Prospective Activity of PLG0206, an Engineered Antimicrobial Peptide, on Chronic Periprosthetic Joint Infection Total Knee Arthroplasty Components Ex Vivo: The Knee Explant Analysis (KnEA) Study

David Huang,a Dana M. Parker,b Jonathan B. Mandell,b Kimberly M. Brothers,b Charles G. Gish,b John A. Koch,b Nicholas Pachuda,a Despina Dobbins,a Jonathan Steckbeck,a Kenneth L. Urisb,c,d,e

aPeptilogics, Pittsburgh, Pennsylvania, USA
bArthritis and Arthroplasty Design Group, Department of Orthopedic Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, USA
cThe Bone and Joint Center, Magee-Womens Hospital of the University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA
dDepartment of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania, USA
eClinical and Translational Science Institute, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

ABSTRACT PLG0206 is an engineered antimicrobial peptide that has completed phase 1 clinical studies. A prospective study was completed on explanted implants from chronic periprosthetic joint infections (n = 17). At a concentration of 1 mg/mL for 15 min, there was a mean 4-log10 reduction (range, 1 to 7) in the bacterial CFU identified from the implants.

IMPORTANCE Chronically infected prosthetics of the knee were exposed to PLG0206, an engineered antimicrobial peptide, at a concentration of 1 mg/mL for 15 min. A mean 4-log10 reduction (range, 1 to 7) in the number of bacteria occurred, which may translate to improved clinical outcomes for persons with prosthetic joint infection of the knee.

KEYWORDS PJI, PLG0206, biofilms

Periprosthetic joint infection (PJI) is the leading cause of revision total knee arthroplasty, with high morbidity and mortality. The incidence of PJI is 1 to 3% in primary arthroplasty (1) and 3 to 10% in revision arthroplasty (2, 3). The incidence of PJI is projected to increase to 10,000 cases per year by 2030 (2). Debridement, antibiotics, and implant retention (DAIR) is a conservative treatment option for acute perioperative infection or an acute hematogenous infection of the knee. Unfortunately, this treatment is associated with failure rates of approximately 60% (4–10).

PLG0206 is a rationally designed, engineered antimicrobial peptide that is broad spectrum, rapidly acting, and active against antibiotic-tolerant biofilm (11, 12). The purpose of this study was to prospectively evaluate the ex vivo activity of PLG0206 at an expected clinical concentration of at least 1 mg/mL for 15 min on explanted components from total knee arthroplasty (TKA) PJI. The primary objective was to determine the reduction in bacterial count compared to that in untreated explants.

From 25 January 2021 to 5 August 2021, 17 adult patients presented with chronic bacterial total knee arthroplasty (TKA) PJI at the University of Pittsburgh Medical Center Healthcare System (UPMC); despite receiving chronic suppressive oral/intravenous antibiotics, they required a 2-stage revision procedure for explantation of components. All patients met the criteria for a diagnosis of PJI as defined by the 2018 International Consensus Meeting (13). The infected prosthetics were removed and deidentified. PLG0206 was diluted in phosphate-buffered solution (PBS) at a concentration of 1 mg/mL and adjusted to pH 7.40. The removed implant prosthetics were
infected prosthetics treated at least one antibiotic (Table 1). Ten out of seventeen samples (59%) of the chronically Escherichia coli (2/17; 12%). The majority (11/17; 65%) of the bacteria were resistant to Staphylococcus epidermidis for chronic bacterial PJI. The most common bacteria identified in CFU per milliliter. The remaining explanted implant material from the same blood agar plates to determine the antibiotic sensitivity and bacterial burden determination in CFU per milliliter. The remaining explanted implant material from the same patient was sonicated and served as an untreated control. Quantitative culture was directly performed on the untreated sonicate, and when this was not possible, the CFU were estimated from the reported clinical value in the medical record. If a sample was directly performed on the untreated sonicate, and when this was not possible, the CFU were estimated from the reported clinical value in the medical record. If a sample was estimated from the removed prosthetics during the 2-stage revision procedure (14). Previous in vitro studies using the same sonication protocol did not demonstrate significant improvements in PLG0206 activity (11). The sonicated solution was then serially diluted and plated onto Trypticase soy agar (TSA) II sheep blood agar plates to determine the antibiotic sensitivity and bacterial burden determination in CFU per milliliter. The remaining explanted implant material from the same patient was sonicated and served as an untreated control. Quantitative culture was directly performed on the untreated sonicate, and when this was not possible, the CFU were estimated from the reported clinical value in the medical record. If a sample was deemed “too numerous to count,” the CFU were determined by serially diluting the sonicate. Fourteen of seventeen (82.4%) patients received antibiotics prior to the 2-stage revision procedure (Table 1). Both Gram-positive and Gram-negative bacteria were identified from the removed prosthetics during the 2-stage revision procedure for chronic bacterial PJI. The most common bacteria identified from the prosthesis were Staphylococcus epidermidis (6/17; 35%), Staphylococcus aureus (3/17; 18%), and Escherichia coli (2/17; 12%). The majority (11/17; 65%) of the bacteria were resistant to at least one antibiotic (Table 1). Ten out of seventeen samples (59%) of the chronically infected prosthetics treated ex vivo with 1 mg/mL PLG0206 became culture negative. The infected prosthetics exposed to PLG0206 demonstrated a mean 4-log10 reduction (range, 1 to 7), whereas those not exposed to PLG0206 did not demonstrate any reduction in the bacterial burden. There were 7 samples, primarily at the beginning of the study, where estimates of the untreated bacterial burden were used because quantitative cultures were not available. All but one of these samples were culture negative after treatment with PLG0206. If these estimates are excluded from analysis, there was a mean 2.5-log10 reduction (range, 1 to 4).

**TABLE 1 Culture and CFU log reduction among bacteria identified from periprosthetic knee joints exposed and not exposed to PLG0206**

| Prosthetic no. | Preoperative antibiotics? | Culture | Resistance pattern | CFU/mL untreated | CFU/mL treated |
|---------------|---------------------------|---------|--------------------|------------------|----------------|
| 1             | Yes (cephalexin)          | Staphylococcus epidermidis | Clindamycin, erythromycin, gentamicin, oxacillin | 5 × 10⁶        | Culture negative |
| 2             | Yes (cephalexin)          | Staphylococcus epidermidis | Clindamycin, erythromycin, gentamicin, oxacillin | 5 × 10⁶        | Culture negative |
| 3             | No                        | Staphylococcus aureus (MRSA) | Oxacillin, erythromycin | 5 × 10⁶        | Culture negative |
| 4             | Yes                       | Staphylococcus haemolyticus | Clindamycin, gentamicin, oxacillin, rifampin, TMP/SMX | 7.3 × 10² | Culture negative |
| 5             | Yes (TMP/SMX)             | Staphylococcus aureus (MSSA) | Susceptible | 5 × 10⁶        | 12.5 × 10³ |
| 6             | Yes                       | Staphylococcus caprae      | Susceptible | 5 × 10⁷        | Culture negative |
| 7             | Yes (cefuroxime)          | Escherichia coli           | Ampicillin, ampicillin/sulbactam | 3.5 × 10⁴ | 6 × 10⁴ |
| 8             | Yes (cefuroxime)          | Escherichia coli           | Ampicillin, ampicillin/sulbactam | 3.5 × 10⁴ | 3 × 10⁴ |
| 9             | No                        | Staphylococcus epidermidis | Susceptible | 1.9 × 10⁳      | 9 × 10⁷ |
| 10            | Yes (doxycycline)         | Haemophilus parainfluenzae | Susceptible | 5 × 10⁷        | Culture negative |
| 11            | Yes (doxycycline)         | Haemophilus parainfluenzae | Susceptible | 5 × 10⁷        | Culture negative |
| 12            | Yes (ciprofloxacin)       | Enterococcus faecalis      | Susceptible | 1.3 × 10³      | 1 × 10⁷ |
| 13            | Yes (vancomycin)          | Staphylococcus aureus (MRSA) | Oxacillin, erythromycin | 1.1 × 10⁷ | Culture negative |
| 14            | Yes (vancomycin) and cefepime | Streplococcus dysgalactiae | Susceptible | 6 × 10⁷       | Culture negative |
| 15            | No                        | Staphylococcus epidermidis | Penicillin | 3.2 × 10⁴      | Culture negative |
| 16            | Yes (cephalexin)          | Staphylococcus epidermidis | Oxacillin, tetracycline, TMP/SMX | 3.2 × 10⁴ | Culture negative |
| 17            | Yes (cephalexin)          | Staphylococcus epidermidis | Oxacillin, tetracycline, TMP/SMX | 3.2 × 10⁴ | 1 × 10⁷ |

*MRSA, methicillin-resistant S. aureus; MSSA, methicillin-sensitive S. aureus; TMP/SMX, trimethoprim-sulfamethoxazole.

*Estimate of the CFU in untreated samples.
The microorganisms identified from the implant prosthetics are consistent with previously published studies (15, 16), in which *S. aureus* and coagulase-negative staphylococci (CoNS) contribute to between 50 and 60% of PJs. CoNS species, of which *S. epidermidis* was the most frequently identified pathogen of this group, are ubiquitous members of the human microbiome found on the skin. The relative pathogenicity of these microorganisms is unclear. However, both *S. aureus* and CoNS cause PJ primarily through their ability to adhere to prosthetic materials, produce biofilm, and produce virulence factors. In most studies, the most commonly isolated aerobic Gram-negative bacillus is *E. coli* (15, 16).

Many of the bacteria identified from the infected prosthetics were susceptible to the antibiotic the patient was prescribed prior to prosthetic removal. Biofilm formation may have protected the bacteria from the antibiotics as well as the host immune system, making treatment of the infection difficult without a biofilm-directed treatment strategy. Given the limitations of treatments currently available, this mandates surgical intervention, in many cases including complete removal of the prosthesis, in order to achieve infection control. The limited susceptibility of bacteria in biofilm is related to their low growth rate, the presence of resistant bacterial subpopulations, and a micro-environment within the biofilm that impairs antimicrobial activity (17–19). Biofilm formation may also explain why some normal floral organisms traditionally considered “harmless” (e.g., coagulase-negative staphylococci) become pathogens when they are grown in the presence of foreign bodies.

In general, antimicrobial therapy should be pathogen directed and guided by the results of antimicrobial susceptibility testing, where applicable. However, most antimicrobials do not have antibiofilm activity. PLG0206 has broad-spectrum activity, including activity against multidrug-resistant bacteria that cause PJ, has potent activity against antibiotic-resistant biofilm, does not have significant local or systemic toxicity in the therapeutic range of dosing in animal models, and has pharmacokinetics with a half-life of more than 12 h (11, 12). In this study, after 15 min of exposure to an expected clinical concentration of 1 mg/mL, a mean 4-log_{10} reduction in CFU counts was observed among the prosthetics exposed to PLG0206 in comparison to those that were not. These findings support the development of PLG0206 as a local irrigation solution of at least a 1 mg/mL concentration in the wound cavity for 15 min for patients undergoing treatment of a PJ occurring after TKA or total hip arthroplasty (THA).

**ACKNOWLEDGMENTS**

This study was designed, conducted, analyzed, and written up by Peptilogics. K.L.U. has equity and serves as a consultant to Peptilogics. CARB-X funding for this research was sponsored through cooperative agreement number 4500003336/IDSEP160030 from ASPR/BARDA and by an award from Wellcome Trust.

The content is solely the responsibility of the authors and does not necessarily represent the official views of CARB-X or any of its funders.

**REFERENCES**

1. Konigsberg BS, Hartman CW, Hewlett AL, Garvin KL. 2014. Current and future trends in the diagnosis of periprosthetic hip infection. Orthop Clin North Am 45:287–293. https://doi.org/10.1016/j.ocl.2014.03.002.
2. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. 2007. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am 89:780–785. https://doi.org/10.2106/JBJS.F00222.
3. Kurtz SM, Ong KL, Schmier J, Mowat F, Saleh K, Dybvik E, Kärnholm J, Garellick G, Havelin LI, Furnes O, Malchau H, Lau E. 2007. Future clinical and economic impact of revision total hip and knee arthroplasty. J Bone Joint Surg Am 89:144–151. https://doi.org/10.2106/JBJS.G.00587.
4. Urish KL, Bullock AG, Kreger AM, Shah NB, Jeong K, Rothenberger SD. 2018. A multicenter study of irrigation and debridement in total knee arthroplasty periprosthetic joint infection: treatment failure is high. J Arthroplasty 33:1154–1159. https://doi.org/10.1016/j.arth.2017.11.029.
5. Hartman MB, Fehring TK, Jordan L, Norton HJ. 1991. Periprosthetic knee sepsis: the role of irrigation and debridement. Clin Orthop Relat Res 273:113–118.
6. Bradbury T, Fehring TK, Taunton M, Hanssen A, Azzam K, Parvizi J, Odum SM. 2009. The fate of acute methicillin-resistant *Staphylococcus aureus* periprosthetic knee infections treated by open debridement and retention of components. J Arthroplasty 24:101–104. https://doi.org/10.1016/j.arth.2009.04.028.
7. Fehring TK, Odum SM, Berend KR, Jiranek WA, Parvizi J, Bozic KJ, Della Valle CJ, Goeo TJ. 2013. Failure of irrigation and debridement for early postoperative periprosthetic infection. Clin Orthop Relat Res 471:250–257. https://doi.org/10.1007/s11999-012-2373-9.
8. Bryan AJ, Abdel MP, Sanders TL, Fitzgerald SF, Hansen AD, Berry DJ. 2017. Irrigation and debridement with component retention for acute infection after hip arthroplasty: improved results with contemporary management. J Bone Joint Surg Am 99:2011–2018. https://doi.org/10.2106/JBJS.16.01103.
9. Deirmengian C, Greenbaum J, Lotke PA, Booth RE, Lonner JH, Booth Lonner RE. 2003. Limited success with open debridement and retention of components in the treatment of acute Staphylococcus aureus infections after total knee arthroplasty. J Arthroplasty 18:22–26. https://doi.org/10.1016/S0883-5403(03)00288-2.

10. Marculescu CE, Berbari EF, Hanssen AD, Steckelberg JM, Harmsen SW, Mandrekar JN, Osmon DR. 2006. Outcome of prosthetic joint infections treated with debridement and retention of components. Clin Infect Dis 42:471–478. https://doi.org/10.1086/499234.

11. Mandell JB, Deslouches B, Montelaro RC, Shanks RMQ, Doi Y, Urish KL. 2017. Elimination of antibiotic resistant surgical implant biofilms using an engineered cationic amphipathic peptide WLBU2. Sci Rep 7:18098. https://doi.org/10.1038/s41598-017-17780-6.

12. Deslouches B, Montelaro RC, Urish KL, Di YP. 2020. Engineered cationic antimicrobial peptides (eCAPs) to combat multidrug-resistant bacteria. Pharmaceutics 12:501. https://doi.org/10.3390/pharmaceutics12060501.

13. Parvizi J, Tan TL, Goswami K, Higuera C, Della Valle C, Chen AF, Shohat N. 2018. The 2018 definition of periprosthetic hip and knee infection: an evidence-based and validated criteria. J Arthroplasty 33:1309–1314. https://doi.org/10.1016/j.arth.2018.02.078.

14. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, Mandrekar JN, Cockerill FR, Steckelberg JM, Greenleaf JF, Patel R. 2007. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 357:654–663. https://doi.org/10.1056/NEJMoa061588.

15. Tande A, Patel R. 2014. Prosthetic joint infection. Clin Microbiol Rev 27:302–345. https://doi.org/10.1128/CMR.00111-13.

16. Pulido L, Ghanem E, Joshi A, Purtill JJ, Parvizi J. 2008. Periprosthetic joint infection: the incidence, timing, and predisposing factors. Clin Orthop Relat Res 466:1710–1715. https://doi.org/10.1007/s11999-008-0209-4.

17. Molina-Manso D, del Prado G, Ortiz-Perez A, Marrubia-Cobo M, Gomez-Barrena E, Cordero-Ampuero J, Esteban J. 2013. In vitro susceptibility to antibiotics of staphylococci in biofilms isolated from orthopaedic infections. Int J Antimicrob Agents 41:521–523. https://doi.org/10.1016/j.ijantimicag.2013.02.018.

18. del Pozo JL, Patel R. 2007. The challenge of treating biofilm-associated bacterial infections. Clin Pharmacol Ther 82:204–209. https://doi.org/10.1038/sj.clpt.6100247.

19. Stoodley P, Nistico L, Johnson S, Lasko LA, Baratz M, Gahlot V, Ehrlich GD, Kathju S. 2008. Direct demonstration of viable Staphylococcus aureus biofilms in an infected total joint arthroplasty. A case report. J Bone Joint Surg Am 90:1751–1758. https://doi.org/10.2106/JBJS.G.00838.