CMTX-101 Treatment in a Mouse Model of Periprosthetic Joint Infection

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INTRODUCTION: Periprosthetic joint infection (PJI) is a devastating complication of arthroplasty, largely due to biofilms. No adequate treatment exists to address biofilms in bacterial infections. Biofilms are the preferred bacterial lifestyle, protecting bacteria from antibiotic and immune clearance. CMTX-101 is a clinical-stage monoclonal antibody that targets a key structural component of biofilm, DNABII proteins, leading to rapid biofilm collapse **(Figure 1A)**(1). IV infusion of CMTX-101 was safe and tolerated in a Phase 1 study in healthy volunteers and patients with pneumonia; it is being evaluated in a Phase 1b/2a study in people with cystic fibrosis chronically infected with *Pseudomonas aeruginosa* (ClinicalTrials.gov: NCT06159725). ***Objective:*** Determine if adjunctive IV CMTX-101 improves bacterial clearance compared with antibiotic (vancomycin) alone in a mouse PJI model.

METHODS: Procedures were IACUC-approved. Mice were housed in SPF conditions on a 12 h light/dark cycle with chow and water ad libitum and monitored daily. 12-week C57BL/6 male mice (n=95) received surgically placed right femoral intramedullary implants (0.6 mm diameter × 7 mm length stainless steel K-wire; Zimmer Biomet) under 2% isoflurane and buprenorphine (0.1 mg/kg) **(Figure 1B–1C)**(2,3). Proper implant placement was confirmed with X-ray (**Figure 1C**); mice with malpositioned implants (n=3) were excluded and euthanized, leaving 92 mice. In this model, male mice of this age have been used since 2010 (2,3), providing reproducible femoral canal geometry for implant placement. Future work will validate the model in females with sex-specific reaming tools and implant dimensions. At the open implant surgical site, 72 mice received 1×10³ CFUs of bioluminescent *Staphylococcus aureus* (*S. aureus)* Xen36; n=20 served as non-infected controls. Inoculum was prepared from overnight culture, quantified by OD_{600}, diluted to target concentration, and verified by back-titer plating. After 7 days, infected mice were randomized by cage (n=24 group) for treatment between Day 7-14 into the following ***groups***: ***(i)*** vehicle (0.9% saline subcutaneous (SC), twice daily), ***(ii)*** vancomycin (110 mg/kg SC, twice daily; Santa Cruz Biotech), or ***(iii)*** vancomycin + CMTX-101 (30 mg/kg IV via tail vein on Days 7 and 10; Clarametyx Biosciences). All except the CMTX-101 group received IV IgG1 isotype control (Bio X Cell). Also, ***(iv)*** non-infected controls received vehicle + isotype IgG. Treatment ended Day 14; mice were followed to Day 28. ***Outcomes*** included: ***(A)*** bioluminescence imaging (BLI; all mice; n=92) and ***(B)*** colony-forming unit (CFU) recovery from peri-implant tissue and implants (n=14–15 per infected group; non-infected n=10). Group sizes of 15 were chosen based on prior work with this model, which demonstrated that this number is sufficient to detect ≥1-log differences in bacterial burden with >80% power (3). For CFU assays, peri-implant bone (distal ¼ femur to proximal ¼ tibia/fibula) and surrounding soft tissue, excluding skin, were homogenized in collagenase D. Implants were removed from the femur, vortexed, sonicated, and re-vortexed. Both tissue homogenates and implant suspensions were subjected to serial dilution, plating, and overnight culture, with recovery of bioluminescent *S. aureus* Xen36 confirmed by BLI. ***(C)*** SEM, histology, and µCT (n=9–10/group) assessments are ongoing. ***Statistics:*** CFU counts assessed for normality (Shapiro–Wilk). Normally distributed data analyzed by one-way ANOVA with Tukey's post-hoc test; non-normal data by Kruskal–Wallis with Dunn's correction. Extreme outliers (>3× IQR) excluded. Culture-negativity (no growth from implant or tissue) compared using Fisher's exact or chi-squared test as appropriate. BLI analyzed by repeated-measures ANOVA with Tukey's post-hoc test. Analyses performed in SPSS v29 and Prism v10. Data presented as mean ± SEM; p<0.05 considered significant.

RESULTS: All 92 mice completed treatment and follow-up to Day 28. ***BLI:*** At Day 14, both the vancomycin and vancomycin + CMTX-101 groups showed suppressed BLI signal, while the vehicle group BLI signal remained high. By Day 28, the vancomycin group BLI signal relapsed to vehicle-like levels (20,067 ± 5,050 Δ radiance), whereas the vancomycin + CMTX-101 group maintained near-baseline suppression of BLI signal (5,150 ± 1,801; p=0.04) (**Figure 2A–B**). ***CFU:*** Vancomycin + CMTX-101 reduced bacterial burden compared with vancomycin alone by 1.6 log (98%) in tissue (82,417 ± 54,005 vs. 2,038 ± 777 CFUs/g; p=0.04) **(Figure 2C)** and 2.0 log (99%) on implants (796 ± 377 vs. 8 ± 4 CFUs; p=0.02) (**Figure 2D**). Tissue CFU culture-negativity was observed with vancomycin + CMTX-101 (1/15; 7%) compared with none in the vancomycin group (0/14; *ns*), while implant culture-negativity occurred in 5/15 (33%) with vancomycin + CMTX-101 versus 0/14 (0%) with vancomycin alone (p=0.04). ***SEM:*** Vehicle and vancomycin implants showed dense *S. aureus* biofilm, whereas vancomycin + CMTX-101 implants exhibited no or minimal biofilm with leukocytes lining the implant surface (**Figure 3**).

DISCUSSION: Adjunctive CMTX-101 reduced bacterial burden versus vancomycin alone, achieving implant culture-negativity in one-third of mice after only 1 week of therapy. Clinical PJI therapy typically requires ≥ 6 weeks of antibiotics; extended regimens with CMTX-101 may further improve eradication. In *S. aureus* infections, abscess formation and potential host cell intracellular persistence represent barriers to drug delivery and efficacy, indicating that adjunctive CMTX-101 may be most effective with extended duration therapy or combined with PJI surgical management (e.g., DAIR or 1-2 stage revision).

SIGNIFICANCE / CLINICAL RELEVANCE: This first in vivo evaluation of CMTX-101 in PJI demonstrates biofilm disruption and enhanced bacterial clearance, supporting its potential as the first-in-class systemic biofilm dismantling therapy, which could potentially improve PJI treatment outcomes.

REFERENCES: (1) Rogers JV. *Antibiotics.* 2022;11(1); (2) Bernthal NM. *PLoS One.* 2010;5:e12580; (3) Hamilton JL. *J Orthop Res.* 2025;43:671–81.

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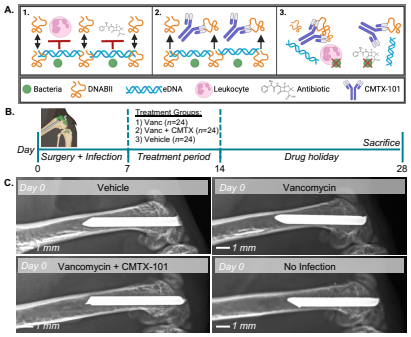

Figure 1. **(A)** Mechanism of CMTX-101: DNABII proteins stabilize biofilm by binding extracellular DNA; CMTX-101 disrupts this interaction, leading to collapse of the biofilm scaffold and enhanced clearance by host defenses and antibiotics. **(B)** Study timeline: surgery and infection on Day 0; treatment with vancomycin ± CMTX-101 on Days 7–14; drug-free period Days 14–28; sacrifice at Day 28. Groups included vancomycin (n=24), vancomycin + CMTX-101 (n=24), and vehicle (n=24); an additional non-infected group (n=20, not shown). **(C)** Representative X-rays confirming femoral implant placement on Day 0.

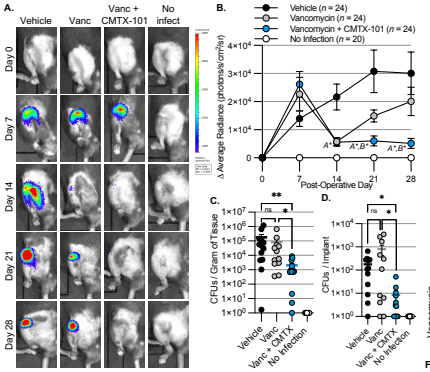

Figure 2. **(A)** Representative bioluminescence imaging (BLI) of infected knees. **(B)** Quantification of change in BLI signal over time from baseline (Day 0). **(C)** Bacterial burden (CFU) recovered from peri-implant tissue. **(D)** Bacterial burden (CFU) recovered from the implant surface. Vanc = vancomycin; Data shown as mean ± SEM. *p < 0.05; **p < 0.01.

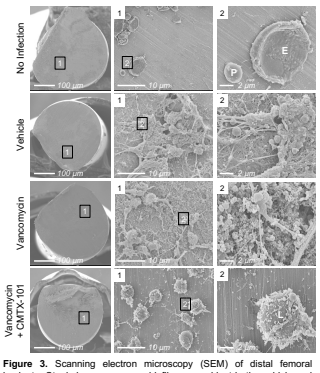

Figure 3. Scanning electron microscopy (SEM) of distal femoral implants. *Staphylococcus aureus* biofilm was evident in the vehicle and vancomycin groups, characterized by 0.5–1.5 µm cocci in grape-like clusters embedded within extracellular polymeric substance (EPS) and associated EPS projections. E = erythrocyte; P = platelet; L = leukocyte.