

# The 2022 Military Health System Research Symposium (MHSRS)

## **Focus Area:**

### ***Antimicrobial Development to Counter Wound Infections in Military Personnel Infectious***

#### **Abstract Title:**

***D-PLEX, a doxycycline releasing antimicrobial product for surgical site wounds, reduces surgical site infection rates and drug resistant-bacteria in surgical trauma injuries***

#### **Introduction**

Combat trauma injuries are characterized with high levels of bacterial contamination in both soft tissues and bone, which can result in infection. These infections can become complicated, lead to multiple surgeries, and result in amputation of infected extremities, reducing combat military personnel readiness and significantly increasing the cost of medical treatment and recovery time. It is estimated that infections complicate ~25% of Gustilo and Anderson grade III open tibia fractures in US combat casualties even with standard of care (SOC) treatment with systemic antibiotics. This is attributed to low penetration of antibiotics from the blood stream into the contaminated/infected wound. Therefore, adjunct local antibiotic administration to the SOC for this wound class may provide optimal antibiotic exposure at the wound area and decrease wound infection rates and associated complications.

#### **Materials**

D-PLEX is a local bio-absorbable antibiotic delivery platform which can complement the SOC treatment with constant exposure to local high concentrations of doxycycline over a period of four weeks at the wound site. D-PLEX is applied once, directly into the wound at the surgical site in a paste form to provide a localized, and well-controlled release of high dose doxycycline for prevention of trauma and surgical related wound infections, including infections by the most common surgical site infection (SSI) bacteria and multi-drug resistant (MDR) strains. D-PLEX was granted two fast track and two Qualified Infectious diseases designations (in abdominal and sternotomy procedures) by the FDA and a Breakthrough Therapy in abdominal surgeries. D-PLEX was evaluated in both bone and soft tissues phase 2 studies and is currently being evaluated in a phase 3 study for the prevention of soft tissue surgical site infections in patients undergoing colorectal resection abdominal surgery.

#### **Methods & Results**

In vitro and in vivo activity of D-PLEX was evaluated against common bacteria associated with SSIs, including drug resistant strains. In clinical settings, D-PLEX safety and efficacy in SSI prevention was evaluated in a phase 2 randomized clinical trial in patients undergoing contaminated abdominal colorectal resection surgeries.

**D-PLEX *In Vitro* Antimicrobial Activity.** We evaluated: *Klebsiella aerogenes* (ATCC #13048), *Staphylococcus epidermidis* coagulase negative (CoN) (ATCC #35984), doxycycline resistant *Klebsiella*

*pneumoniae* (ATCC #700603), vancomycin-resistant *Enterococcus faecium* (ATCC #700221), *Escherichia coli* (ATCC #25922) and doxycycline resistant *Staphylococcus aureus* (MRSA) (ATCC #33591). Bacterial Suspensions ( $5 \times 10^5$  CFU/mL) were individually challenged with two-fold serial dilutions of D-PLEX in 10 mL of 50% Mueller-Hinton Broth. The minimum bactericidal concentration (MBC) of D-PLEX ranged between 0.975 and 31.3 mg/mL, demonstrating >3-log reduction relative to untreated control at 24 hours in all the tested bacteria. The MBC for doxycycline-sensitive bacteria i.e., *E. coli*, *S. epidermidis*, and Vancomycin-resistant enterococci, was much lower than the MBC for doxycycline-resistant bacteria *K. pneumoniae* and MRSA (7.8, 3.9, 0.975 vs 31.2, 15.6 mg/mL respectively).

**D-PLEX Antibacterial Activity in Rabbit Sternal and Abdominal Wound Infection Models.** Animals underwent surgery to produce either a median sternal incision (involving both soft and bone tissues) or a partial thickness abdominal incision (soft tissue). D-PLEX or placebo were administered into the incision site and challenged with different bacterial inoculum prior to incision surgical closure. D-PLEX significantly reduced the bacterial loads of all clinically relevant bacteria tested in both models compared to control animals, regardless of sensitivity to doxycycline. In the sternal wound model, the Log<sub>10</sub> reduction (LogR) of bacterial load for doxycycline-resistant bacteria *K. pneumoniae* (MIC=31.3 ug/mL) and MRSA (MIC=15.6 ug/mL) was even greater than doxycycline-sensitive bacteria *S. epidermidis* (MIC=0.49 ug/mL), (LogR of 2.14, 3.09, 1.75, p<0.0001 respectively). D-PLEX was similarly effective against MRSA in the abdominal wound model (LogR >3, p<0.0001). Significant reductions were also demonstrated in sternal and abdominal models with *E. coli* (LogR 8.84, 5.52, p<0.0001). Local pharmacokinetic measurements of doxycycline released from D-PLEX in both wound models showed higher concentrations than the MIC values in doxycycline-sensitive-bacteria, and similar MIC levels in doxycycline-resistant bacteria. Plasma values were 3 orders of magnitudes lower than local values.

Gross necropsy and histopathology evaluations were assessed in animals that were challenged with the doxycycline resistant MRSA for 7 days. These observations revealed purulent white matter discharge in all the untreated animals but not in the animals treated with D-PLEX. Histopathology sternal samples of the control animals indicated early fibrosis (granulation tissue) with neovascularization and chronic-active inflammation in all animals. In the D-PLEX treated group, the number of acute inflammatory cells (polymorphonuclear cells) was drastically reduced compared to the untreated samples.

**D-PLEX Antimicrobial activity in Phase 2 studies.** A phase 2 clinical trial in patients undergoing elective colorectal surgery evaluated SSI prevention by D-PLEX. Patients were randomized 1:1 to D-PLEX plus SOC or SOC alone (**ClinicalTrials.gov identifier NCT03633123**). The SOC included prophylactic IV antibiotics 30-60 minutes prior to surgery, and D-PLEX was applied to the surgical incision at the time of surgical closure. The study results indicated a 64% reduction in SSI rate in the D-PLEX group (N=7/88 [7.9%]) vs SOC alone (N=20/91 [21.9%]); p<0.05. There were no mortalities in the D-PLEX arm, and in the SOC, 3 (3.0%) within 30 days (p=0.1213) and total 5 (5%) within 60 days (p=0.029). D-PLEX was well tolerated. No serious adverse events were deemed related to D-PLEX. Minimal systemic exposure from D-PLEX was confirmed in patients treated with D-PLEX (126 mg doxycycline hyclate) where doxycycline exposure (mean C<sub>max</sub>) was 183ng/mL, significantly lower than the reference standard of 200mg doxycycline oral dose, 3160 ng/mL.

The SSI causative organisms isolated from the infected wound sites were evaluated between the treatment arms. The percentage of MDR organisms (MDRO) were very high (~70% of the isolated bacteria, some were

resistant to doxycycline) and similar between the study arms suggesting that D-PLEX reduction of SSI rate was independent of the enriched microenvironment that included doxycycline resistant bacteria. To determine if prolonged D-PLEX exposure may select for doxycycline resistance or MDROs, patients were assessed by rectal swab tests before and after D-PLEX treatment. There was no significant difference in rectal swab colonization with MDROs between groups.

D-PLEX safety was also demonstrated in cardiac surgery patients undergoing median sternotomy where D-PLEX was applied directly over the sternum wound (**ClinicalTrials.gov identifier NCT03633123**, Kachel E, et al 2020). Negligible plasma concentration of doxycycline was observed following the administration of different D-PLEX doses, further supporting the minimal systemic exposure from local D-PLEX treatment. There were no sternal infections in the D-PLEX group (0/60) while there was one patient with a sternal infection in the control group (1/21, 4.8%).

## Conclusions

Unlike systemic antibiotic treatment, localized treatment can exceed the MIC and MBC levels of drug resistant bacteria without high systemic exposure and without affecting the incidence of postoperative colonization by MDROs. Additionally, preliminary clinical data support the hypothesis, and additional studies are warranted. As such, D-PLEX may be a promising addition to combat trauma SSI prevention bundles for reducing SSIs without the risks associated with systemic antibiotic exposure.

**Abstract Disclaimer: Limit of 350 characters (includes spacing)**

This document contains confidential, proprietary, or privileged information that is exempt from public disclosure. Noam Emanuel is an employee and a stockholder of PolyPid Ltd. These studies were funded by PolyPid, Ltd, Petach Tikva, Israel.

**Learning Objectives: Three (3) are required.** These should answer the question - What do you expect the attendee to be able to do at the end of the session? Each learning objective should start with an action verb (e.g., Describe, Analyze, Discuss, etc). Each learning objective has a limit of 255 characters (includes spacing).

Describe the advantages of local, extended, antibiotic exposure in terms of safety and efficacy in preventing infections by drug-resistant pathogens in surgical trauma wounds.

Analyze anti-microbial efficacy data from in vitro, in vivo, and clinical reports in trauma wound treatment.

Discuss the benefits of adding a local anti-microbial drug such as D-PLEX to the trauma wound SOC in significantly reducing military-personnel soft tissue and bone infections.