human leukocyte antigen; Vanc, vancomycin; DRESS, drug reaction with eosinophilia and systemic symptoms; SFU, spot-forming units; IM-ADR, immune-mediated adverse drug reaction.

Figure 2. Time-to-event analysis demonstrating that only HLA-A*32:01 positive patients developed DRESS. Kaplan-Meier estimates were used to determine time to DRESS or possible DRESS development during vancomycin treatment stratified by carriage of HLA-A*32:01. Cases of DRESS occurred in HLA-A*32:01 positive subjects between 1 and 4 weeks of vancomycin therapy but not in HLA-A*32:01 negative subjects. The estimated risk of DRESS prior to 4 weeks of treatment was 19.2% in those carrying the HLA-A*32:01 allele.

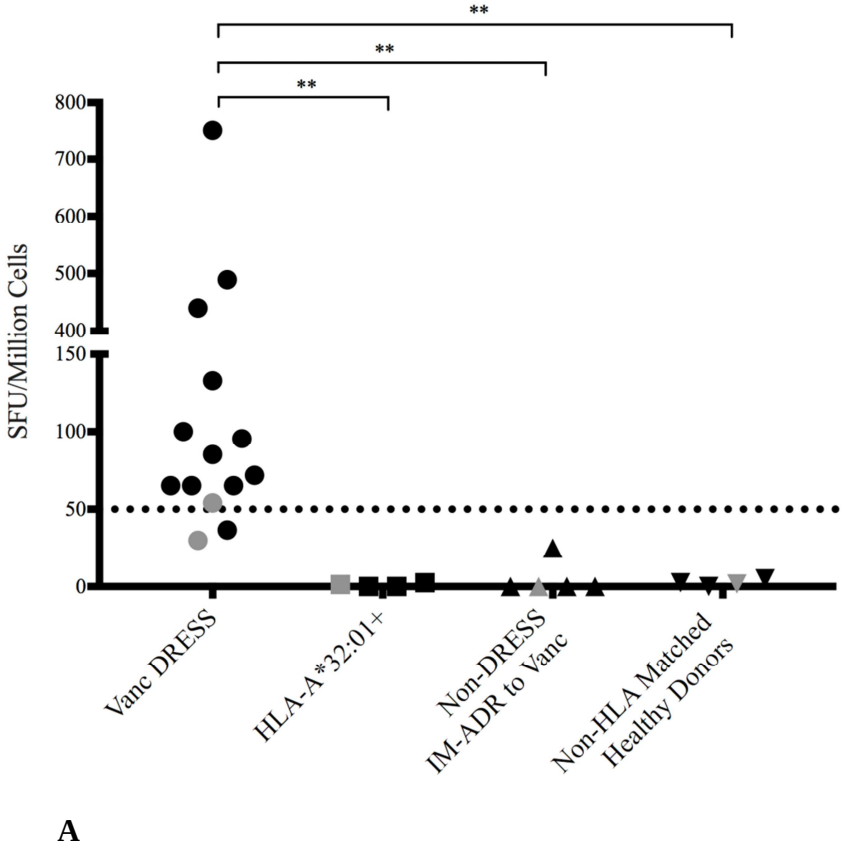
Figure 3. Vancomycin skin testing, acute DRESS skin eruption and skin biopsy histology. A and **B**. Vancomycin intradermal testing (IDT) results in patient 18 approximately 6.5 months after developing vancomycin DRESS and control C50, an HLA-A*32:01 positive, vancomycin-naïve healthy donor. IDT was performed on the volar forearm of the skin with 0.02 mL of vancomycin at concentrations of 0.05, 0.5, 5 and 50 mg/mL. The positive histamine and negative saline controls worked as expected. Vancomycin produced a strong immediate histamine

response at 20 minutes in both control C50 and patient 18, but only patient 18 with a history of HLA-A*32:01 positive DRESS developed a concentration dependent induration of the skin at 48 hours at the 0.5, 5 and 50 mg/ml concentrations. Additionally, patient 18 had negative IDT to levofloxacin (not shown) and was successfully rechallenged with levofloxacin, a drug that, at the time of reaction, was administered with vancomycin. **C**. A representative example of the skin eruption from patient 18 during acute vancomycin DRESS. She had a diffuse morbilliform exanthema with facial involvement and facial edema (not shown). **D**. Hematoxylin and eosin staining of punch biopsies of skin from patient 18. Acute DRESS histology from a skin biopsy taken three days following onset of symptoms (upper panel) and skin test histology from the 5 mg/ml vancomycin positive intradermal skin test at 48 hours (lower panels) demonstrate papillary dermal edema, epidermal spongiosis and a dense lymphocytic infiltrate. Rare eosinophils are present on both biopsies. The skin test histology mirrors the results from the acute biopsy. **E.** Immunohistochemistry of T-cell subsets from acute DRESS and positive skin test biopsies from patient 18. Healthy skin is shown for comparison (upper panel). There was no discernable difference in the distribution of CD4 and CD8 positive cells in the dermal infiltrate between the acute (middle panel) and skin test (lower panel) biopsies. The number of intraepidermal CD8 positive cells was substantially higher in the acute biopsy. There was no appreciable exocytosis of CD4 T cells in the acute biopsy or CD4 or CD8 T cells in the biopsy from vancomycin IDT. Notably, dermal FOXP3+, regulatory T cells were present in the skin test biopsy, but absent in the acute biopsy.

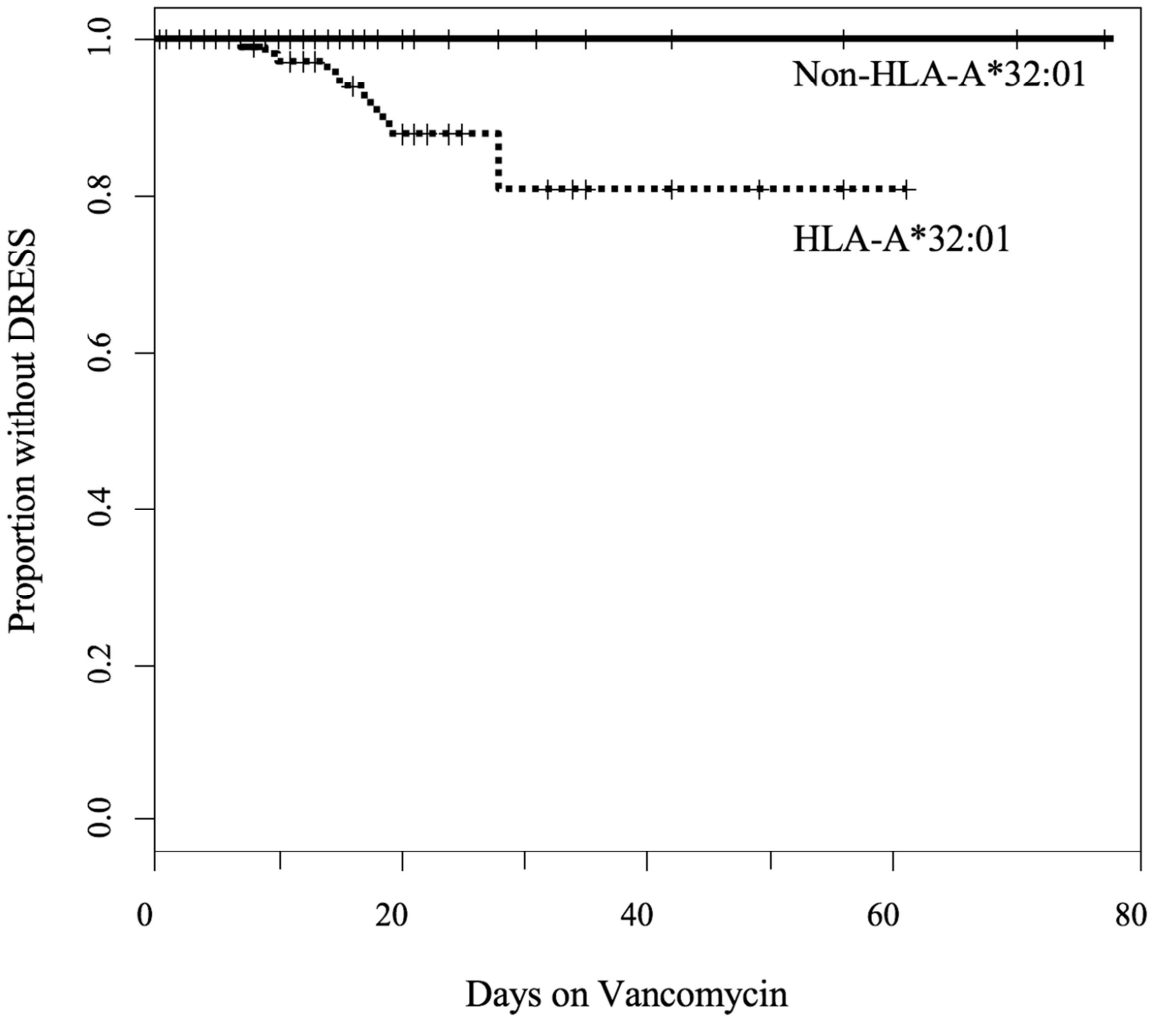
Figure 4. Molecular docking prediction of vancomycin binding HLA-A*32:01. Vancomycin is shown as sticks, white for carbon, blue for nitrogen, red for oxygen. Vancomycin atoms that mediate intermolecular contacts with D-Ala-D-Ala (in PDB 1FVM) are shown in cyan. A homology model of HLA-A*32:01 is shown in yellow as a ribbon diagram. Polymorphic positions that distinguish the associated HLA-A*32:01 allele from the closely related HLA-A*29:02 allele are shown in magenta. Intermolecular contacts between vancomycin and HLA-A*32:01 predicted by molecular docking are shown as black dashes.


***
***
***
***
100
80
60
40
20
0
% HLA-A*32:01 Positive
Vanc DRESS (n = 23)
Vanc Tolerant (n = 46)
Immunologically Confirmed Vanc DRESS (n = 12)
Vanc Tolerant (n = 24)
BioVU Population (n = 54,249)

A

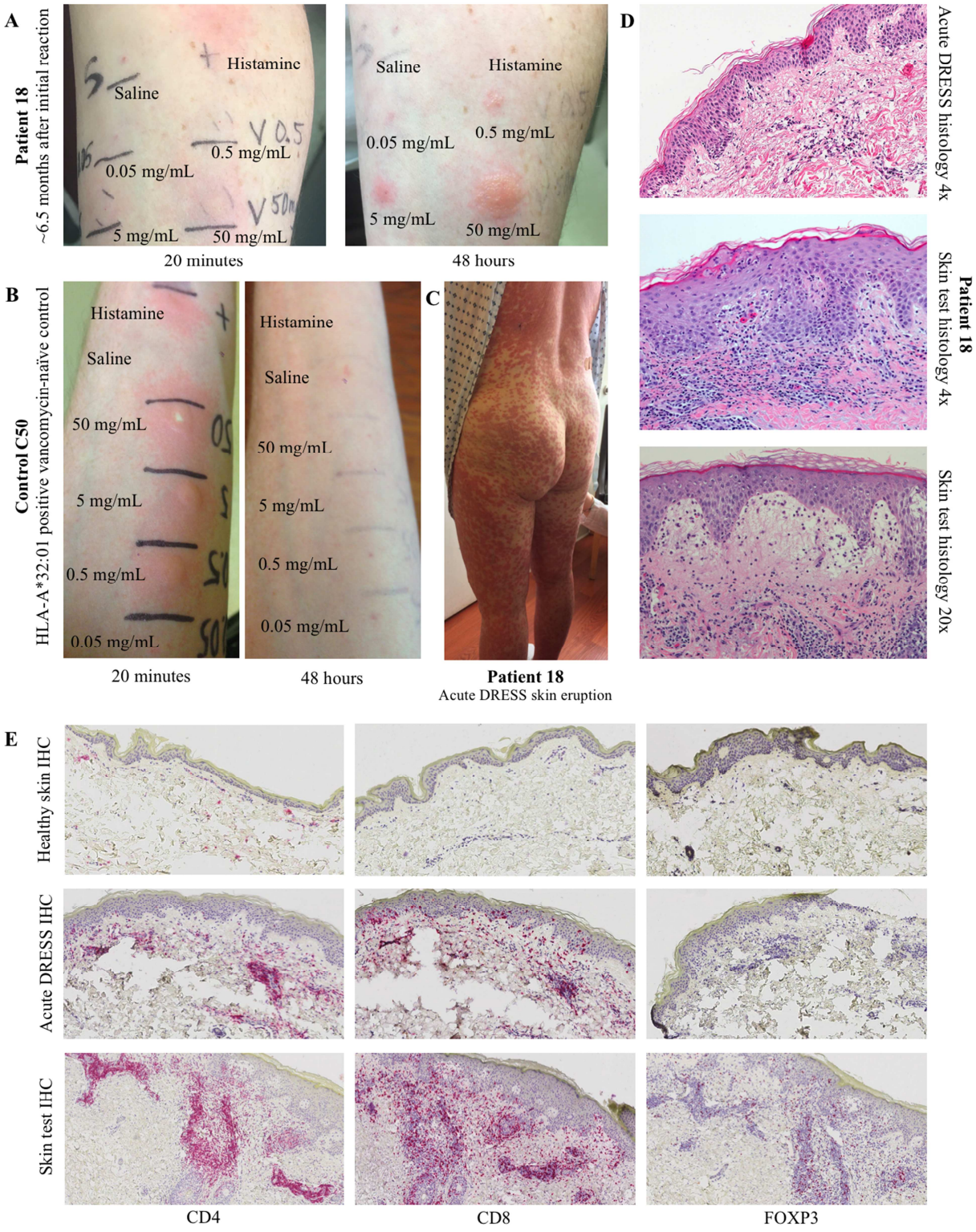

**
**
**
800
700
600
500
400
150
100
50
0
SFU/Million Cells
Vanc DRESS
HLA-A*32:01+
Non-DRESS IM-ADR to Vanc
Non-HLA Matched Healthy Donors

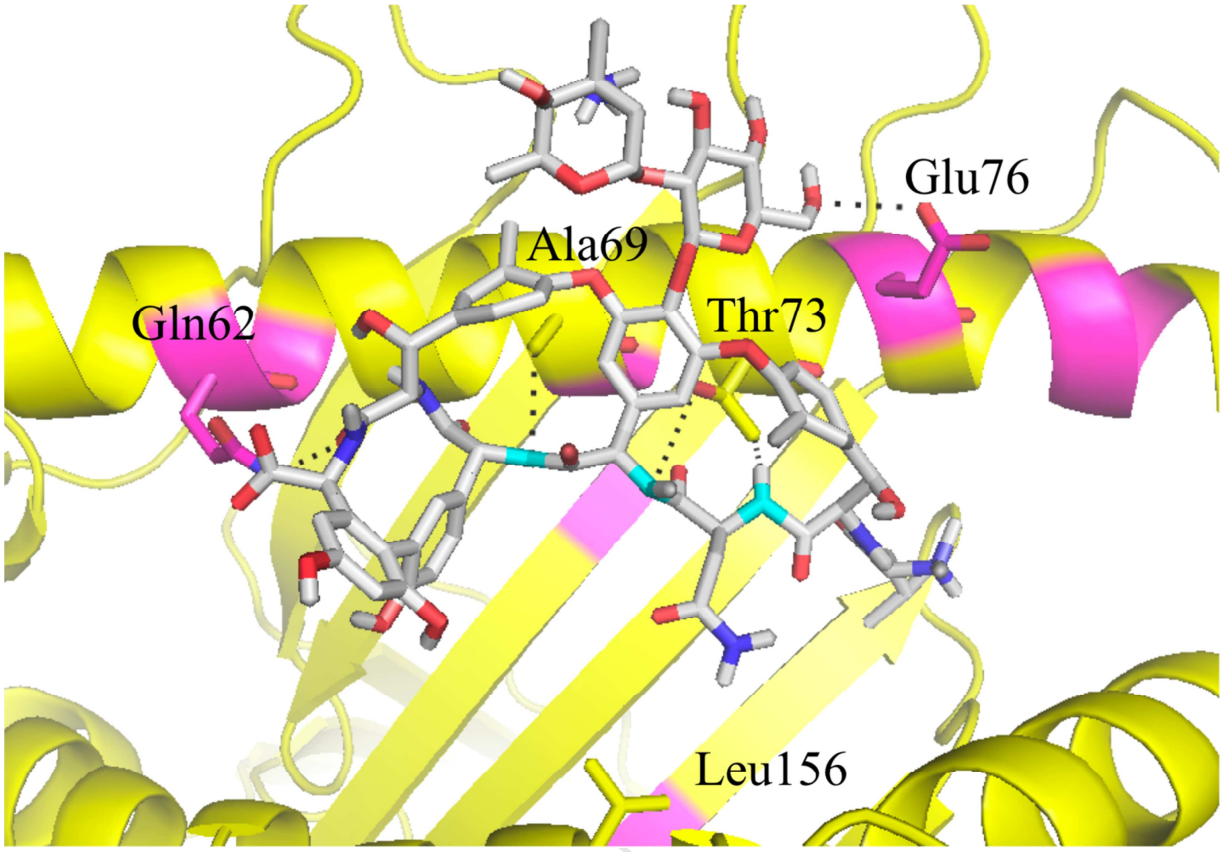
B

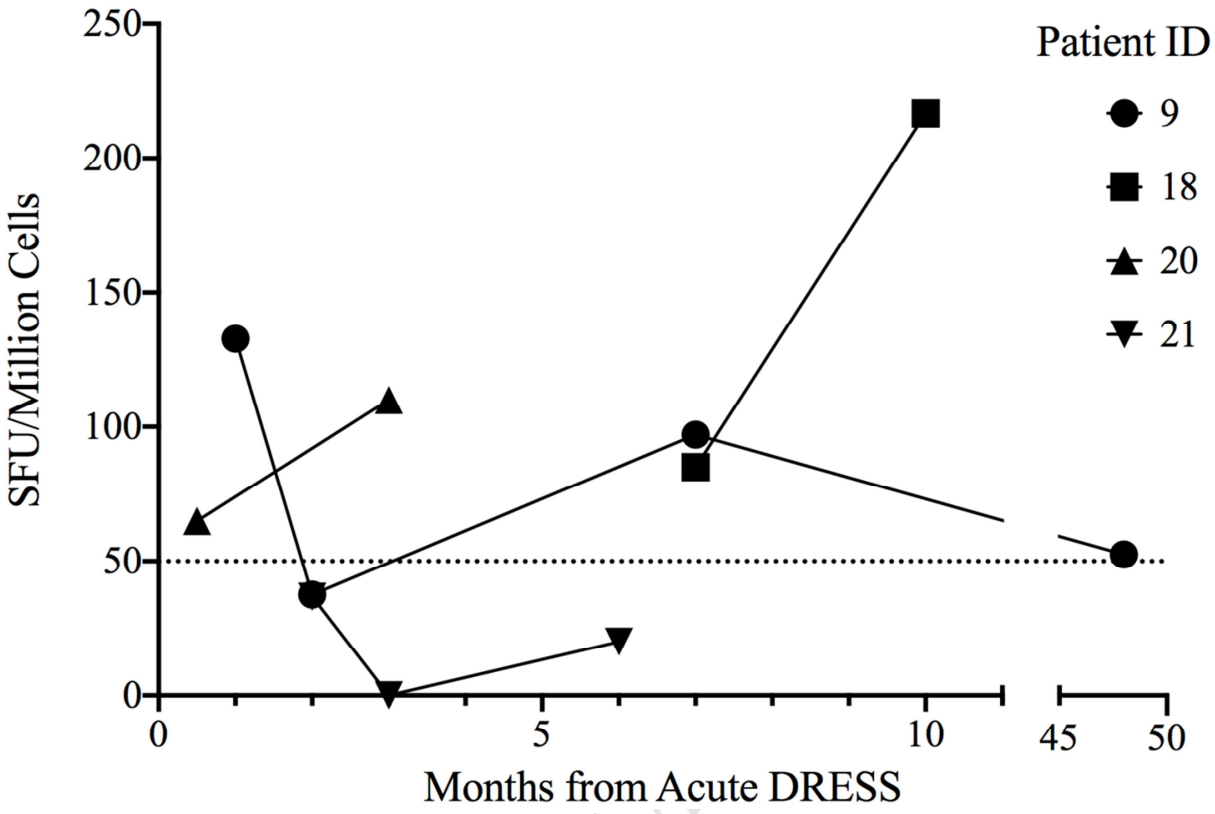


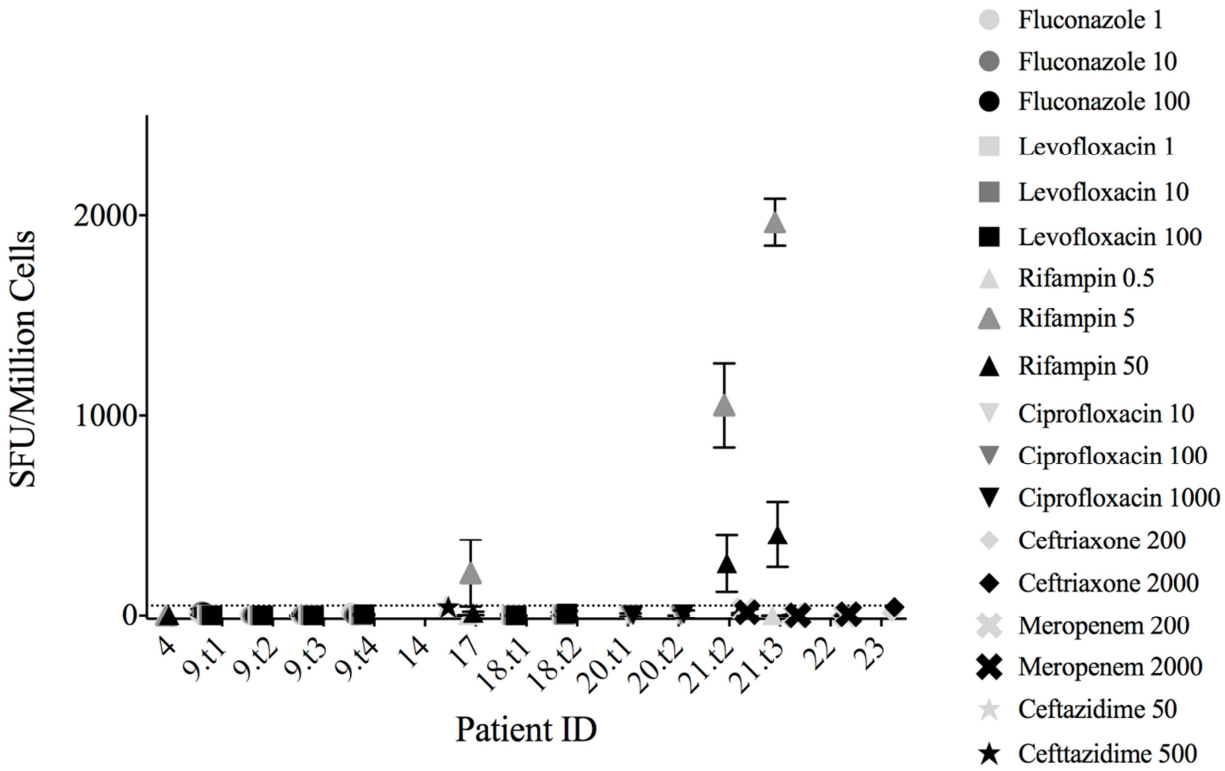
Numbers on Vancomycin:

137	99	41	18	16	5	HLA-A*32:01
137	104	50	32	28	4	Non-HLA-A*32:01


A
Patient 18
~6.5 months after initial reaction
Histamine
Saline
0.5 mg/mL
0.05 mg/mL
5 mg/mL
50 mg/mL
20 minutes
Saline
Histamine
0.05 mg/mL
0.5 mg/mL
5 mg/mL
50 mg/mL
48 hours
B
Control C50
HLA-A*32:01 positive vancomycin-naïve control
Histamine
Saline
50 mg/mL
5 mg/mL
0.5 mg/mL
0.05 mg/mL
20 minutes
Histamine
Saline
50 mg/mL
5 mg/mL
0.5 mg/mL
0.05 mg/mL
48 hours
C
Patient 18
Acute DRESS skin eruption
D
Acute DRESS histology 4x
Patient 18
Skin test histology 4x
Skin test histology 20x
E
Healthy skin IHC
Acute DRESS IHC
Skin test IHC
CD4
CD8
FOXP3

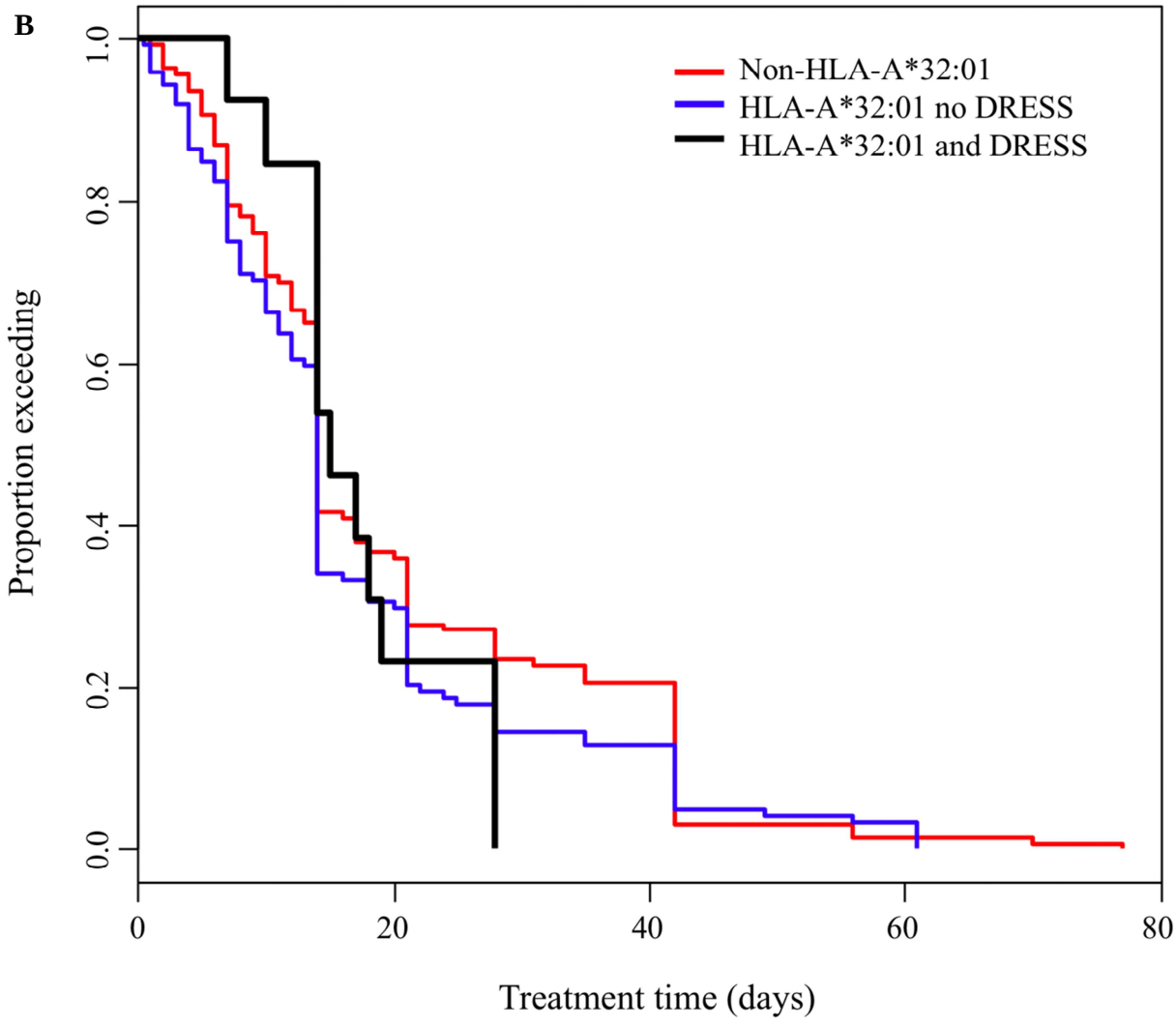






A		HLA-A*32:01 Positive (n=137)	HLA-A*32:01 Negative (n=137)	p-value
Age (years)				
	Mean (SD)	52.6 (21.0)	47.7 (22.3)	0.061
Longest treatment length (days)				
	Mean (SD)	17.3 (13.5)	20.0 (15.0)	0.12
Sex	**n (%)**			
Female		50 (36.5)	61 (44.5)	
Male		87 (63.5)	76 (55.5)	0.22
Race	**n (%)**			
European American		125 (91.2)	120 (87.6)	
Other		12 (8.8)	17 (12.4)	0.43
Adverse reaction	**n (%)**			
Yes		20 (14.6)	12 (8.8)	
No		117 (85.4)	125 (91.2)	0.19
Possible or definite DRESS	**n (%)**			
Yes		13 (9.5)	0 (0.0)	
No		124 (90.5)	137 (100)	0.00018

B



ID	Fever (Y/N)	Extent of Rash >50% BSA (Y/N)	Facial Edema (Y/N)	Lymphadenopathy (Y/N)	Peak Liver Enzymes >2x Normal (Y/N)	Peak Creatinine (mg/dL)	Absolute Eosinophil Count (Cells/μL)	Resolution >15 days (Y/N)	Biopsy Supporting DRESS (Y/N)	Prior Exposure to Vancomycin >7 Days (Y/N)	Steroid Treatment Course
1	Y	Y	Y	N	Y	1.10	17.4% (no absolute)	Y	Y	N	“1 dose of high dose steroids”
2	Y	Y	Y	Y	Y	2.19	2450	Y	Y	N	Slow taper starting at 60mg/day of prednisone
3	Y	Y	N	N	N	1.10	680	Y	Y	N	Slow taper starting at 80mg/day of prednisone
4	Y	Y	U	U	Y	1.38	990	Y		N	None
5	Y	Y	Y	N	Y	0.98	5480	Y	Y	N	Slow taper starting at 60mg/day of prednisone
6	Y	Y	N	N	Y	1.69	3610	Y	Y	N	1mg/kg methylprednisolone, then slow taper starting at 80mg/day of prednisone
7	Y	Y	Y	N	Y	1.67	2770	Y	Y	N	Slow taper starting at 40mg/day of prednisone
8	Y	Y	Y	N	Y	6.98	2400	Y	N	N	None
9	Y	Y	Y	N	Y	1.44	5290	Y	Y	N	Slow taper starting at 60mg/day of prednisone; 60 mg/day restarted 11 months after reaction for DRESS colitis
10	Y	Y	Y	U	Y	2.24	2280	Y	Y	U	None
11	Y	Y	Y	U	Y	2.10	5100	Y	Y	N	Slow taper starting at 60mg/day of prednisone
12	Y	Y	N	N	N	2.12	1840	N	N	N	None
13	Y	Y	Y	N	N	2.12	1030	Y	Y	N	Slow taper starting at 40mg/day of prednisone
14	Y	Y	Y	U	Y	1.35	1950	Y	Y	N	250 mg methylprednisolone, then slow taper starting at 50mg/day of prednisone
15	Y	Y	Y	N	Y	1.65	2150	Y	Y	N	None
16	Y	Y	Y	N	Y	2.22	3810	Y	Y	N	Slow taper starting at 60mg/day of prednisone
17	Y	Y	Y	Y	N	1.12	710	Y	Y	N	Slow taper starting at 30mg/day of prednisone
18	Y	Y	Y	U	Y	0.71	1470	Y	Y	N	Slow taper starting at 80mg/day of prednisone
19	Y	Y	Y	U	Y	6.19	3180	Y	N	U	“High dose steroids"
20	Y	Y	N	N	Y	5.12	6550	Y	N	N	Slow taper starting at 80mg/day of prednisone
21	Y	Y	N	N	Y	3.77	1230	Y	Y	N	Slow taper starting at 12mg/day of dexamethasone
22	Y	Y	Y	U	Y	1.39	1200	N	Y	N	None
23	Y	Y	Y	U	Y	"Normal"	6000	Y	Y	N	100mg/day hydrocortisone, then slow taper starting at 75mg/day of prednisolone

Supplemental Table S1. Additional hypersensitivity syndrome characteristics for vancomycin DRESS cases. The length of the steroid tapers ranged from 4 weeks to >6 months. Data in quotes were taken directly from the electronic health records when laboratory values or medication records were not available. Where relevant creatinine values have been converted from μmol/L to mg/dL. Legend: Y, yes; N, No; BSA, body surface area; U, Unknown.

Vancomycin DRESS Cases				Vancomycin Tolerant Controls			
ID	**Age**	**Sex**	**Race**	**ID**	**Age**	**Sex**	**Race**
1	56	F	W	C1	57	F	W
				C2	56	F	W
2	52	M	W	C3	53	M	W
				C4	51	M	W
3	59	M	W	C5	57	M	W
				C6	60	M	W
4	66	M	W	C7	66	M	W
				C8	65	M	W
5	27	M	W/H	C9	22	M	W
				C10	28	M	W
6	33	M	W	C11	33	M	W
				C12	37	M	W
7	52	M	W	C13	50	M	W
				C14	50	M	W
8	48	M	B	C15	52	M	B
				C16	43	M	B
9	53	F	W	C17	54	F	W
				C18	57	F	W
10	29	M	W	C19	32	M	W
				C20	26	M	W
11	58	M	W	C21	59	M	W
				C22	56	M	W
12	62	M	W	C23	62	M	W
				C24	62	M	W
13	17	M	W	C25	18	M	W
				C26	17	M	W
14	51	F	W	C27	51	F	W
				C28	51	F	W
15	57	M	W	C29	57	M	W
				C30	56	M	W
16	24	F	W	C31	22	F	W
				C32	25	F	W
17	38	M	W	C33	37	M	W
				C34	40	M	W
18	47	F	W	C35	49	F	W
				C36	48	F	W
19	76	M	W	C37	78	M	W
				C38	80	M	W
20	61	F	W	C39	62	F	W
				C40	60	F	W
21	71	F	W	C41	66	F	W
				C42	74	F	W
22	47	F	W	C43	47	F	W
				C44	49	F	W
23	47	F	W	C45	49	F	W
				C46	45	F	W

Supplemental Table S2. Results of the 1:2 case to control match. Controls were identified using Vanderbilt's BioVU, a deidentified electronic medical record database linked to a DNA biobank. Patients were matched on sex, race and age within five years. Legend: ID, patient

identification; F, Female; M, Male; W, Caucasian; W/H, Caucasian/Hispanic; B, African American.

ID	Age	Sex	Race	HLA-A*32:01	Indication for vancomycin treatment
C1	57	F	W	Negative	Right orbital cellulitis with interconal and extraconal abscess
C2	56	F	W	Negative	*MRSA*-infected failed aortobifemoral bypass graft
C3	53	M	W	Negative	Tongue and pulmonary lesions concerning for infection in setting of AML
C4	51	M	W	Negative	*Saccharomyces cerevisiae* pneumonia post bone marrow transplant
C5	57	M	W	Negative	Scrotal abscess and probable rectus sheath hematoma infection
C6	60	M	W	Negative	Osteomyelitis with abscess right heel
C7	66	M	W	Negative	Osteomyelitis and sepsis secondary to diabetic foot infection
C8	65	M	W	Negative	Mediastinal infection status post coronary artery bypass grafting
C9	22	M	W	Negative	Pneumonia and neutropenic with persistent fevers in the setting of AML
C10	28	M	W	Negative	Prosthetic aortic valve endocarditis with perivalvular abscess
C11	33	M	W	Negative	Pneumonia and skin lesion in setting of neutropenia and CLL
C12	37	M	W	Negative	Coagulase negative *Staphylococcus* native aortic valve endocarditis and septicemia
C13	50	M	W	Negative	Native valve *MSSA* endocarditis with embolization to skin, brain and kidney and enterococcus in blood culture
C14	50	M	W	Negative	Sepsis secondary to contaminated decubiti
C15	52	M	B	Negative	Fever and altered mental status in the setting of HIV/AIDS
C16	43	M	B	Negative	*MRSA* pneumonia in the setting of AIDS and end stage renal disease
C17	54	F	W	Negative	*MRSE* infection of right total knee arthroplasty after liner exchange
C18	57	F	W	Negative	*MRSE*-infected left chest port in setting of AML
C19	32	M	W	Negative	Persistent fevers in the setting of Ewing's sarcoma on chemotherapy
C20	26	M	W	Negative	Pneumonia in the setting of relapsed AML
C21	59	M	W	Negative	*MRSA* bacteremia in the setting of chronic renal failure
C22	56	M	W	Negative	Empiric vancomycin for cellulitis of foot with chronic non-healing wound following nail puncture
C23	62	M	W	Negative	*MRSE* empyema in the setting of a single lung transplant
C24	62	M	W	Negative	Donor-derived surgical culture growing *Staphylococcus aureus* after double lung transplant
C25	18	M	W	Negative	Empiric therapy in patient with cystic fibrosis exacerbation and history of growing *MSSA* and Pseudomonas
C26	17	M	W	Negative	Coagulase-negative staphylococcal bacteremia in the setting of hypoplastic left heart syndrome status post failed Fontan
C27	51	F	W	Negative	Inferior ischiopubic ramus osteomyelitis
C28	51	F	W	Negative	*MRSA* bacteremia and endocarditis of the atrioventricular valves with evidence of septic embolization
C29	57	M	W	Negative	Coagulase-negative staphylococcal bacteremia status post

					autologous peripheral blood stem cell transplant
C30	56	M	W	Negative	*MRSA* bacteremia in the setting of cellulitis of lower abdomen and possible endocarditis
C31	22	F	W	Negative	Poly-Gram negative rod septicemia in the setting of orthotopic liver transplantation complicated
C32	25	F	W	Negative	*MRSA* pneumonia in the setting of severe end stage cystic fibrosis
C33	37	M	W	Negative	Left femur osteomyelitis and surrounding soft tissue infection at stump site of above knee amputation
C34	40	M	W	Negative	Supracystic abscess communicating with the sigmoid colon after failed kidney/pancreas transplant status post explant of failed grafts
C35	49	F	W	Negative	Probable pneumonia in the setting of splenic rupture and AML
C36	48	F	W	Negative	Right toe osteomyelitis with overlying abscess positive for *MRSA, Enterococcus faecalis, and Providencia* status post amputation
C37	78	M	W	Negative	*MRSA*-infected graft and right iliac region
C38	80	M	W	Negative	Enterococcal septicemia
C39	62	F	W	Negative	*Enterococcus faecalis* positive sacral decubitus ulcer with associated osteomyelitis
C40	60	F	W	Negative	Fever and altered mental status in the setting of AML
C41	66	F	W	Negative	Tibial osteomyelitis and hardware infection
C42	74	F	W	Negative	*MRSA*-positive right shoulder prosthetic septic arthritis and sepsis
C43	47	F	W	Negative	Necrotic anal mass with associated draining abscess
C44	49	F	W	Negative	*Staphylococcal aureus* positive post-surgical meningitis and sepsis
C45	49	F	W	Negative	Parapharyngeal abscess and cervical spine osteomyelitis with epidural abscess
C46	45	F	W	Negative	*MRSA* osteomyelitis of the spine

Supplemental Table S3. Demographics, HLA risk allele carriage and indication for vancomycin treatment for case-matched vancomycin tolerant individuals. Legend: ID, patient identification; Age, age at time of vancomycin treatment; HLA, human leukocyte antigen; F, female; M, male; W, Caucasian; B, African American; *MRSA*, Methicillin-resistant *Staphylococcus aureus*; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphoblastic leukemia; *MSSA*, Methicillin-sensitive *Staphylococcus aureus*; HIV/AIDS, human immunodeficiency virus/acquired immune deficiency syndrome; *MRSE*, Methicillin-resistant *Staphylococcus epidermidis.*

ID	HLA-A **Allele 1/2**	HLA-B **Allele 1/2**	HLA-C **Allele 1/2**	HLA-DPB1 **Allele 1/2**	HLA-DQA1 **Allele 1/2**	HLA-DQB1 **Allele 1/2**	HLA-DRB1 **Allele 1/2**
1	02:01/**32:01**	07:02/44:02	05:01/07:02	04:02/04:02	NT	03:01/06:02	04:08/15:01
2	02:01/**32:01**	51:01/51:01	02:02/16:02	04:02/06:01	01:01/03:01	03:02/05:01	01:01/04:04
3	**32:01**	40:01	02:02/03:04				
4	01:01/**32:01**	08:01/44:02	05:01/07:01	04:01/19:01	NT	05:03/06:02	14/15
5	02:06/**32:01**	39:05/40:02	02:02/07:02	04:02/04:02	01:03/03:01	03:02/06:03	04:07/13:01
6	02:01/**32:01**	44:02/44:02	05:01/05:01	03:01/04:02	03:01/05:01	03:01/03:01	04:01/11:04
7	01:01/**32:01**	08:01/27:05	02:02/07:01	01:01/15:01	03:01/05:01	02:01/03:02	03:01/04:01
8	23:01/23:01	08:01/15:16	07:02/14:02	01:01/85:01	01:02/05:01	02:03/05:02	03:02/16:02
9	03:01/**32:01**	07:02/18:01	07:02/07:41	03:01/04:01	NT	02:01/06:02	03:01/15:01
10	23:01/**32:01**	44:02/49:01	05:01/07:01	02:01/04:02	05:01/05:01	03:01/03:01	11:01/12:01
11	**32:01/32:01**	15:01/35:01	03:03/04:01	04:02/10:01	01:01/01:01	05:01/05:03	01:01/11:13
12	02:01/26:01	14:01/45:01	06:02/08:02	02:01/04:01	02:01/04:01	02:02/04:02	07:01/08:01
13	01:01/**32:01**	44:02/44:03	04:01/05:01	04:01/04:01	02:01/03:01	02:02/03:01	04:01/07:01
14	24:02/**32:01**	35:03/35:08	04:01/04:01	04:01/14:01	01:02/01:02	05:02/05:02	16:01/16:02
15	01:01/02:01	08:01/44:02	05:01/07:01	01:01/04:02	03:01/05:01	02:01/03:01	03:01/04:01
16	03:01/**32:01**	07:02/35:01	04:01/07:02	04:01/04:01	05:01/05:01	03:01/03:01	11:04/12:01
17	03:01/**32:01**	07:02/07:02	07:02/07:02	03:01/04:01	01:02/03:01	03:01/06:02	04:07/15:01
18	03:01/**32:01**	07:02/14:01	07:02/08:02	02:01/05:01	03:01/03:01	03:02/03:02	04:04/04:04
19	01:01/**32:01**	13:02/51:01	06:02/14:02	01:01/04:01	02:01/02:01	02:02/02:02	07:01/07:01
20	68:01/**32:01**	13:02/51:01	02:02/06:02	02:01/04:01	02:01/03:01	02:02/03:03	07:01/09:01
21	01:01/03:01	07:02/08:01	07:01/07:02	03:01/04:01	01:02/05:01	03:01/06:02	11:01/15:01
22	03:01/**32:01**	15:01/44:02	03:04/05:01	02:01/11:01	01:01/02:01	02:02/05:02	01:01/11:01
23	24:02/**32:01**	08:01/40:01	03:04/07:01	02:01/04:02	03:01/05:01	02:01/03:02	03:01/04:04

Supplemental Table S4. Full HLA typing results of potential vancomycin DRESS cases. Empty wells could not be imputed. Legend: ID, patient identification; HLA, human leukocyte antigen; NT, not typed.

ID	HLA-A Allele 1/2	HLA-B Allele 1/2	HLA-C Allele 1/2	HLA-DPB1 Allele 1/2	HLA-DQA1 Allele ½	HLA-DQB1 Allele 1/2	HLA-DRB1 Allele 1/2
C1	01:01/24:02	08:01/08:01	07:01/07:01	01:01/13:01	01:02/05:01	02:01/06:02	03:01/15:01
C2	01:01/02:01	08:01/44:02	07:01/07:04	04:01/11:01	02:01/05:01	02:02/03:01	07:01/11:01
C3	03:01/11:01	07:02/07:02	07:02/07:02	03:01/04:01	03:01/03:01	03:01/03:02	04:01/04:04
C4	02:01/29:02	07:02/44:03	07:02/16:01	04:01/04:02	01:01/02:01	02:02/05:01	07:01/10:01
C5	03:01/26:01	35:01/45:01	04:01/06:02	04:01/09:01	01:01/01:01	05:01/05:01	01:01/01:01
C6	01:01/02:01	18:01/57:01	06:02/07:01	04:01/04:01	01:02/02:01	03:03/06:02	07:01/15:01
C7	01:01/03:01	07:02/57:01	06:02/07:02	04:01/04:01	01:02/02:01	03:03/06:02	07:01/15:01
C8	01:01/02:01	18:01/50:01	06:02/07:01	03:01/14:01	01:01/02:01	02:02/05:01	01:01/07:01
C9	02:01/03:01	15:18/40:01	03:04/07:04	02:01/04:01	01:01/01:02	05:01/06:02	01:02/15:01
C10	01:01/24:02	37:01/55:01	03:03/06:02	02:01/02:01	01:02/05:01	03:01/06:04	12:01/13:02
C11	25:01/29:02	15:01/44:02	03:04/05:01	02:01/03:01	03:01/03:01	03:02/03:02	04:01/04:01
C12	02:01/11:01	44:02/50:01	05:01/06:02	04:01/04:01	01:02/03:01	03:01/06:02	04:08/15:01
C13	01:01/03:01	18:01/40:02	02:02/07:01	04:01/04:01	01:01/05:01	03:01/05:01	01:01/11:04
C14	02:01/03:01	44:02/51:01	01:02/07:04	03:01/03:01	01:01/05:01	03:01/05:01	01:01/11:01
C15	30:02/66:02	07:02/07:02	07:01/15:05	02:01/17:01	01:01/01:02	05:01/05:01	10:01/11:01
C16	23:01/30:01	07:02/18:01	02:10/07:02	01:01/18:01	01:02/01:02	06:02/06:02	11:01/15:03
C17	01:01/02:01	08:01/08:01	07:01/07:01	01:01/01:01	05:01/05:01	02:01/02:01	03:01/03:01
C18	26:01/68:02	15:01/15:07	03:03/05:01	04:01/11:01	01:02/01:03	06:02/06:03	13:01/15:01
C19	02:01/02:01	15:82/44:02	03:03/05:01	04:01/04:02	03:01/05:01	03:02/03:02	04:01/11:01
C20	01:01/68:01	08:01/39:01	07:01/07:02	03:01/04:01	01:02/03:01	03:02/06:02	04:07/15:01
C21	01:01/11:01	40:02/51:01	01:02/02:02	04:01/04:01	01:01/05:01	03:01/05:03	11:01/14:01
C22	03:01/03:01	07:02/15:01	03:03/07:02	03:01/04:01	01:01/03:01	03:02/05:01	01:03/04:01
C23	02:01/68:01	40:01/55:01	03:03/03:04	03:01/05:01	01:01/03:01	03:01/05:03	04:01/14:01
C24	02:01/11:01	44:02/52:01	05:01/12:02	04:01/04:01	01:01/01:02	05:03/06:02	14:04/15:01
C25	01:01/01:01	15:01/44:02	03:03/05:01	04:01/19:01	01:03/01:03	06:03/06:03	13:01/13:02
C26	02:01/24:02	40:02/44:02	01:02/02:02	02:01/04:01	01:01/05:01	03:01/05:01	01:03/11:01
C27	03:01/03:01	07:02/51:01	01:02/07:02	03:01/04:01	01:01/01:02	05:01/06:02	01:01/15:01
C28	23:01/24:02	49:01/NT	03:03/07:01	01:01/04:01	02:01/05:01	02:02/03:01	07:01/11:01
C29	25:01/29:02	18:01/44:02	05:01/12:03	03:01/04:01	01:02/03:01	03:01/06:02	04:01/15:01
C30	02:01/02:01	15:01/44:02	03:04/05:01	04:01/20:01	01:01/03:01	03:02/05:03	04:01/14:01
C31	02:01/02:01	15:01/44:02	03:04/05:01	03:01/04:01	01:02/01:03	06:02/06:03	13:01/15:01
C32	03:01/11:01	35:01/56:01	04:01/04:01	04:01/04:02	01:02/01:02	06:02/06:02	15:01/15:01
C33	02:01/02:01	27:02/44:02	02:02/05:01	04:02/04:02	03:01/03:01	03:02/03:02	04:01/04:04
C34	01:01/23:01	08:01/49:01	07:01/07:01	01:01/01:01	03:01/05:01	02:01/03:02	03:01/04:05
C35	01:01/01:01	14:01/57:01	06:02/06:02	05:01/13:01	02:01/02:01	02:02/03:03	07:01/07:01
C36	01:01/02:01	08:01/57:01	06:02/07:01	03:01/04:01	02:01/05:01	02:01/03:03	03:01/07:01
C37	02:01/29:02	44:03/44:03	16:01/16:01	01:01/03:01	02:01/02:01	02:02/02:02	07:01/07:01
C38	02:01/11:01	08:01/55:01	03:03/07:01	02:01/04:01	03:01/05:01	02:01/03:01	03:01/04:07
C39	03:01/25:01	07:02/18:01	07:02/12:03	04:01/04:01	01:02/01:02	06:02/06:02	15:01/15:01
C40	01:01/02:01	08:01/08:01	07:01/07:01	01:01/03:01	05:01/05:01	02:01/02:01	03:01/03:01
C41	02:01/02:02	15:01/44:02	03:03/05:01	02:01/04:01	03:01/05:01	03:01/03:01	04:01/12:01
C42	02:01/03:01	07:02/07:02	07:02/07:02	01:01/04:01	05:01/05:01	02:01/02:01	03:01/03:01
C43	03:01/30:01	07:02/40:02	02:02/07:02	04:01/04:01	01:02/05:01	03:01/06:02	11:01/15:01
C44	01:01/26:01	08:01/38:01	07:01/12:03	01:01/04:01	01:02/05:01	02:01/06:04	03:01/13:02
C45	01:01/31:01	08:01/40:01	03:04/07:01	03:01/04:01	01:02/05:01	02:01/06:02	03:01/15:01
C46	29:02/31:01	07:02/49:01	07:01/07:02	03:01/03:01	01:02/03:01	03:02/06:02	04:05/15:01

Supplemental Table S5. Full HLA typing results of vancomycin tolerant controls. Legend: ID, patient identification; HLA, human leukocyte antigen; NT, not typed.

ID	Age	Sex	Race	Adverse Reaction	HLA-A*32:01
C51	57	F	W	Delayed rash	Negative
C52	51	M	W	Linear IgA Bullous Dermatosis	Negative
C53	47	M	W	Linear IgA Bullous Dermatosis	Negative
C54	28	F	W	Fixed Drug Eruption	Negative
C55	64	F	W	Acute Generalized Exanthematous Pustulosis	Positive

Supplemental Table S6. Demographics and HLA risk allele carriage for vancomycin ELISpot negative patients who developed non-DRESS adverse reactions to vancomycin.
Legend: ID, patient identification; Age, age at time of vancomycin treatment; HLA, human leukocyte antigen; F, female; M, male; W, Caucasian.

Supplemental Figure S1. T-cell Responses to Vancomycin Appear Persistent Months to Years after Acute Reaction. IFN-γ release ELISpot results after overnight stimulation at a vancomycin concentration of 500 μg/mL using peripheral blood mononuclear cells (PBMCs) from vancomycin DRESS patients. Vancomycin DRESS patients 9, 18 and 20 had multiple blood draws at time points distant from the initial reaction with repeat positive results. Counting from the start of DRESS symptoms, sample time points were at one month, two months, seven months and four years for patient 9, seven months and ten months for patient 18, twelve days and three months for patient 20, and two months, three months and six months for patient 21. Blood from time point 2 on patient 9 was drawn during steroid treatment, which likely dampened the ELISpot response. Patient 21 does not carry HLA-A*32:01 and had a persistently negative vancomycin ELISpot. Means of the replicates are plotted. Positive results are those above the dotted line intersecting the y-axis at 50 SFU/million cells.

Supplemental Figure S2. IFN-γ release ELISpot results using peripheral blood mononuclear cells from DRESS patients after overnight stimulation with all medications taken concurrently with vancomycin. All drugs were tested at μg/mL concentrations. Counting from the start of DRESS symptoms, sample time points were at one month, two months, seven months and four years for patient 9, seven months and ten months for patient 18, twelve days and three months for patient 20, and three months and six months for patient 21. Cells from Patient 21 who did not respond to vancomycin stimulation and does not carry the HLA-A*32:01 risk allele had a strong response to rifampin stimulation. Cells from Patient 17 who does carry the risk allele and did respond to *ex vivo* vancomycin stimulation also had a positive response to rifampin. However, cells from Patient 4 and other healthy donors did not respond to rifampin stimulation. No other patient samples tested released IFN-γ in response to stimulation from any other medication. Means of the replicates are plotted. Error bars indicate standard deviations of the mean after background subtraction. Positive results are those above the dotted line intersecting the y-axis at 50 SFU/million cells. Legend: Patient ID, patient identification; SFU, spot-forming units.

Supplemental Figure S3. Summary of vancomycin-exposed BioVU Cohort. A. None of the factors including age, longest treatment length, sex, race and overall adverse drug reaction rate differed significantly between the HLA-A*32:01 positive and HLA-A*32:01 negative groups (t-tests or Fisher exact tests as appropriate). In a joint logistic regression with vancomycin DRESS as response, none of the other factors including age, treatment length, sex and race were significant individually ($p>0.4$) or jointly ($p=0.95$) after adjusting for HLA-A*32:01, nor did they abrogate the significance of HLA-A*32:01 ($p=0.00014$, all based on likelihood ratio tests). 3/13 vancomycin DRESS cases overlapped with the previously identified BioVU cases (Patients 1, 6 and 7 from Table 1). **B.** The maximal treatment periods were similar for HLA-A*32:01 negative patients and HLA-A*32:01 positive patients who did not develop DRESS.