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BRIEF REPORT

Targeting Dalbavancin Inoculum Effect: Adjunctive Single Dose of Daptomycin

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ABSTRACT

Introduction: Daptomycin (DAP) has proven to be a viable alternative amid vancomycin resistance; however, the use of DAP post vancomycin treatment has led to the development of DAP non-susceptible (DNS) strains. Dalbavancin (DAL), a novel single-dosed lipoglycopeptide, has shown enhanced activity against highly resistant *Staphylococcus aureus* strains. However, on the basis of previous reports and our observations, DAL does not demonstrate similar activity at high versus low inoculum levels. Therefore, we hypothesized that addition of DAP even at minimal concentrations (single dose on day 1) will lower the inoculum to the level that can be cleared by dalbavancin.

Methods: Isolates from methicillin-resistant *S. aureus* (MRSA)-infected patients with varying susceptibility profiles were evaluated using broth microdilution methods. Two DNS–VISA strains (vancomycin intermediate resistant *S. aureus*) and one MRSA strain were further evaluated in a one-compartment PK/PD model using a high starting initial inoculum of 10^9 CFU/mL as well as low initial inoculum of 10^7 CFU/mL over 168 h to assess the activity of DAL and DAP monotherapy and in combination.

Results: Single therapies were not bactericidal when evaluated in the 168 h in vitro one-com-

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partment model with an initial inoculum of 10^9; however, the combination of DAL plus single dose of DAP resulted in enhanced killing at the end of the 168-h exposure. DAL single therapy caused reduction in colony counts down to detection limit (2 $\log_{10}$ CFU/ml) at a lower inoculum but did not show enhancement (< 2 $\log_{10}$ CFU/ml) at higher initial inoculums ($P < 0.01$) for all three strains. Similarly, DAP caused initial bacterial reduction up to 4 $\log_{10}$ CFU/ml with regrowth at about 32 h of exposure, which stayed at initial inoculum levels for the duration of the model for all three strains.
Conclusions: Dalbavancin inoculum effect is a major issue in bacterial infections with high bacterial loads and the combination of DAL plus single dose of DAP showed promise in eradicating resistant *S. aureus* strains at high inoculums.

Keywords: Dalbavancin; Combination therapy; High inoculum

Key Summary Points
Daptomycin monotherapy is not effective at high bacterial loads/inoculums.
Dalbavancin leads to complete clearance of infection at low bacterial loads/ inoculums.
Dalbavancin is not effective at high bacterial loads /inoculums.
A single dose of daptomycin adjunctive to a single dose of dalbavancin can lead to clearance of high bacterial loads.
This combination is a great choice for outpatient therapies associated with high bacterial loads.

INTRODUCTION

Antibiotic resistant strains of *Staphylococcus aureus* both in community and hospital settings continue to pose a serious public health threat [1–3]. In this context, methicillin-resistant *S. aureus* (MRSA) infections with reduced susceptibility to glycopeptide antibiotics, specifically vancomycin (VAN), have become more frequently identified [4, 5]. To mitigate this problem, the use of daptomycin (DAP) amid VAN resistance has become a viable option over the past decade [6].

Utilizing the lipopeptide pharmacophore allows DAP to evade the mechanisms of resistance that bacteria orchestrate to decrease VAN activity, making it a premiere antimicrobial selection for eradication of MRSA. However, DAP resistance after VAN exposure is very common especially in cases with a high bacterial load such as infective endocarditis (IE) or complicated bacteremia [7, 8]. As a result of continued DAP exposure, bacteria are able to acquire hetero-resistant subpopulations propagating the development of DAP resistance [9–11]. Similarly, emergence of DAP resistance after exposure to VAN is very common [12]. This may be in part due to cell wall thickening upon emergence of VAN intermediate resistance phenotype in VISA isolates. Cell wall thickening can potentially block DAP from reaching the cytoplasmic membrane [12].

Dalbavancin (DAL) is a novel, second-generation lipoglycopeptide antibiotic that exhibits concentration-dependent bactericidal activity against a variety of Gram-positive organisms, including MRSA [13, 14]. The long elimination half-life of dalbavancin allows for once-weekly dosing and, more importantly, maintains the serum concentrations of DAL above the minimum inhibitory concentration (MIC) of common pathogens [15].

The data for DAL in combination with other agents utilized for the treatment of MRSA is minimal; nevertheless, DAL has been shown to have synergistic activity with oxacillin and β-lactams against methicillin-resistant *S. aureus* and VISA infections [16–18]. The synergy represented here hints at the possibility of DAL being dosed in combination with DAP, allowing for enhanced eradication of bacterial load at higher inoculums. The standard of care dosage of DAL and DAP is a single dose of 1500 mg and daily dose of 10 mg/kg/day, respectively. In this paper while we are referring to standard of care

dosage of DAL, we only apply one dose of DAP on day 1. Similarly, combination of DAL + DAP refers to a single dose of 1500 mg DAL and a single dose of 10 mg/kg/day DAP.

The main objective of this study was to evaluate the impact of DAL and a single-dose DAP in combination against MRSA strains, in a simulated one-compartment pharmacokinetic/pharmacodynamic (PK/PD) model. We hypothesized that as a result of DAL's inoculum effect, addition of a single dose of DAP will reduce the bacterial load and efficacy of DAL while preventing emergence of resistance. This is especially important from the clinical standpoint regarding outpatient treatments where only one dose of both antibiotics can lead to clearance.

METHODS

Bacterial Strains

Ten patient isolates, characterized by various *S. aureus* phenotypes including MRSA, VISA (VAN intermediate resistant *S. aureus*), and DNS (DAP non-susceptible *S. aureus*)–VISA strains, were used for susceptibility testing and two DNS–VISA strains (8015 and D712) and one MRSA strain (2732) were selected for evaluation in a one-compartment PK/PD model.

Antibacterials and Media

DAL (analytical) was provided by its manufacturer (Allergan, Parsippany NJ) and DAP was purchased through Sigma Chemical Company (St. Louis, MO). In vitro experiments were performed in cation adjusted Mueller–Hinton broth (MHB; Difco, Detroit, MI) supplemented with 25 mg/L calcium and 12.5 mg/L magnesium. According to the recent CLSI guidelines [19], MHB was supplemented with 0.002% polysorbate 80 (Tween; Sigma Chemical Company, St. Louis, MO) for dalbavancin studies owing to the propensity of dalbavancin attachment to plastic (hydrophilic surfaces) present within several in vitro testing modalities. Brain heart infusion agar (BHIA; Difco Laboratories, San Jose, CA, USA) supplemented with vancomycin was used to subculture VISA strains to maintain this phenotype. As a result of the calcium-dependent nature of DAP, MHB supplemented with a total of 50 mg/L of calcium was used for susceptibility tests and PK/PD models [16–18, 20].

Susceptibility Tests

MIC values of DAL and DAP for all isolates were determined in duplicate by broth microdilution in duplicate at approximately $10^{5.5}$ CFU/ml (low inoculum) [19]. In addition, combination MIC testing was performed with DAL in the presence of DAP at 0.5 times the MIC against each respective organism. Further high inoculum MICs for DAL and DAP against DNS–VISA isolates were performed by broth microdilution in duplicate at approximately $10^{7.5}$ CFU/ml (high inoculum).

In Vitro One-Compartment PK/PD Models

Four regimens including a growth control, DAL simulating 1500 mg × day 1 (maximum free-drug concentration in serum), *f*Cmax, 30.1 mg/L; half-life ($t_{1/2}$) 187.4 h, protein binding 93% [15, 21, 22], DAP simulating 10 mg/kg q24h *f*Cmax 14.11 mg/L; $t_{1/2}$ 8 h, protein binding 94% [23]. For the DAL plus DAP combination model, DAL was supplemented as continuous infusion and DAP 10 mg/kg/day was administered as a bolus injection on the first day of the PK/PD model. The models were performed in duplicate to ensure the reproducibility of the results. A starting inoculum of 10^9 was targeted for each high inoculum experiment. To examine the inoculum dependency of DAL, each isolate was tested in a one-compartment in vitro model with an initial inoculum of 10^7 as well. Samples were collected in duplicate from each model and bacterial counts were determined by spiral plating appropriate dilutions using an automatic spiral plater (easy spiral; Interscience, Woburn, MA, lower limit of reliable detection of 100 CFU/ml) [24]. The free antibiotic concentration of each model was estimated by accounting for the proposed protein binding of

DAL and DAP (90% and 90–93%, respectively) [17, 25]. DAP and DAL concentrations were measured using a previously described high-performance liquid chromatography (HPLC) and bioassay method, respectively [18, 26]. The half-life, area under the curve ($AUC_{0–24\ h}$), and peak concentrations were determined using PK Analyst Software (version 1.10; MicroMath Scientific Software, Salt Lake City, UT) using the trapezoidal method to calculate the AUC.

Resistance Tests

Emergence of resistance was evaluated at the end of the models (7 days) by plating 100-µl samples from the model on plates supplemented with DAL or DAP at a concentrations equal to three times the MIC of DAL or DAP, respectively. Plates were examined for growth after 24 and 48 h of incubation at 36 ± 1 °C. Resistant colonies growing on screening plates were evaluated by broth microdilution methods to determine the MIC. If resistance was detected at the end of the model, additional screenings were performed to identify the first occurrence of resistance [27, 28].

Statistical Tests

One-way analysis of variance (ANOVA) with Tukey's post hoc test (Prism 9.1.0) software was applied to compare PD outcomes in the one-compartment models. *P* values of 0.05 or less were considered significant.

Ethical Approval

This study has not received any funding from Allergan or any other company and the ethics committee approval was not required. Additionally, there were no human or animal subjects involved in this study.

Table 1 High and low inoculum MIC values for the three selected MRSA isolates

Isolate	Low inoculum	High inoculum	Fold difference
8015	0.125	2	16
D712	0.063	0.25	4
2732	0.125	0.5	4

RESULTS

Susceptibility Tests

The obtained MIC values (at initial inoculum of $10^{5.5}$ CFU/ml) are listed in Tables 1 and 2. High inoculum ($10^{7.5}$ CFU/ml) MIC values against DAL for 8015 (DAP non-susceptible (DNS) and vancomycin intermediate *S. aureus* (VISA)), D712 (DNS–VISA), and 2732 (MRSA) strains were 2, 0.25, and 0.5 mg/L respectively (i.e., 16-, four-, and four-fold higher than low inoculum ($10^{5.5}$ CFU/ml)). Of note, high and low inoculum MIC values did not vary for DAP against the same strains [26]. Therefore, we did not perform DAP monotherapy against low vs. high initial inoculums [26].

Impact of DAL–DAP Combination Therapy in a PK/PD Model

In vitro PK/PD models with DAL monotherapy against 8015 did not demonstrate considerable reduction from initial inoculum (≤ 2 $\log_{10}$ CFU/ml). However, the combination of DAL plus DAP demonstrated clearance with reduction in colony counts down to detection limits noted at the 144-h time point until the end of exposure (Fig. 1a). DAP monotherapy in this strain caused 3 $\log_{10}$ CFU/ml reduction as early as 4 h after exposure which was accompanied by regrowth up to initial inoculum levels by the end of the exposure time. In the D712 strain, the DAL monotherapy caused less than 1.5 $\log_{10}$ CFU/ml reduction. The DAP monotherapy did demonstrate 4 $\log_{10}$ CFU/ml reduction activity at the 24-h time point which was accompanied

Table 2 List of tested isolates patient isolates with their corresponding MIC values

Phenotype	Drug Isolate #	DAL MIC (mg/L)	VAN	DAP	DAL–DAP
MRSA	2731	0.125	2	0.125	0.008
	2732	0.125	1	0.125	0.016
	2738	0.063	1	0.125	0.004
	2739	0.016	1	0.125	0.008
DNS–VISA	8015	0.125	4	4	0.0031
	6838 (D712)	0.063	4	4	0.063
DNS	3652 (684)	0.016	2	2	0.063
VISA	5989	0.5	4	2	0.25
	5991	0.5	4	0.25	0.25
	5998	1	4	0.5	0.25

VAN vancomycin, *DAP* daptomycin, *DAL* dalbavancin

by regrowth as early as the succeeding 48-h time point continued throughout the conclusion of the model at 168 h (Fig. 1b). The DAL plus DAP combination caused CFU reduction down to detection limit as early as 32 h, which was sustained for the whole exposure time. Regarding 2732 strain, similar to the two aforementioned strains, DAL monotherapy only caused 1.5 $\log_{10}$ CFU/ml reduction from the initial inoculum and DAP monotherapy demonstrated a similar trend of abrupt reduction up to 3 $\log_{10}$ CFU/ml accompanied by regrowth (at 32–48 h) up to and above initial inoculum levels (Fig. 1c). Interestingly, no resistant mutants (or elevated MIC) were recovered from any of the models with aforementioned organisms. We have previously tested these strains with therapeutic doses of DAP (i.e., 10 mg/kg/day for 7 days) and, despite a temporary reduction of initial inoculum, we observed regrowth of the infection up to initial inoculum levels [18].

Impact of the Initial Inoculum on Efficacy of DAL Monotherapy

Dalbavancin single therapy caused reduction in colony counts down to detection limit (2 $\log_{10}$CFU/ml) at a lower inoculum but did not show enhancement at higher initial inoculums ($P < 0.01$) for all three strains. We did note that DAL single therapy was effective at an initial inoculum of 10^7 CFU/mL against all three strains, resulting in a greater than 3 log-reduction in bacterial viability at 8 h, 24 h, and 48 h for D712, 2723, and 8015, respectively, which was sustained throughout the exposure time (Fig. 1d). In contrast, at the higher (10^9 CFU/ml) initial inoculum DAL did not demonstrate bactericidal activity against any of the strains (Fig. 1d).

PK Values

The achieved free peak concentration for DAL was 30.93 ± 1.12 mg/L (targeted value 30.1) and $t_{1/2}$ was 187.6 ± 0.47 h (targeted value 187.33 h) [29]. The bioassay standards had intraday coefficient of variation between 4.2% and 9.8%. The achieved free peak concentration for DAP 10 mg/kg was 13.38 ± 0.45 mg/L (targeted value 14.1) and $t_{1/2}$ was 8.21 ± 0.22 h (targeted value 8 h) [30]. The HPLC standards had intraday coefficient of variation between 0.5% and 5.4%. Table 3 shows the PK values for the two antibiotics. *f*AUC/MIC is calculated separately for each organism since they have

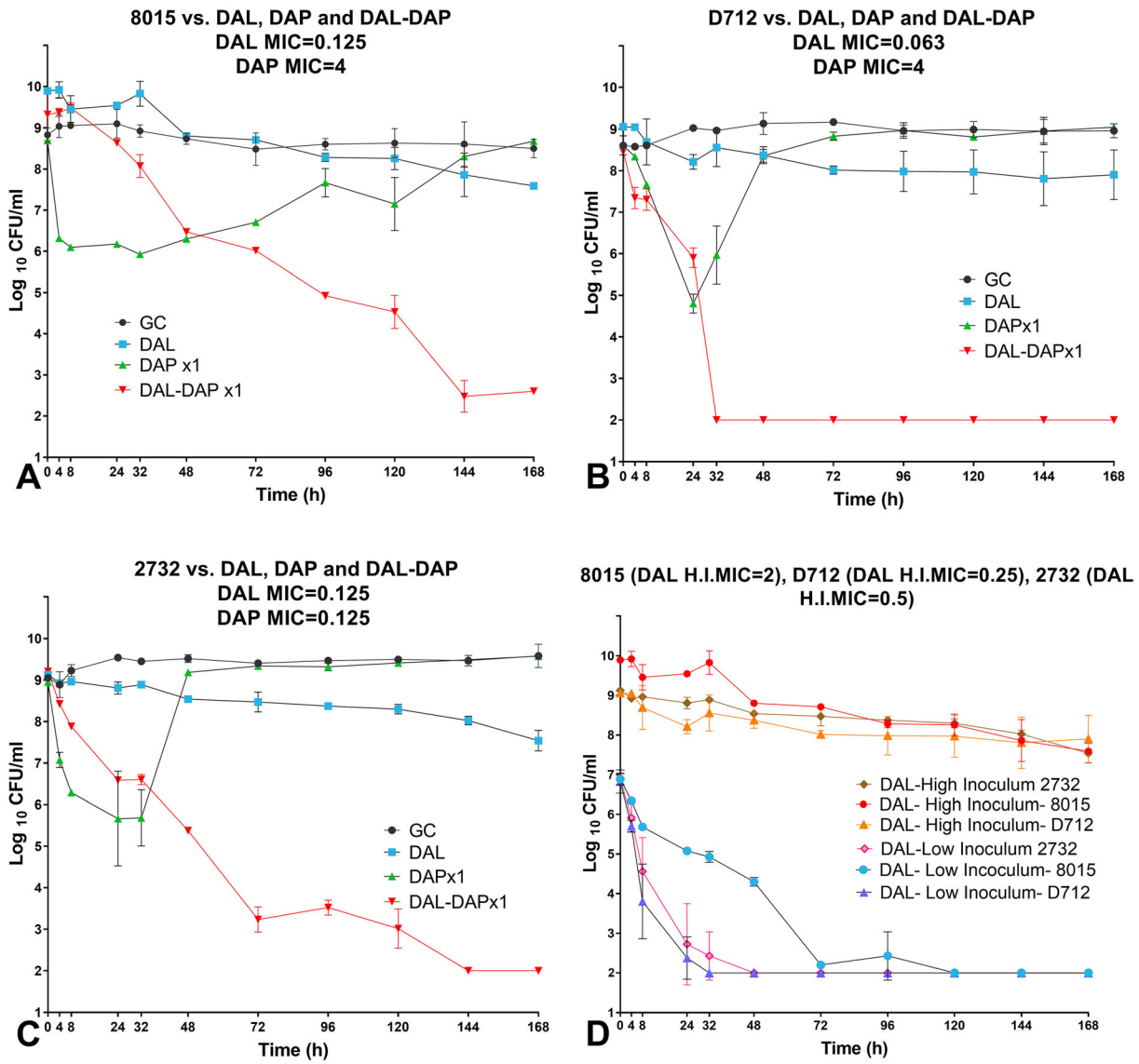

Fig. 1 PK/PD one-compartment models of GC, DAL 1500 mg × 1, DAP 10 mg/kg/day × 1 vs. (**A**) 8015 (**B**) D712 and (**C**) 2732. Comparison between high inoculum and low inoculum shows that DAL alone can kill to detection limit at low inoculum (10^6 CFU/ml) while it causes only 1–2-fold reductions in high inoculum (10^9 CFU/ml) (**D**). None of the single therapies showed enhanced activity vs. the two selected strains (8015 and D712) at high initial inoculum. *GC* growth control, *DAL* dalbavancin, *DAP* daptomycin, *H.I. MIC* high inoculum MIC

Table 3 PK parameters for each regimen

Regimen	fC_{max} (mg/L)	$t_{1/2}$	*f*AUC (mg h/L)
DAL	30.93 ± 1.12 mg/L (30.1)	187.6 ± 0.47 h (187.33 h)	8305.07 ± 0.53
DAP	13.38 ± 0.45 mg/L (14.1)	13.38 ± 0.45 mg/L (14.1)	172.60 ± 2.78

Targeted values are shown in parentheses

different MIC values. Regarding the combination model, DAL was administered by bolus injection while DAP was present in the media (at peak concentration for the first day as continuous infusion), and PK values for DAL were the same as for the single model (Table 3). However, the main difference is in *f*AUC/MIC values since the combination of the two antibiotics demonstrates lower MIC values leading to higher *f*AUC/MIC values.

DISCUSSION

The activity of many antibiotics depends on the initial density of cells in bacterial growth inhibition tests. The inoculum effect (IE) is described as a significant increase in MIC of an antibiotic when the number of organisms inoculated is increased [26, 28, 31, 32]. This phenomenon is detectable using the microbroth dilution test in the laboratory [19, 33]. From the clinical standpoint, bacterial loads in relevant infectious diseases can vary by several orders of magnitude and therefore IE can lead to significantly different therapeutic outcomes. While IE has been investigated for various antibiotics such as β-lactams [32], novel lipoglycopeptides such as DAL require further investigations in both in vitro and in vivo settings.

The favorable PK/PD properties of DAL, including a single weekly administration in addition to low resistance rate, make this antibiotic an excellent candidate for the treatment of high inoculum infections such as endocarditis, osteomyelitis [34], and vascular infection especially in outpatient settings; however, as a result of high volume of bacterial burden, sterilization with only DAL is not always achievable [35]. Here we have proposed a single dose of DAL in addition to a single dose of DAP to accomplish complete bacterial eradication. Previous research [36] used this combination in checker board microbroth dilution MIC tests and reported a synergistic effect in 67% (20/30) of the MRSA strains tested. To our knowledge there is no other report with combination of DAL + DAP in a dynamic in vitro PK/PD model against *S. aureus*; however, Belley et al. investigated the pharmacodynamics of single dose oritavancin (1200 mg) and daily high dose DAP (12 mg/kg/day) regimens against vancomycin resistant *Enterococcus faecium* isolates in an in vitro PK/PD model [37].

This strategy can potentially be beneficial in outpatient treatments, leading to shorter length of hospital stay, reduced antibiotic exposure/side effects, reduction in healthcare costs, and prevention of resistance and complications associated with catheters [38]. Furthermore, this anti-MRSA combination option may be more acceptable to practitioners that express discomfort in using an anti-staphylococcal β-lactam in combination with DAP or VAN when strains are not susceptible to either agent. Targeting inoculum effect is most appealing in conditions such as osteomyelitis and infective endocarditis, which often present with bacterial loads of 10^9 CFU/ml and higher, in which the development of decreased susceptibility to VAN and DAP is more likely because of the extended duration of therapy.

Thus, the ability of the single dose combination treatment to quickly lower the inoculum improves the performance of both agents and reduces the potential for the emergence of resistance. Furthermore, both DAP and DAL are relatively safe alternative agents and the exposure to DAP using this model is brief which aligns with antimicrobial stewardship efforts to shorten the duration of antibiotic exposure where possible.

We understand that there are only limited number of isolates (three strains) tested in PK/PD models and a wider range of isolates/phenotypes are needed in future studies. However, both antibiotics have shown efficacy as monotherapy or combination with other antibiotics in clinical practice [39, 40]. Additionally, it is important to investigate the mechanistic aspects of this synergy in the context of drug-pathogen interactions at the molecular level.

CONCLUSION

Considering the therapeutic enhancement represented by DAL plus DAP (single dose) combination, the pairing of these agents seems to be a

potential therapeutic option in treating infections with high bacterial loads and also offers the benefit of minimal DAP exposure potentially leading to shorter length of stay and prevention of recurrence/relapse. More structured studies are required to examine the efficacy and safety of DAP + DAL (other lipoglycopeptide) combinations in other phenotypes of *S. aureus*.

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Author Contribution. Razieh Kebriaei: drafting the manuscript, experimental design, data collection, data analysis software, PK analysis. Jacinda C. Abdul-Mutakabbir: drafting the manuscript, experimental procedures, assistance with data collection. Kyle C. Stamper: reviewing the manuscript, experimental procedures, assistance with data collection. Katherine L. Lev: reviewing the manuscript, experimental procedures, assistance with data collection. Michael J. Rybak: hypothesis generation, project supervision, reviewing the manuscript, experimental design, data analysis. Note: some of the authors have changed affiliation since completion of this research.

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Data Availability. Raw data regarding susceptibility tests are all included in manuscript draft. Any other datasets generated and/or analyzed during the current study including the CFU counts of the PK/PD models are available from the corresponding author on reasonable request. The software used in this study are publicly available and are stated in methods section.

Declarations

Conflict of Interest. Michael J Rybak has received research grant support, consulted, or provided lectures for Allergan, Bayer, Merck, Spero, Melinta, and Paratek. Michael J Rybak is also supported in part by NIAID R01 AI12400. All other authors have no financial disclosures.

Ethical Approval. This study has not received any funding from Allergan or any other company and the ethics committee approval was not required. Additionally, there were no human or animal subjects involved in this study.

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