In vitro evaluation of chimeric antigen receptor T cells to target implant-associated *Staphylococcus aureus* biofilms

Martijn Riool[1,#], Saima Shaikh[4,#], Raphaëlle Youf[1], Hong Pan[3], Markus Barden[3], Hinrich Abken[3], Volker Alt[1], Markus Feuerer[4,5], Uwe Ritter[4,5]

[1]Department of Trauma Surgery, University Hospital Regensburg, Regensburg, Germany, [2]Division of Genetic Immunotherapy, Leibniz Institute for Immunotherapy, Regensburg, Germany, [4]Division of Immunology, LIT – Leibniz Institute for Immunotherapy, Regensburg, Germany, [5]Chair for Immunology, University of Regensburg, Regensburg, Germany.

Introduction & Aim:

Prosthetic joint infections (PJIs) caused by *Staphylococcus aureus* remain clinically challenging due to the capacity of biofilms to confer antibiotic tolerance and evade immune-mediated clearance. Consequently, immune-related approaches might represent promising alternatives to address PJI-associated disease. We aimed to develop an *in vitro* framework to study the efficacy and delivery potential of chimeric antigen receptor (CAR)-T cells against implant-*associated S. aureus* biofilms.

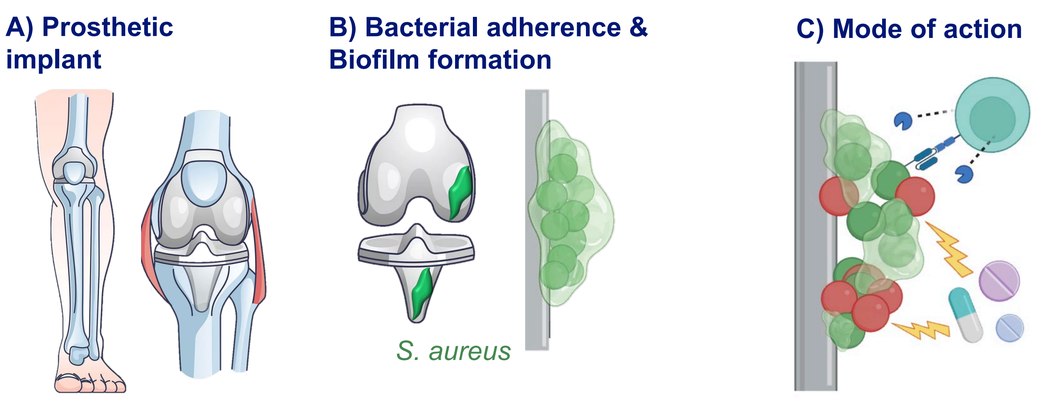

Figure 1: Multi-modal therapeutic strategy targeting biofilm-associated PJI.
(A) Prosthetic implant placement. **(B)** Bacterial adherence & biofilm formation: *Staphylococcus aureus* (green spheres) establishes a biofilm resistant to conventional antibiotics. **(C)** CAR T cell-based intervention in combination with antibiosis: CAR-T cells (teal circle) infiltrate biofilm and deliver antimicrobial peptides and enzymes that disrupt extracellular polysaccharide matrix architecture (red spheres = lysed bacteria), restoring antibiotic penetration. This synergistic approach overcomes biofilm-mediated resistance through immunocellular targeting coupled with matrix-degrading payloads.

Methods:

We established a standardized *in vitro* biofilm model of *S. aureus* grown in 8-well chamber slides under defined conditions. Primary human T cells were transduced with a lipoteichoic acid (LTA)-specific CAR and co-incubated with viable biofilms. LTA-CAR-T activation was assessed by ELISA quantification of IFN-γ and IL-2. To further study biofilm perturbation by CAR-directed release of biofilm-degrading payloads, we developed an automated imaging platform integrating live–dead bacterial staining, automated cellular-density mapping, and microscopy-based assessments of matrix integrity, providing multidimensional quantitative readouts of biofilm treatment response.

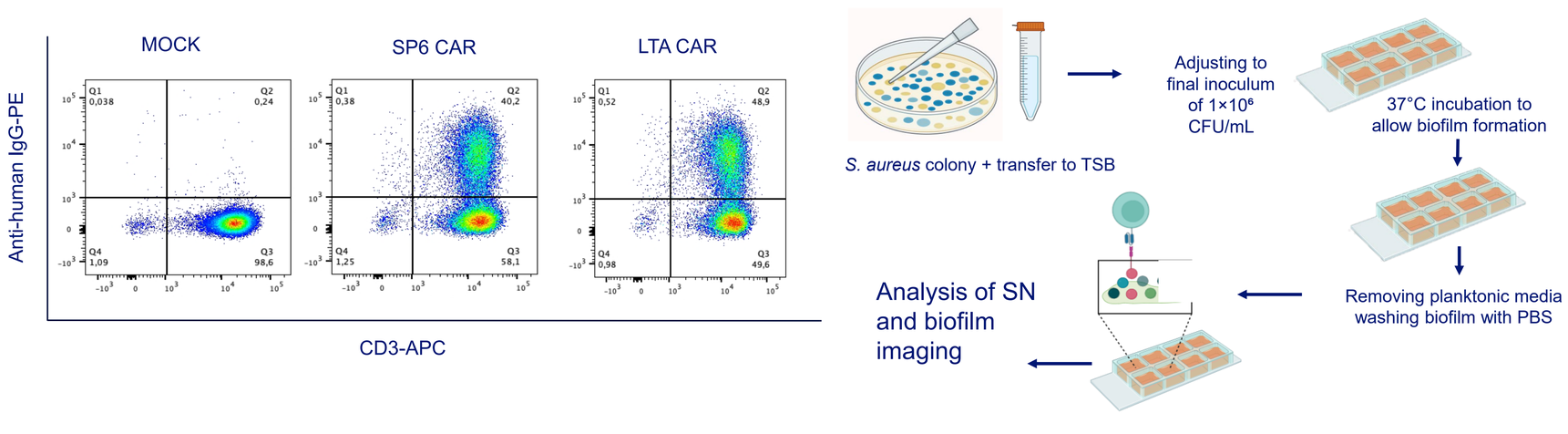

Figure 2: CAR-T cell development and biofilm engagement assay.
(A) CAR-T cell generation: Flow cytometry analysis confirmed successful transduction of SP6 CAR (CAR control, specific for the trinitrophenyl hapten) and LTA CAR constructs, with >40% CAR⁺ cells (upper-right quadrant), compared with MOCK control cells (no CAR expression). **(B)** Biofilm formation and CAR-T cell exposure: *S. aureus* was cultured on 8-well IBIDI slides at 37 °C to generate mature biofilms. After 48 h, supernatants were collected for cytokine analysis. In parallel, biofilms were incubated with candidate bactericidal payloads and stained with ViaGram staining kit for subsequent imaging analysis.

Results: LTA-CAR-T cells demonstrated antigen-dependent functional activation upon contact with *S. aureus* biofilms, as evidenced by increased IFN-γ and IL-2 secretion. The imaging workflow provided high-content metrics of bacterial viability, cellular distribution, and matrix disruption, enabling objective quantification of biofilm-disrupting effects. Preliminary application of candidate biofilm-degrading payloads revealed a measurable reduction in viable bacterial biomass and biofilm matrix integrity.

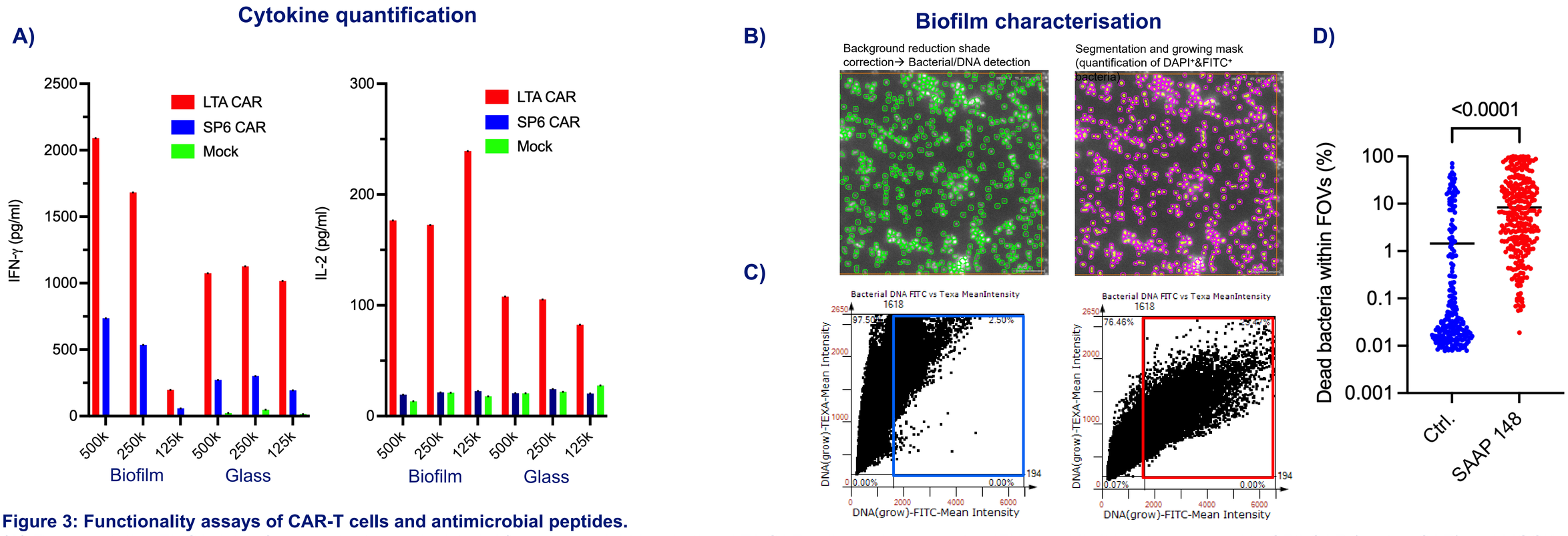

Figure 3: Functionality assays of CAR-T cells and antimicrobial peptides.
(A) Representative ELISA data of supernatants are depicted: After an overnight incubation, LTA CAR cells produce elevated IFN-γ and IL-2 levels compared to SP6 CAR (control CAR) and MOCK (no CAR) controls across different cell densities (125k–500k). **(B)** Imaging-based biofilm analysis pipeline: Representative fluorescence images are shown: Left: machine learning detection of DAPI⁺ bacteria (green marks) with background reduction algorithms. Right: Segmentation and growing mask generation (magenta marks) for quantification of FITC intensities of bacteria (yellow narks). **(C)** *In situ* quantification of dead (DAPI⁺/FITC⁺) bacteria: Left: Control biofilm showing predominantly viable bacteria (blue gate). Right: SAAP148-treated (60 µM) biofilm showing increased dead bacteria (red gate). **(D)** Quantification of dead bacteria: FITC⁺ viability staining demonstrates combined CAR-T cell and SAAP-148 treatment significantly reduces viable bacteria (Mann-Whitney U test; $p<0.0001$). Image acquisition and analysis were performed with TisssueFAXS and StrataQuest software (TissueGnostics, Vienna, Austria).

Conclusions: These preliminary data indicate that LTA-targeting CAR-T cells can function as programmable, antigen-directed carriers to locally concentrate therapeutic agents that destabilize biofilm structure and support bacterial clearance. Combining spatial analysis, controlled *in vitro* evaluation, and rapid CAR/payload iteration may accelerate preclinical optimization of CAR-T-based strategies for biofilm-associated implant infections.

Acknowledgements: This research was partly supported by the HyFiDress consortium, which has received funding from the European Union's Horizon Europe Widening Participation and Spreading Excellence programme under grant agreement No. 101159615. Parts of this work were also supported by the Bavarian Research Network (bayresq.net; Bavarian State Ministry for Science and the Arts).

HyFiDress

Funded by the European Union

Corresponding authors:
Prof. Dr. Martijn Riool: martijn.riool@ukr.de
Prof. Dr. Uwe Ritter: uwe.ritter@lit.eu

Leibniz Institute for Immunotherapy
Cells built to cure

https://lit.eu