Pathogenesis of blinding corneal infections with methicillin-resistant *Staphylococcus aureus* (MRSA)

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University of California, Irvine

Covered Topics

Background

- Microbial keratitis (infections of the cornea)
- Inflammation and treatment

Potential targets

- IL-1β as a potential druggable target in microbial keratitis
- α-hemolysin as a potential target in MRSA keratitis
    - Novel binding residue?

Microbial Keratitis is a leading cause of blindness

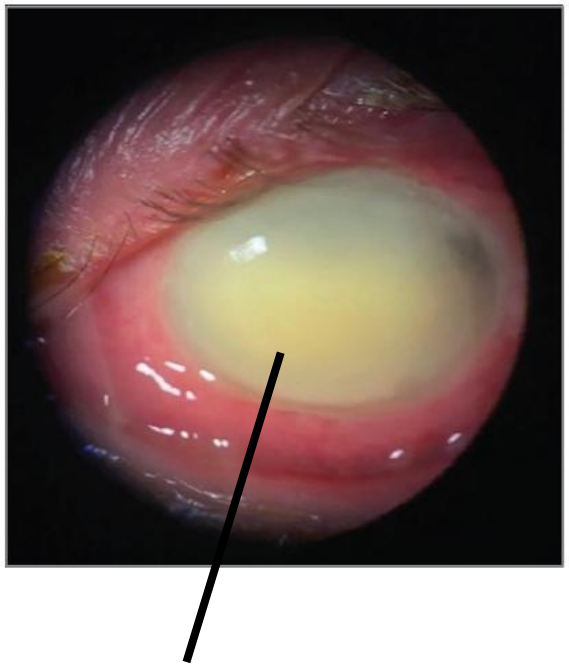
- **1.5 - 2 million** individuals worldwide develop blindness from trauma related infections every year
- Costs United States healthcare system **~$175 million** per year
- Associated with **poor contact lens care**
- Caused by a variety of pathogens including bacterial or fungal
  - *Pseudomonas aeruginosa* and *Staphylococcus aureus* are most common causes of bacterial keratitis
- Increasing incidences of MRSA infections
  - Up to **34.4%** of *S. aureus* keratitis are MRSA

2023 outbreak of multi drug-resistant *P. aeruginosa* from contaminated eye product
81 cases across 18 states

Lee et al. (Clin Med, 2021)
Carrel et al. (Emerg Infect Dis, 2013)
Shoji et al. (JAMA Ophthalmology, 2023)
Chodosh et al. (Surveys in Ophthalmology, 2019)

Severe inflammation damages ocular tissues

- Infection recruits neutrophils to the cornea, causing **inflammation**
  - Neutrophils release proteases that damage surrounding tissues
  - Injury leads to corneal scarring and vision impairment



Immune cells recruited to eye (i.e. pus)

Coll et al. (Nat Med, 2015)
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Putnam & Hoffman (Immunol Rev, 2024)

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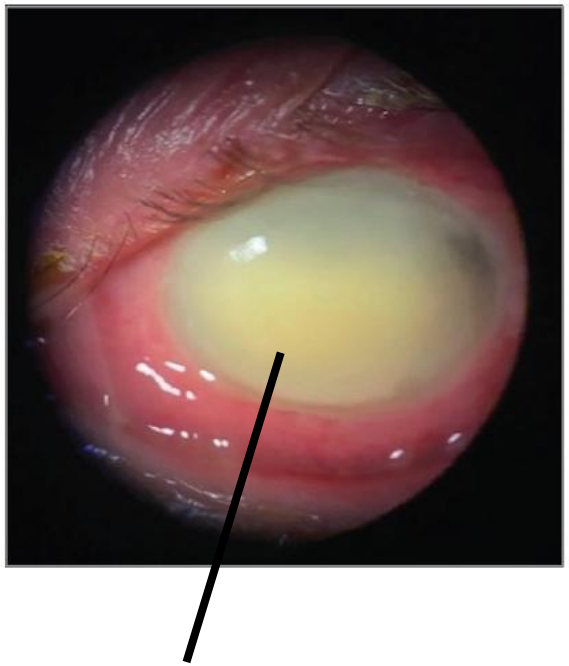
- Microbial keratitis treatment involves antibiotics followed corticosteroids to reduce inflammation and promote wound healing
  - **Corticosteroids risk inducing glaucoma or cataracts**



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- Microbial keratitis treatment involves antibiotics followed corticosteroids to reduce inflammation and promote wound healing
  - **Corticosteroids risk inducing glaucoma or cataracts**
- IL-1β is an important **pro-inflammatory cytokine** that contributes to a variety of inflammatory diseases
  - Inhibitors of IL-1β secretion are under clinical trials
  - IL-1β pathway inhibitors are more targeted than corticosteroids

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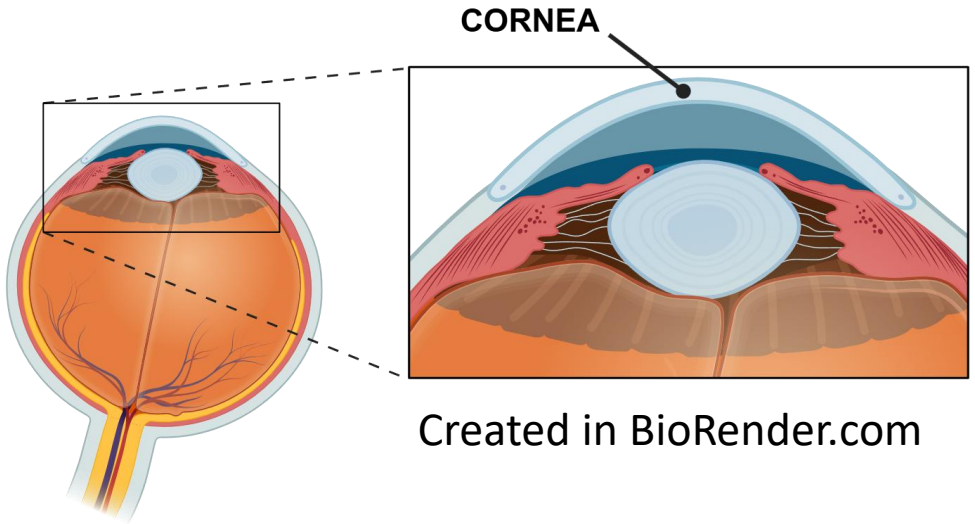
Can targeting IL-1β reduce inflammation during microbial keratitis?

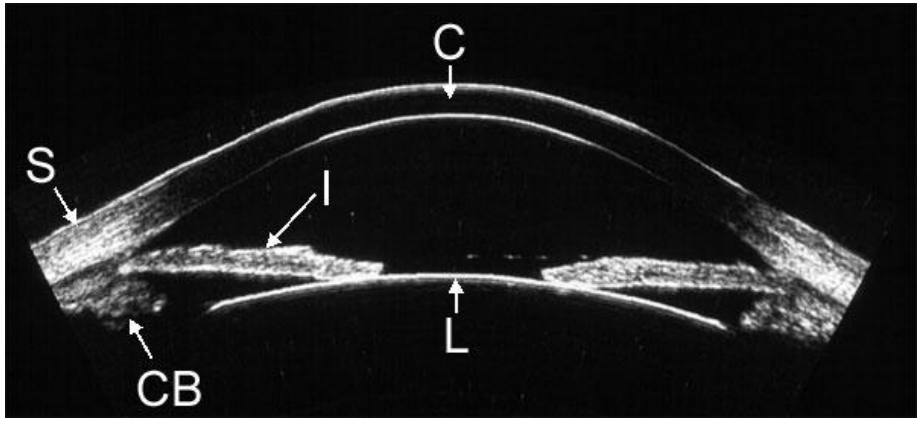
The cornea is the most external part of the eye



Created in BioRender.com

High-resolution ultrasound

Silverman, 2004 (Cornell University)



Replicating human disease with a mouse model of microbial keratitis

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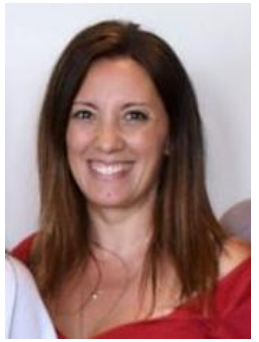
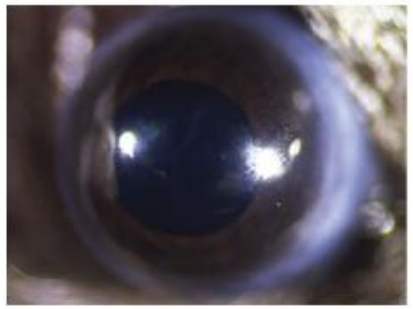


Uninfected



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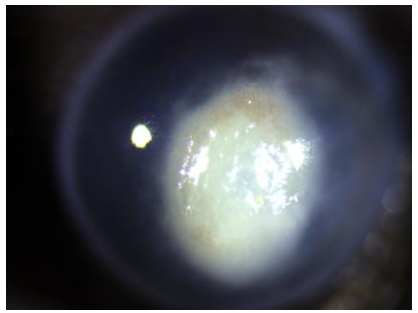
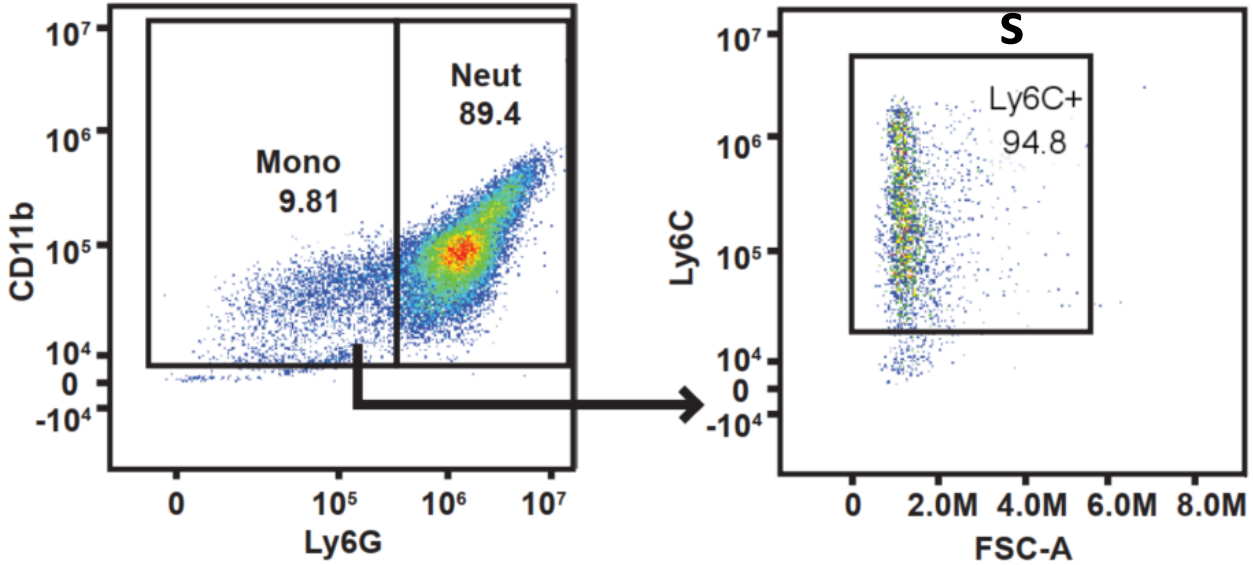


1x10^3 cells of *S. aureus* injected into the stroma of corneas

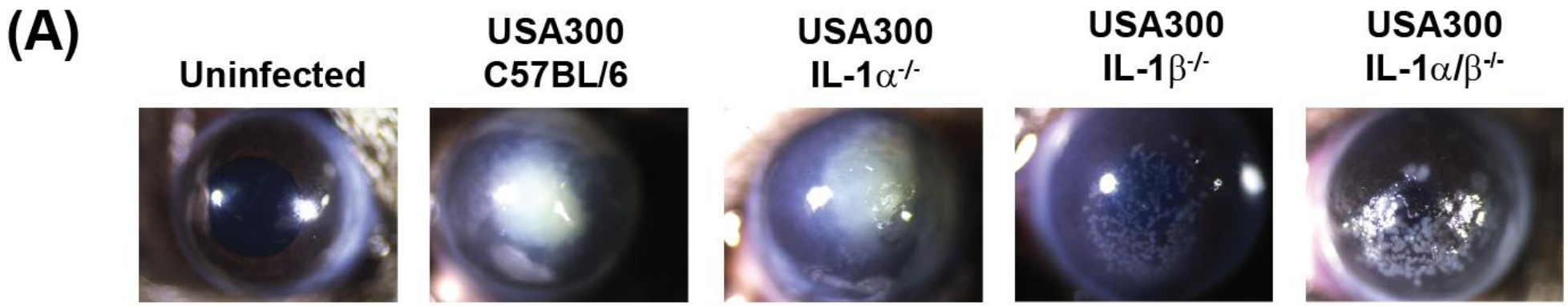
Neutrophils

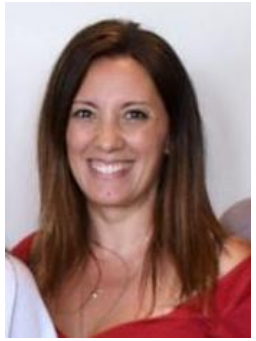
Monocytes



24 h post infection

MRSA USA300 growth in the cornea is dependent on IL-1β





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USA300 is most common clone of MRSA in the U.S.

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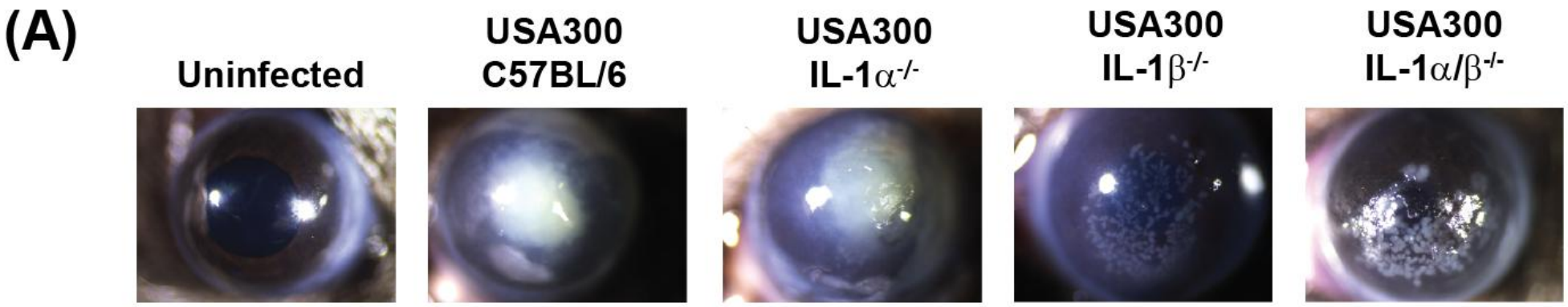

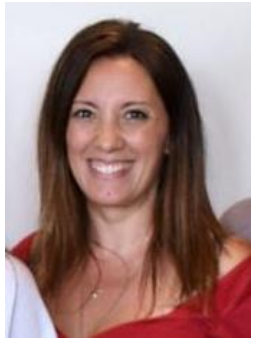

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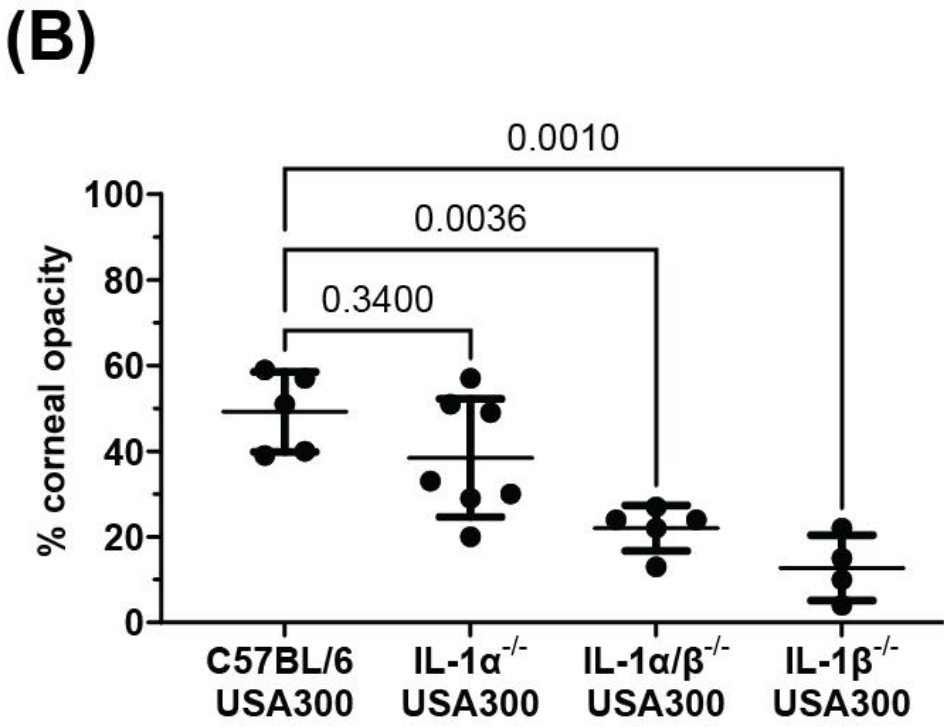

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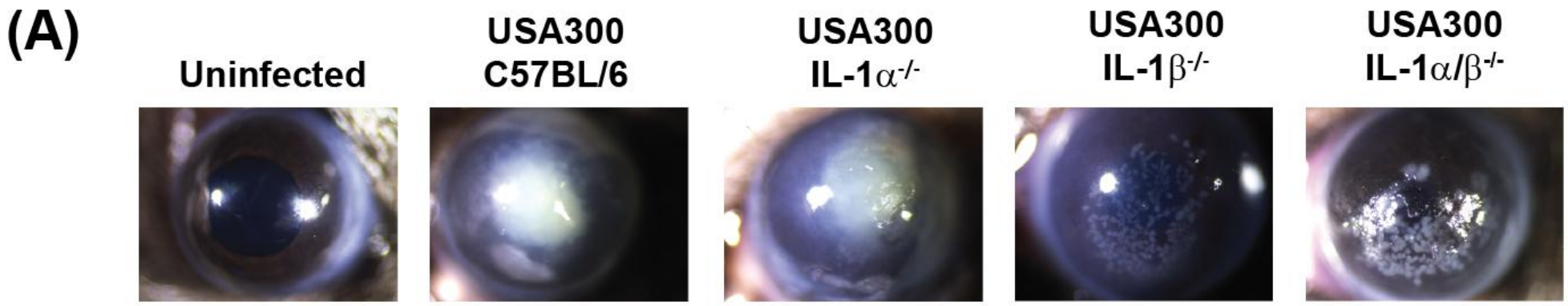

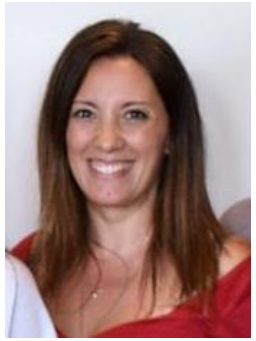

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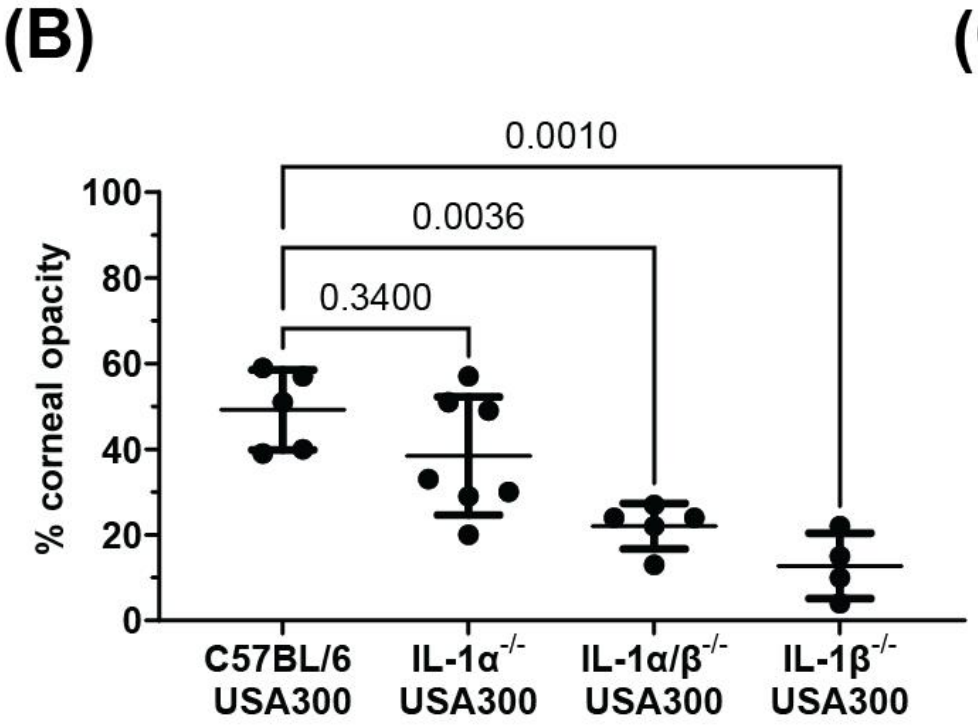
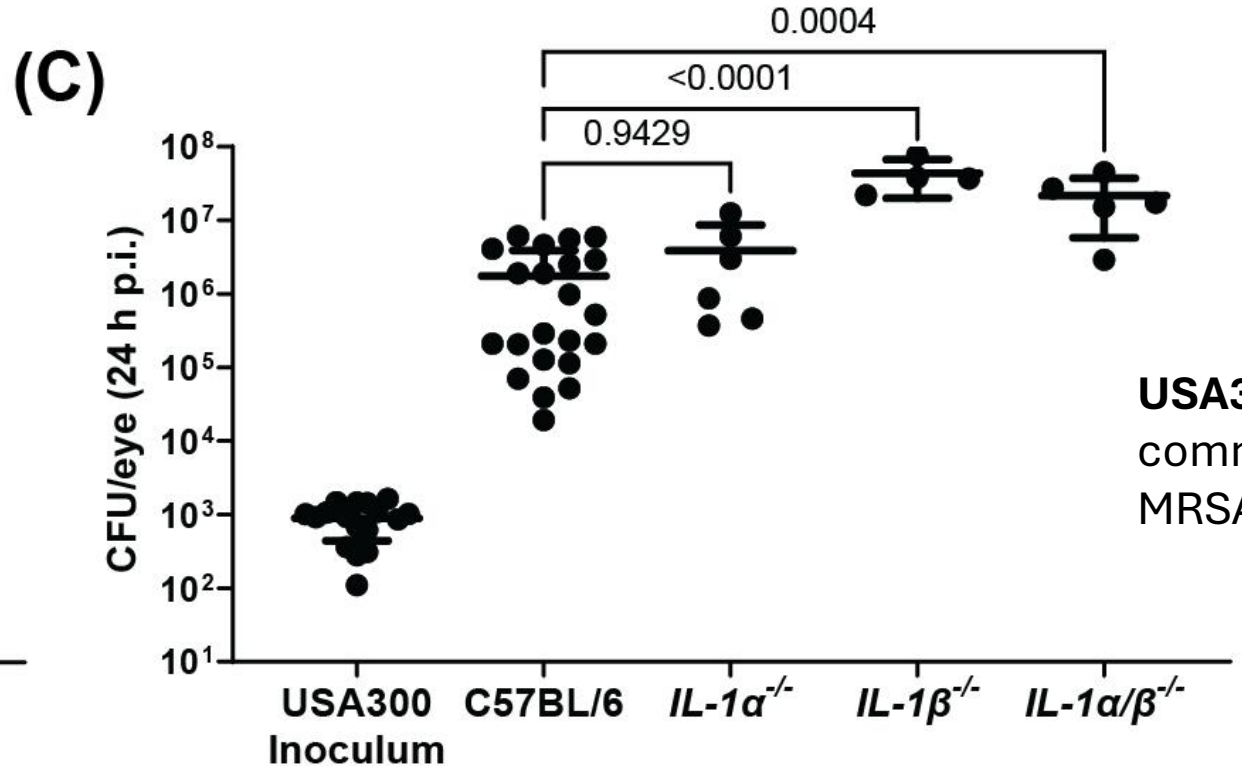
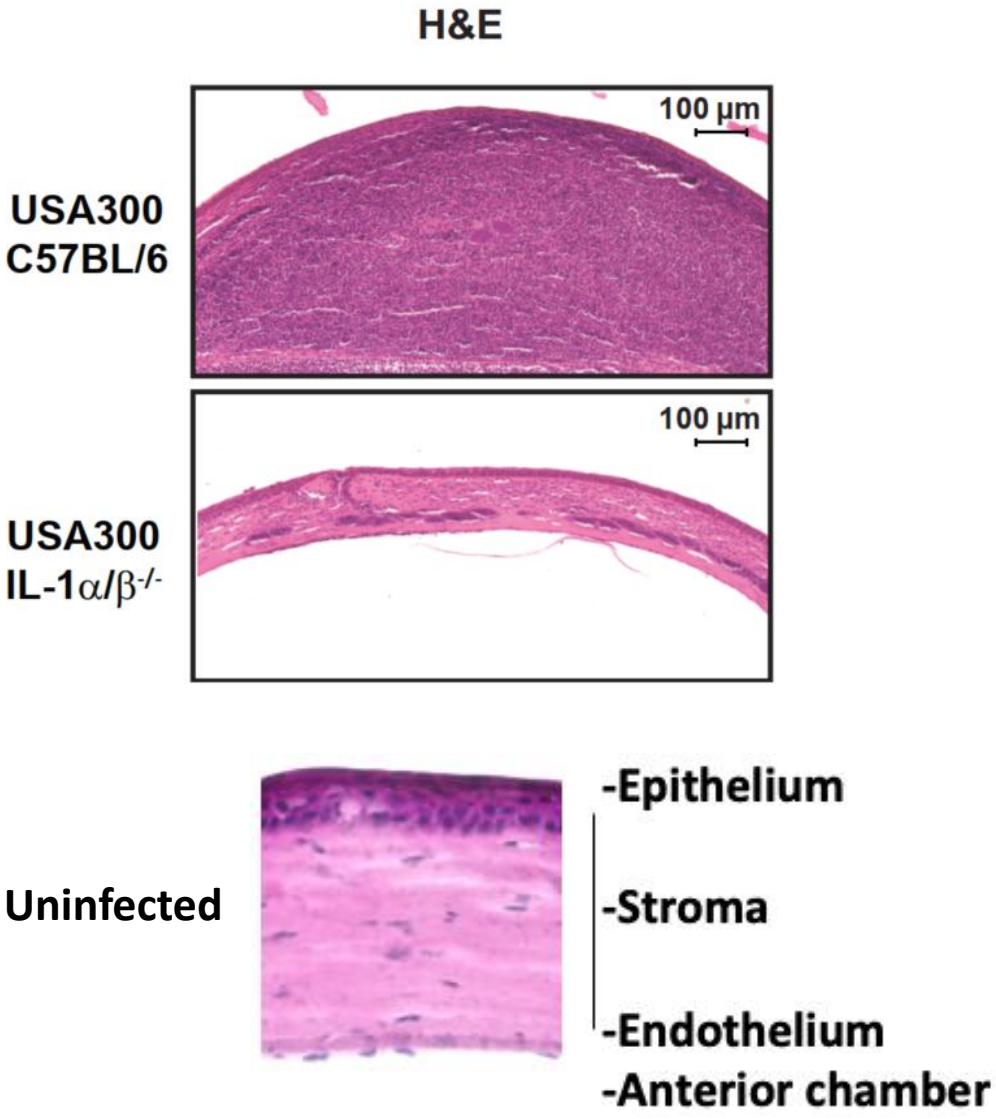
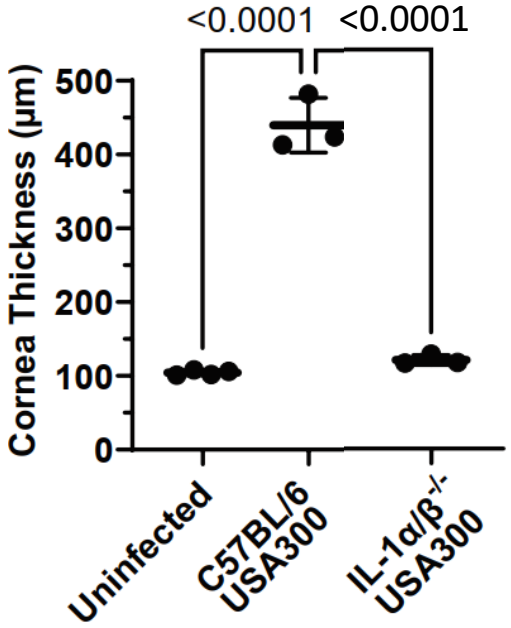




USA300 is most common clone of MRSA in the U.S.

Inflammation is reduced in corneas lacking IL-1β$^{-/-}$





James Begando

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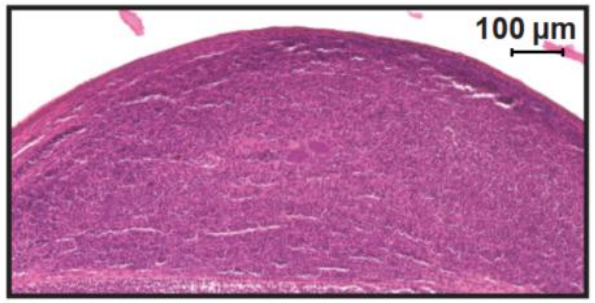
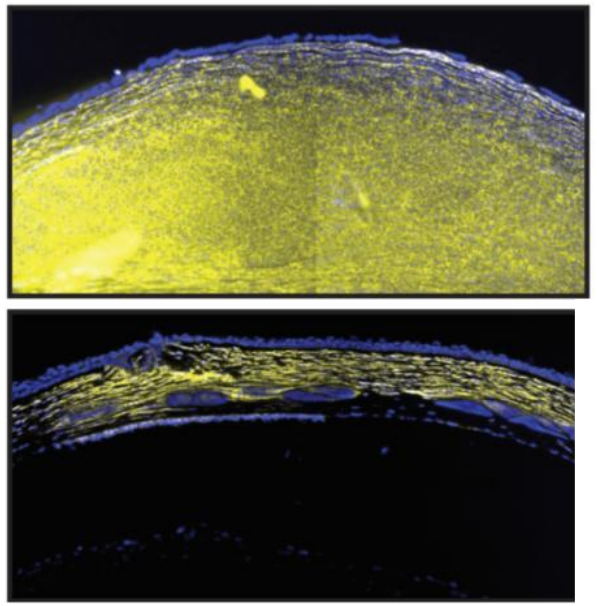
H&E

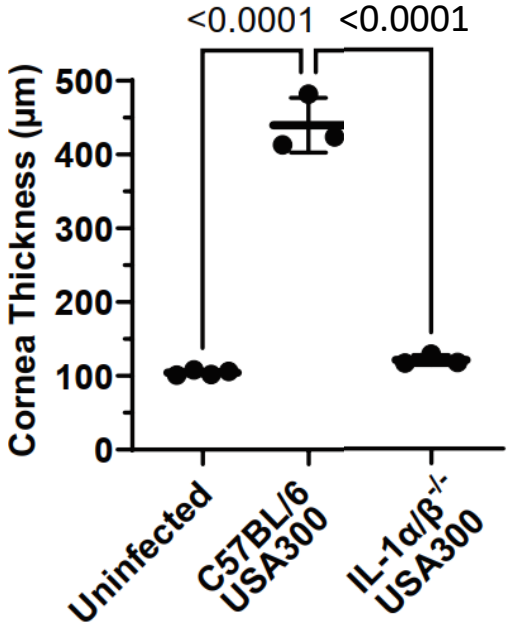
USA300
C57BL/6



USA300
IL-1α/β$^{-/-}$

(Neutrophils)
DAPI / Ly6G





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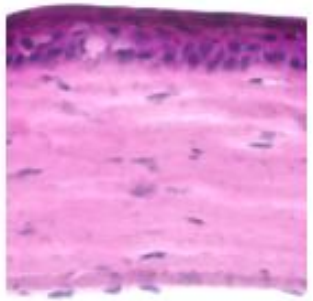

-Epithelium
-Stroma
-Endothelium
-Anterior chamber

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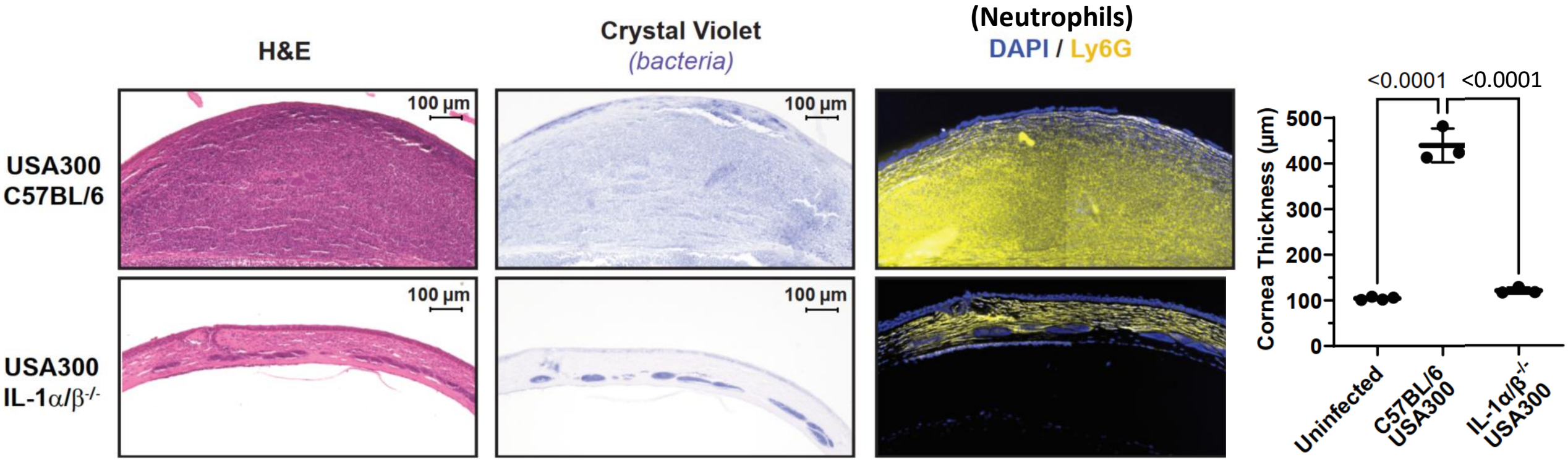

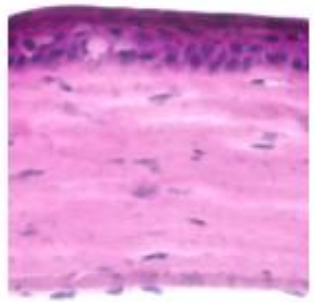

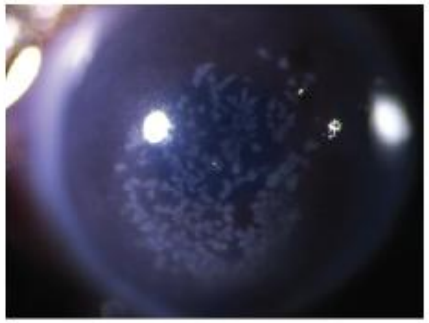

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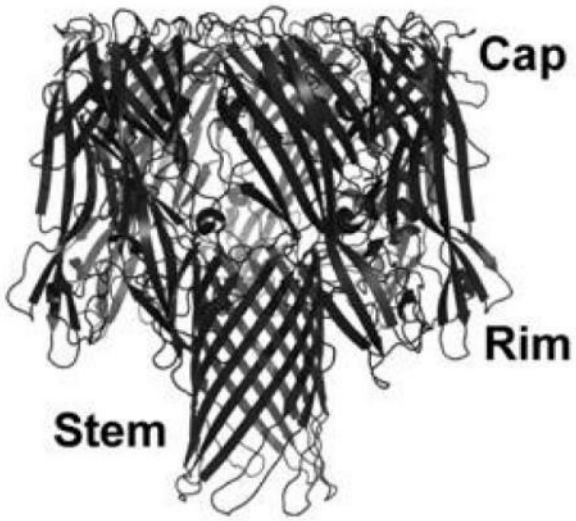
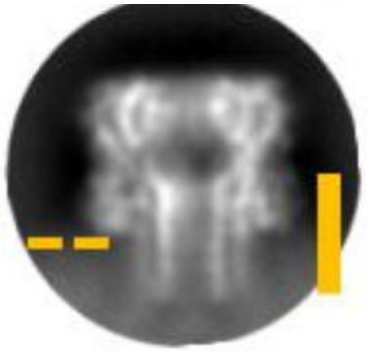
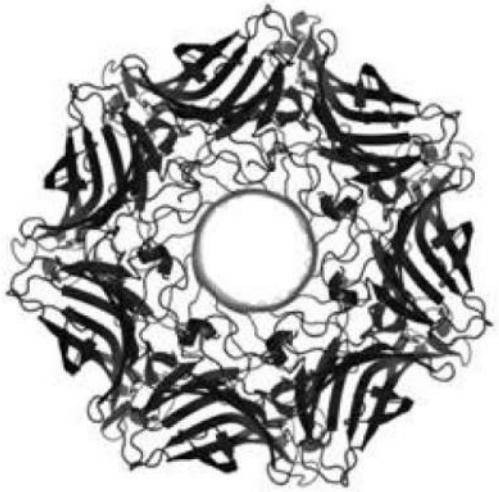
Take home message:

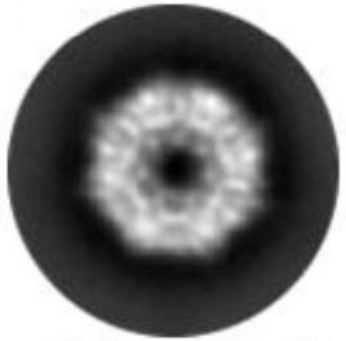
IL-1β knockout reduces inflammation during microbial keratitis

Targeting virulence factors may be the next step to treatments









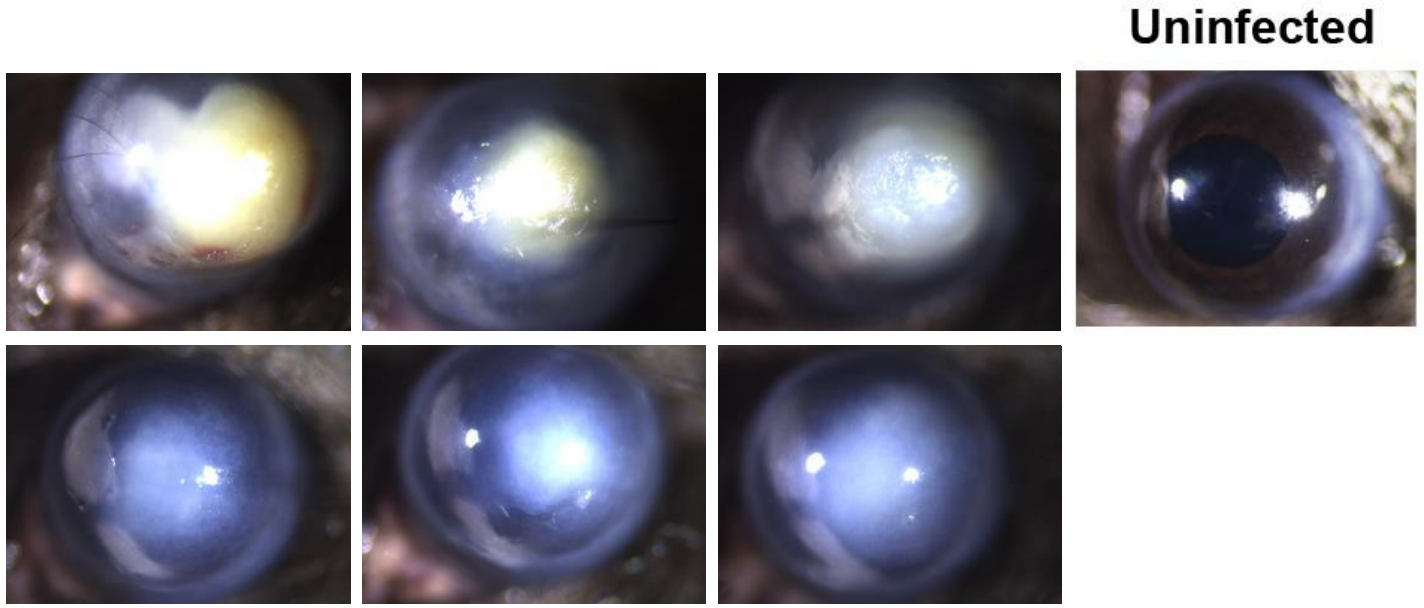
2D class averages of Cryo-EM micrograph
(Chatterjee et al., bioRxiv 2025)

- The emergence of antibiotic resistance necessitates the need for novel treatments
- Targeting specific virulence factors may be an alternative
- α-hemolysin has been investigated as possible target in *S. aureus* infections
  - α-hemolysin is a lytic, pore-forming toxin
  - α-hemolysin induces IL-1β secretion

Berube & Wardenburg (Toxins, 2013)

Can targeting α-hemolysin reduce inflammation during MRSA keratitis?

α-hemolysin is an essential virulence factor in MRSA keratitis



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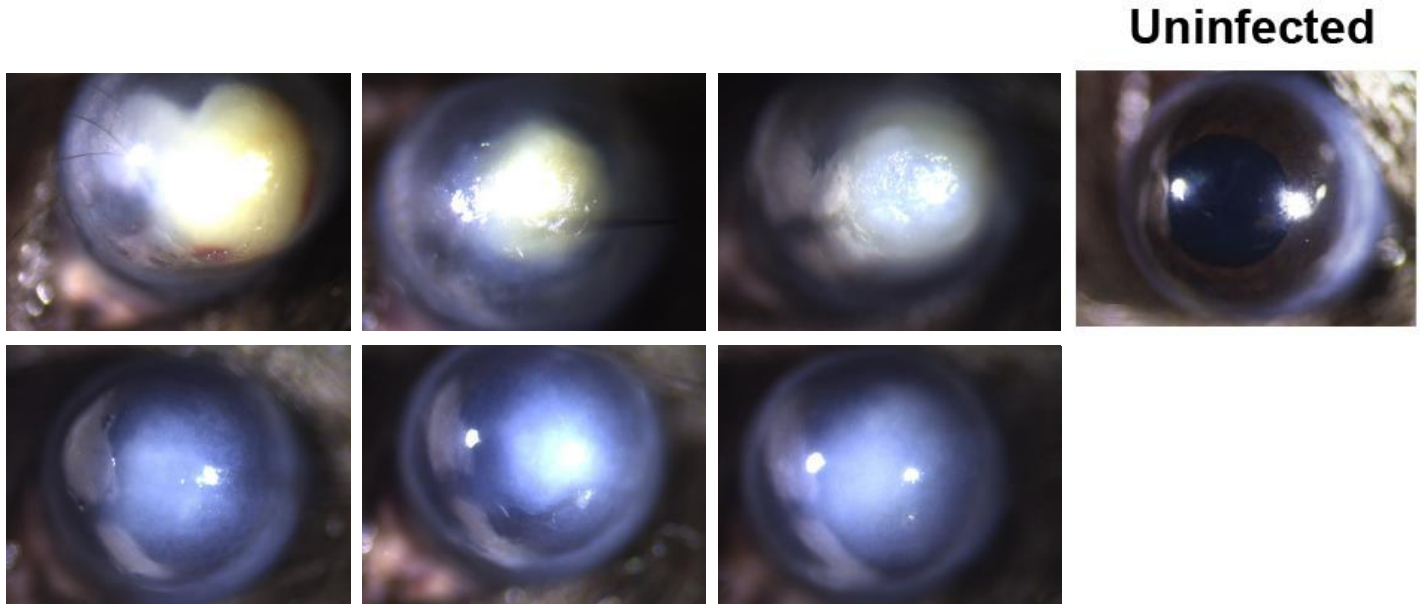

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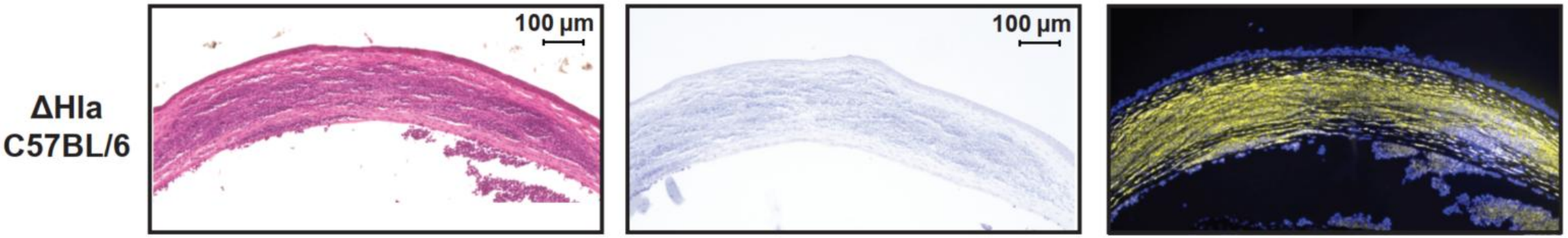
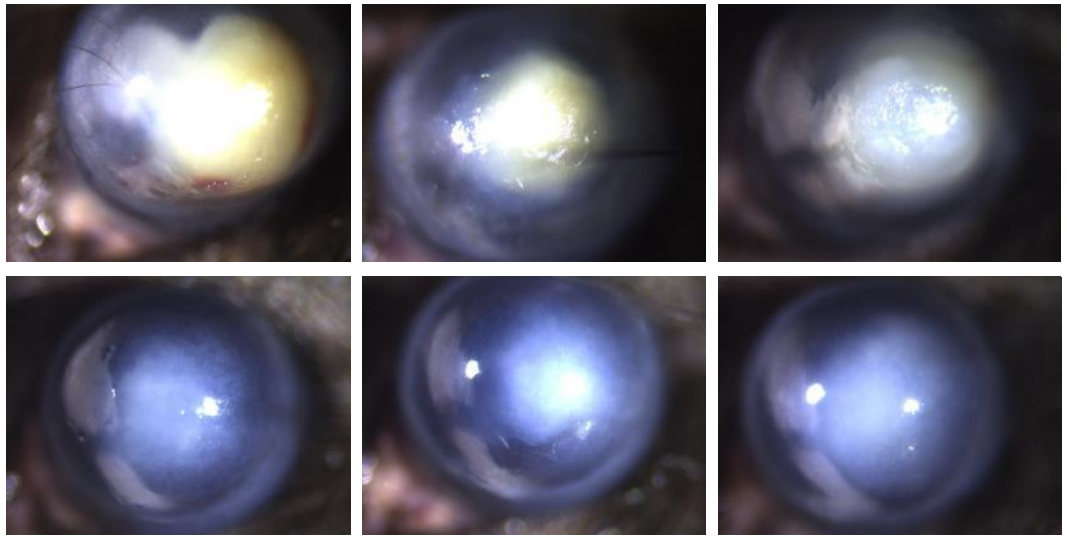


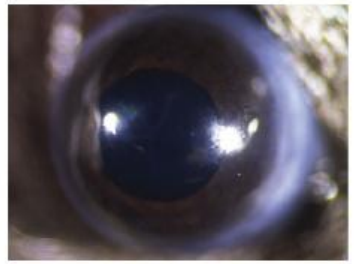
α-hemolysin is an essential virulence factor in MRSA keratitis

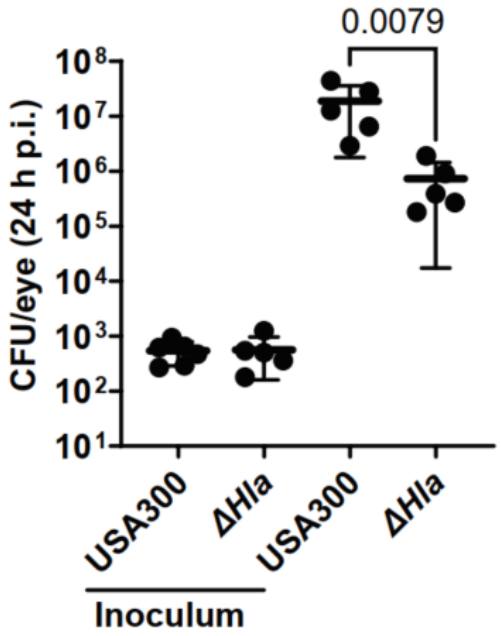
Uninfected

USA300
C57BL/6

ΔHla
C57BL/6







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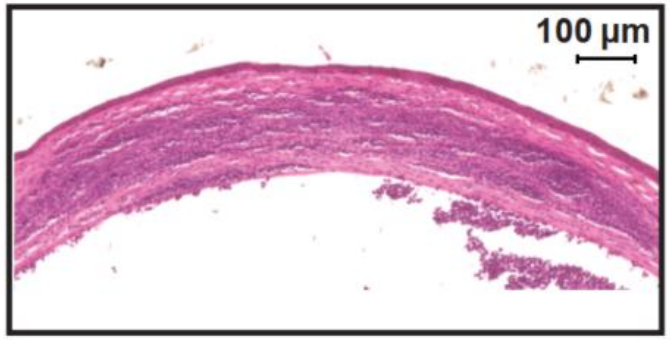
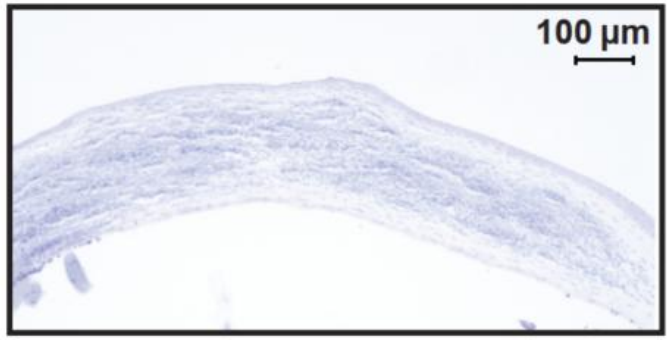
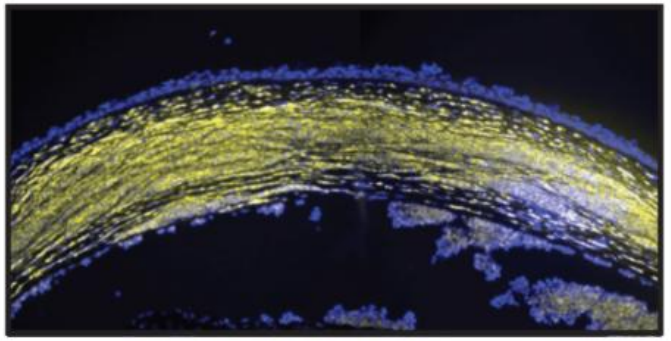
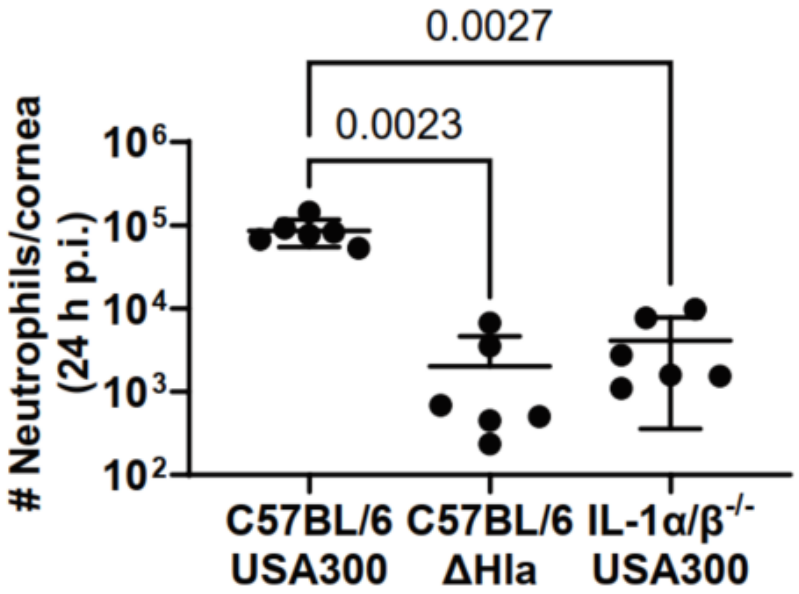
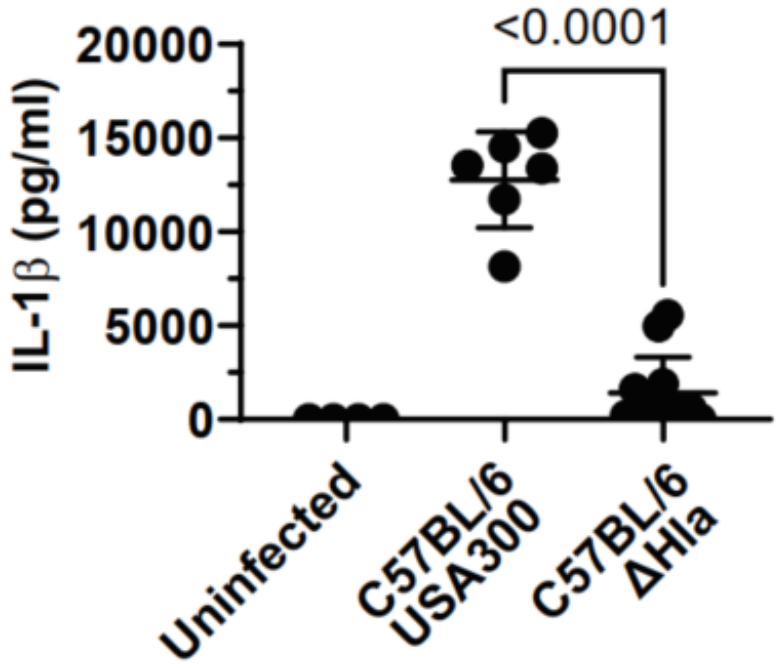






Neutrophil recruitment to corneas is dependent on α-hemolysin and IL-1β

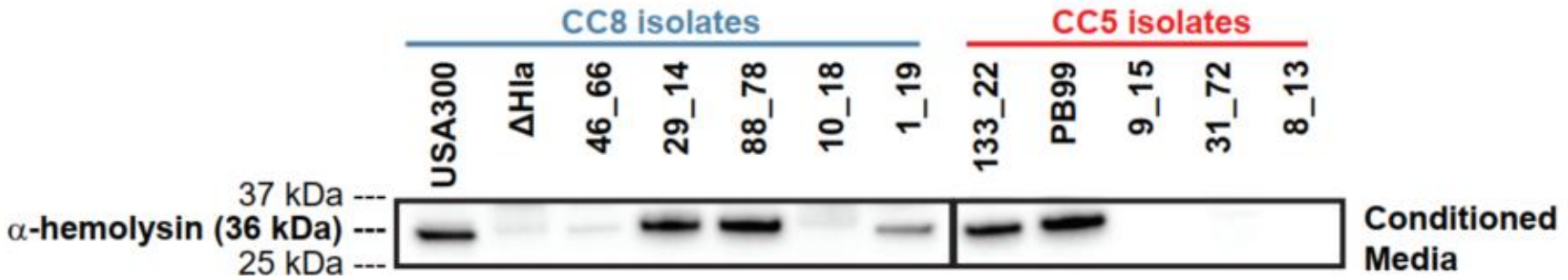




- Collagenase digested corneas 24 h post infection
- **Neutrophils** (CD11b+Ly6G+)
- **Monocytes** (CD11b+Ly6G-Ly6C+)

Neutrophil IL-1β secretion induced by MRSA clinical isolates is associated with α-hemolysin secretion

Jolynn Tran-Chau



Clonal Complex
Grouping genetically similar lineages based on house-keeping genes

Conditioned Media
Bacteria-free supernatants from MRSA (secreted proteins only)

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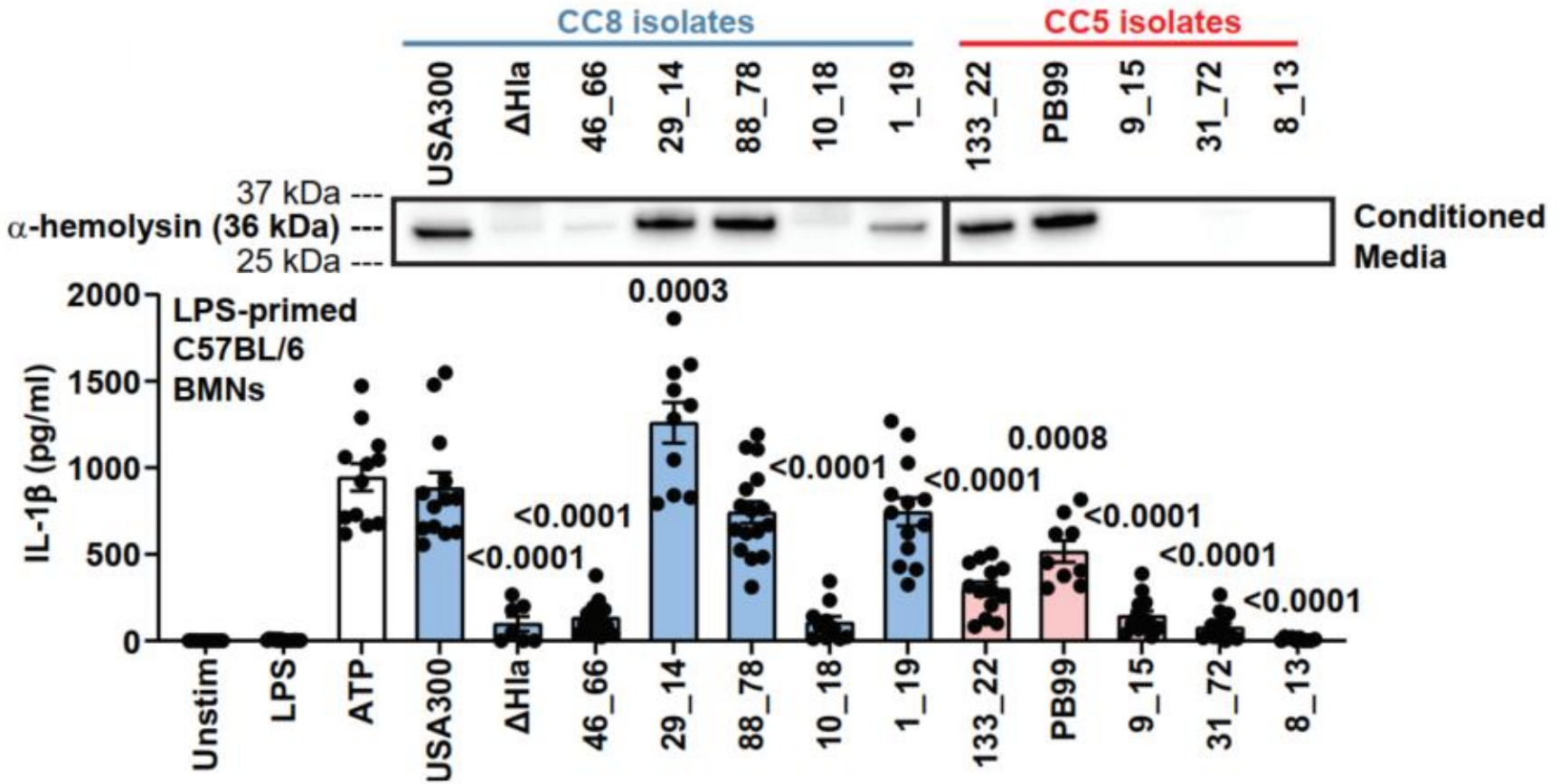

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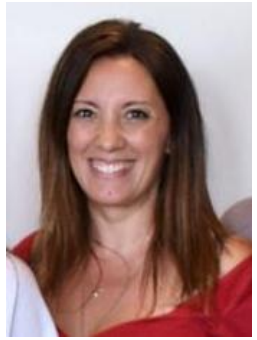
Bacterial growth and disease severity in the cornea is associated with α-hemolysin secretion for CC8s



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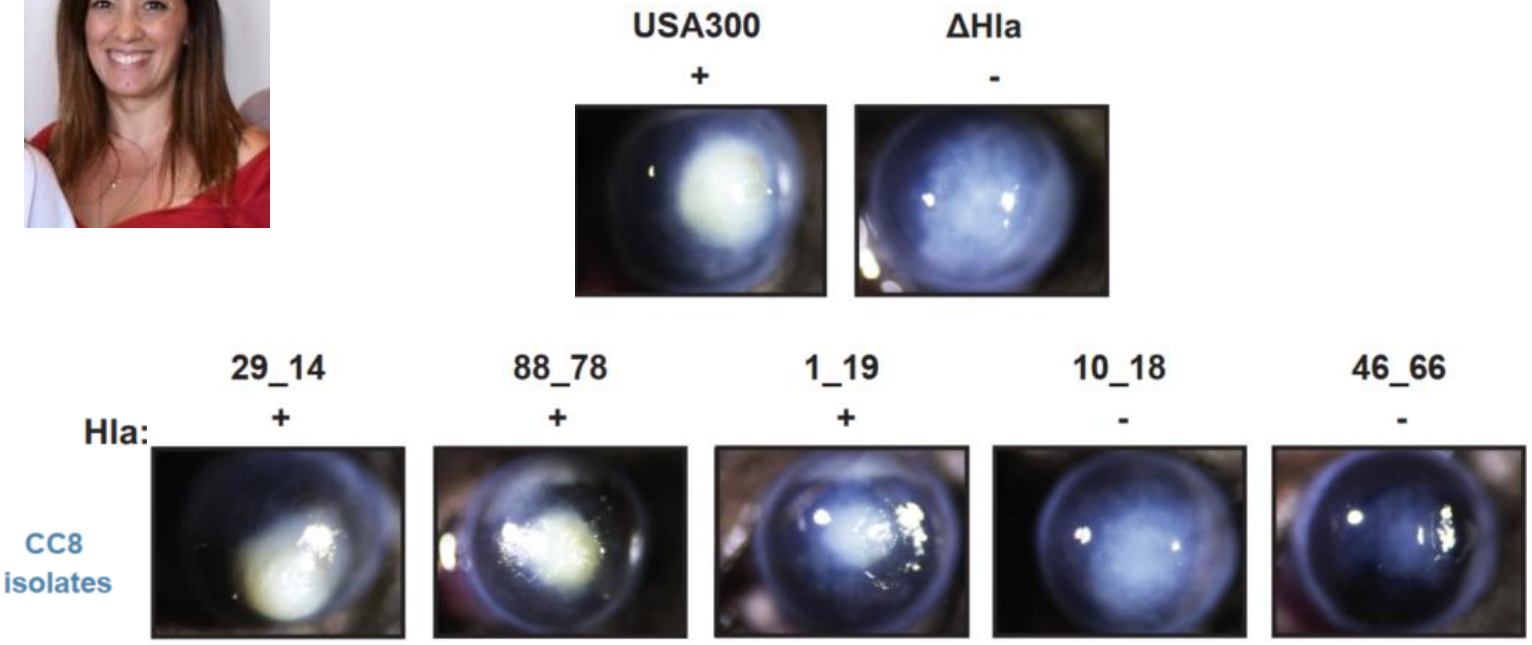

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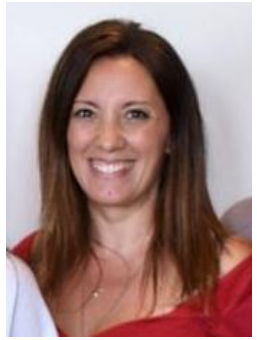

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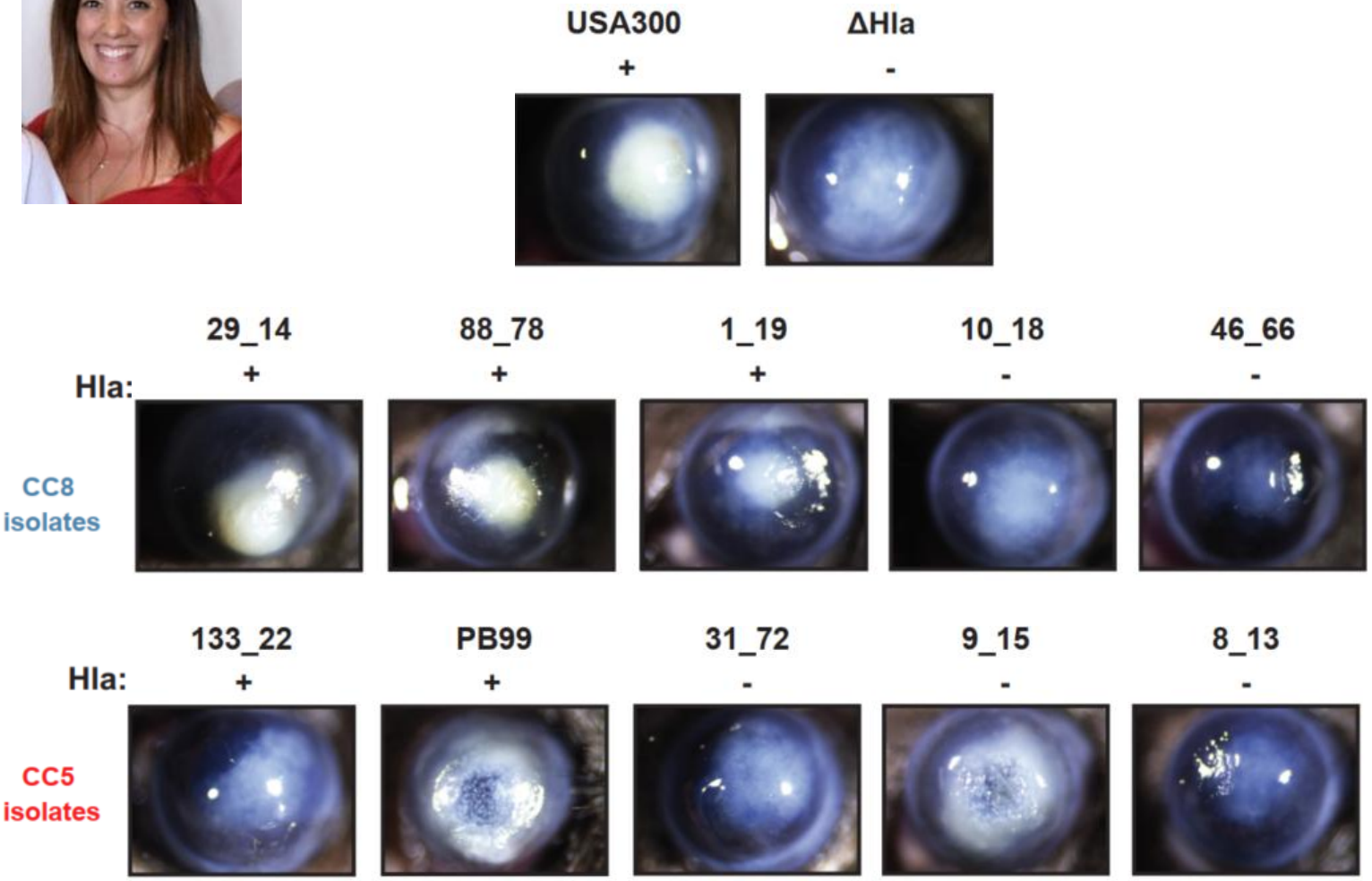

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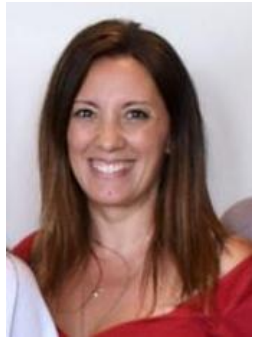

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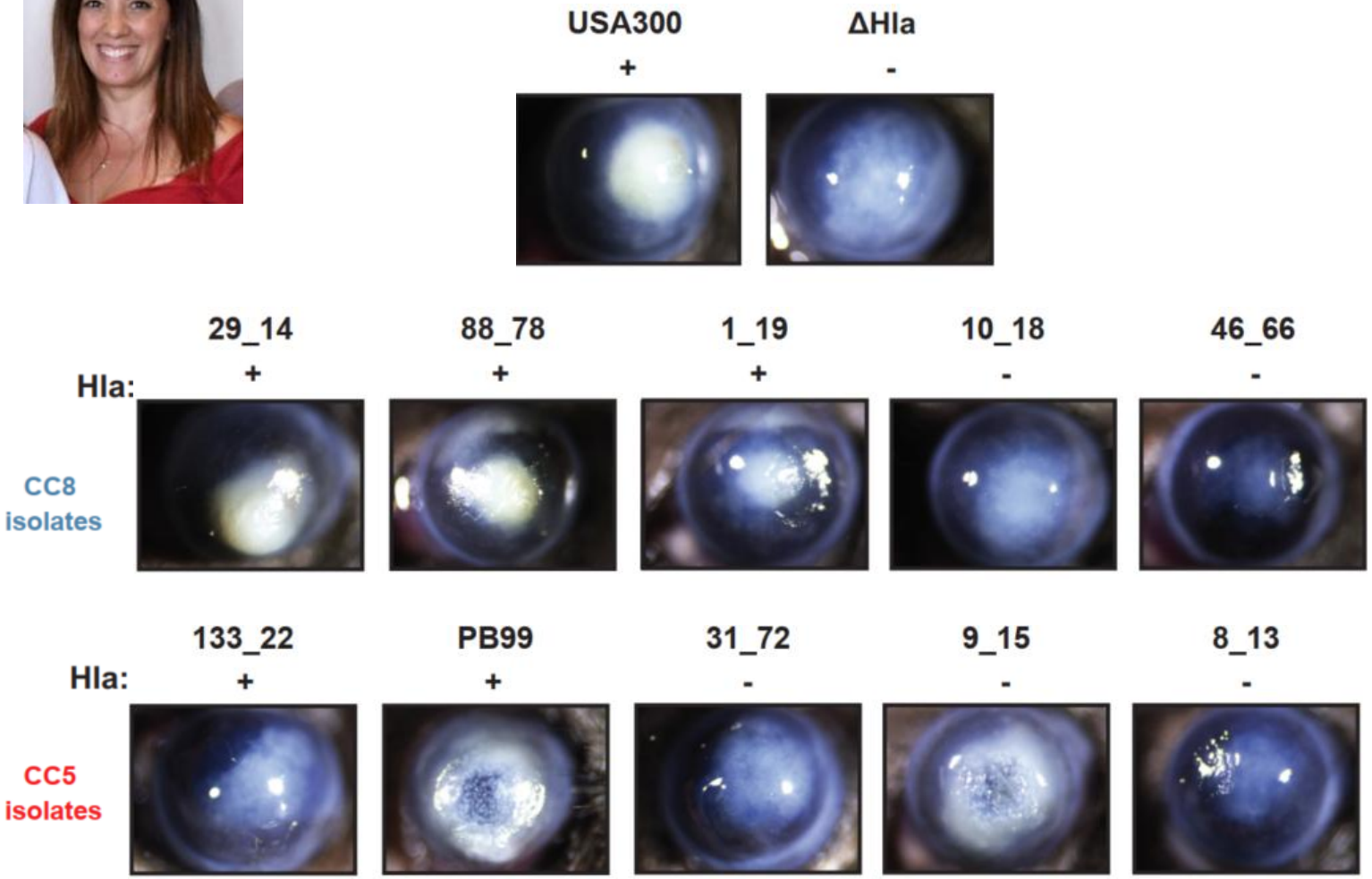
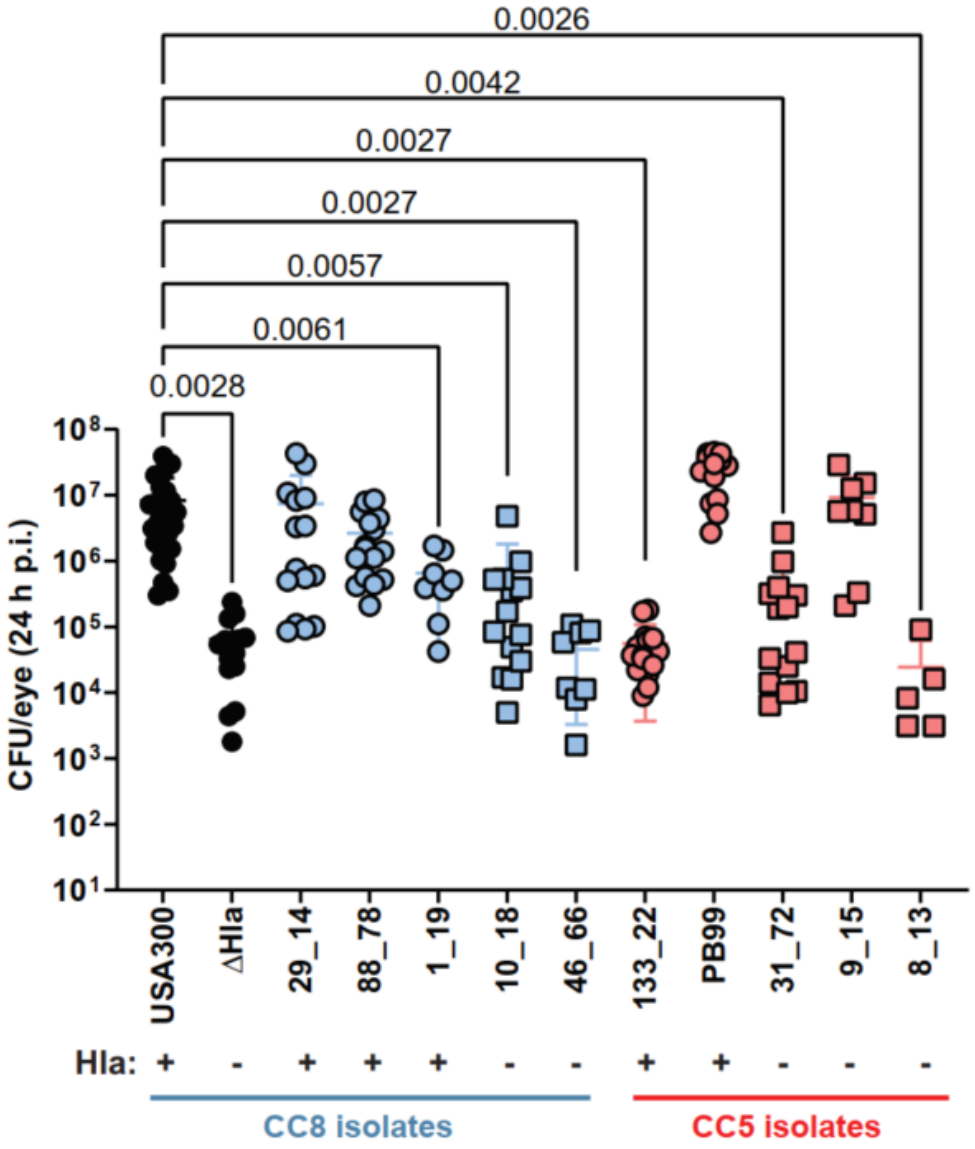




Are there differences in α-hemolysin between CC8 and CC5 clinical isolates?

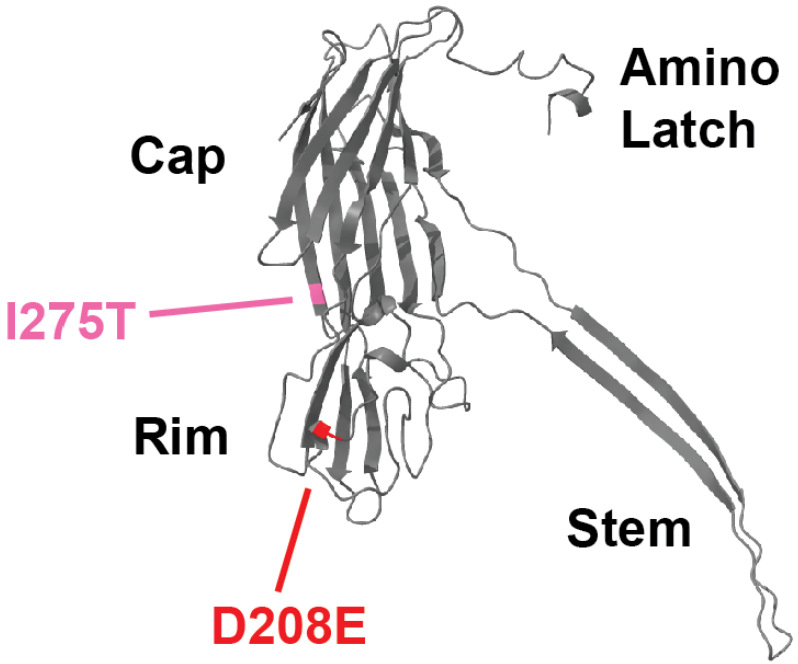
CC5 clinical isolates harbor α-hemolysin mutations

Camille Andre

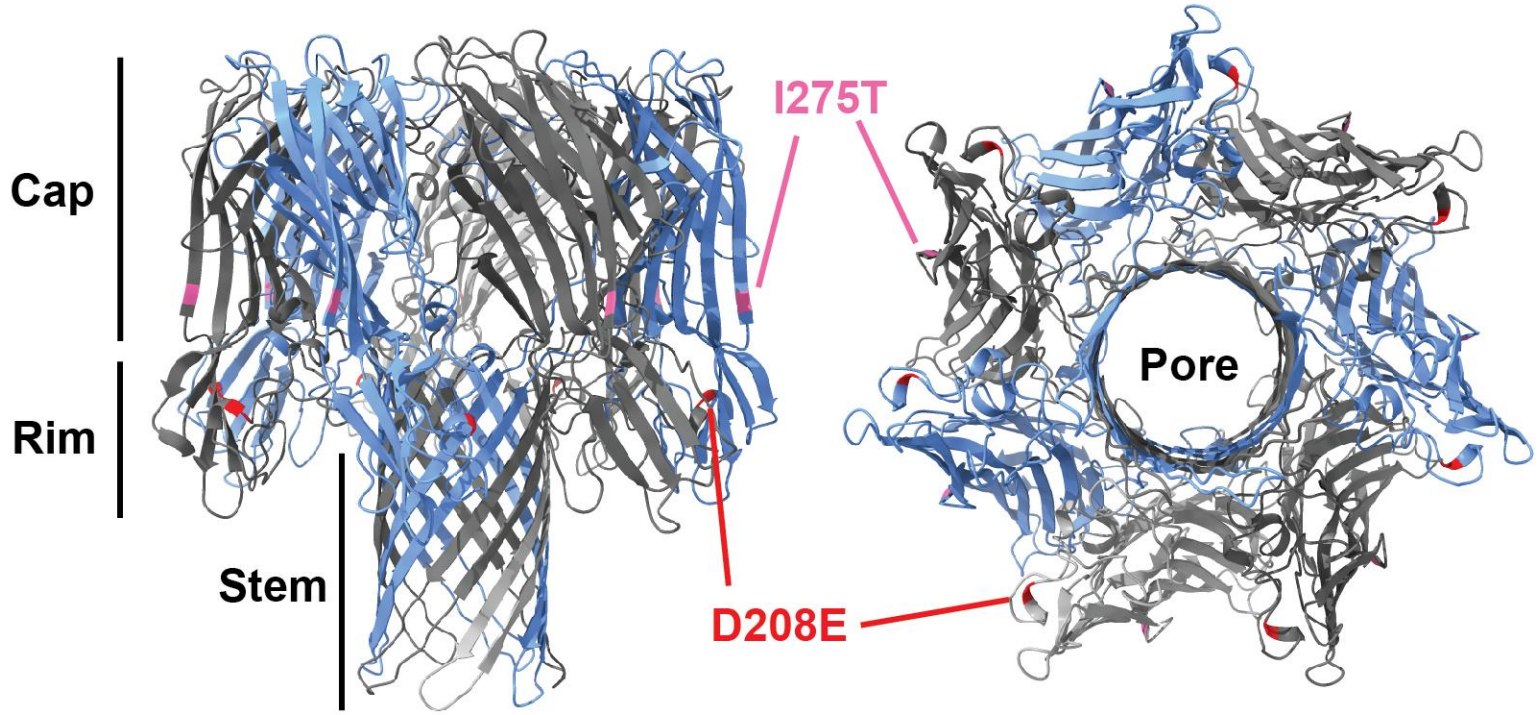
Michael Gilmore

I275T: Isoleucine → Threonine
D208E: Aspartic Acid → Glutamic Acid

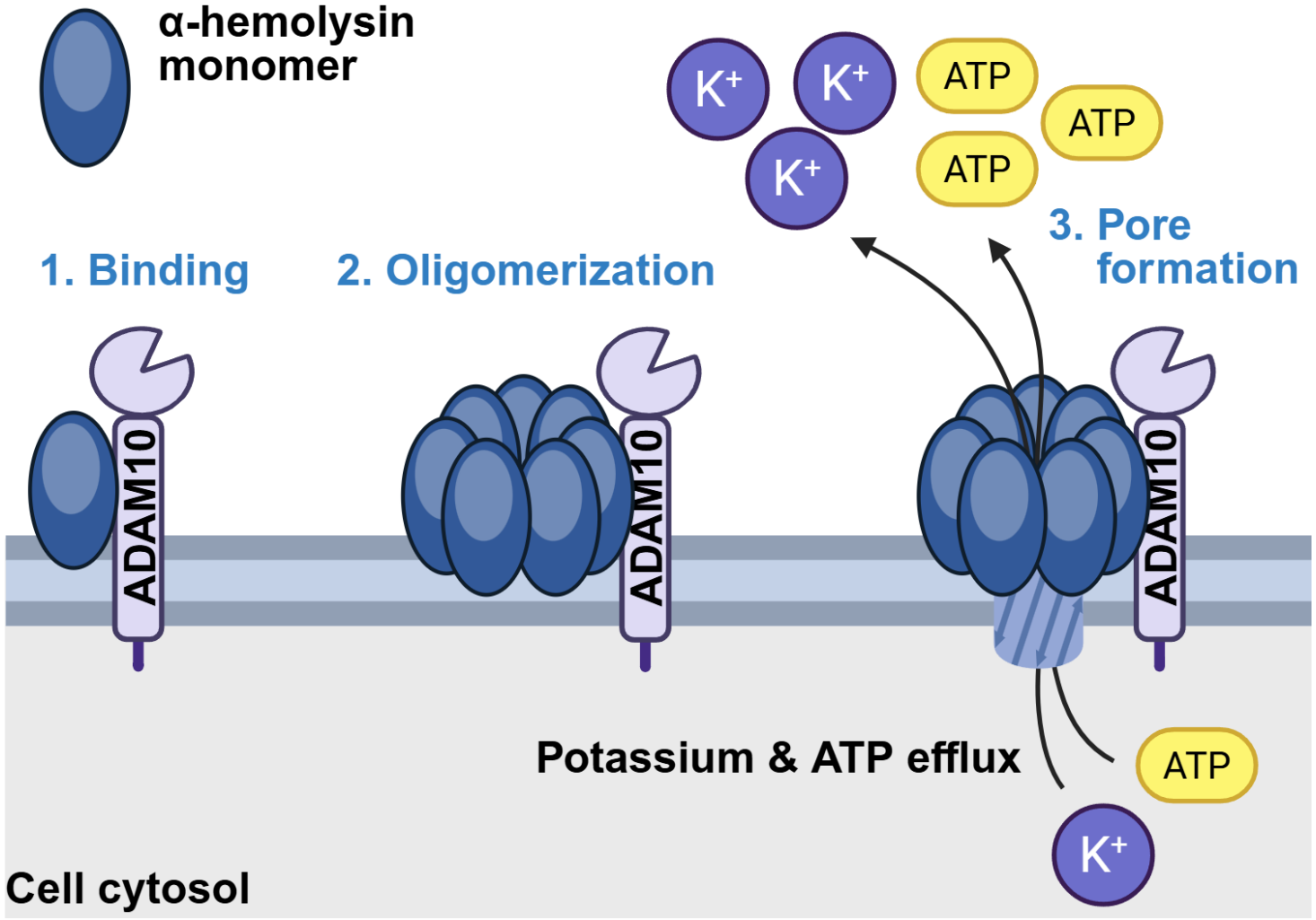
α-hemolysin protomer



Assembled Pore (7 protomers)



ADAM10 is cell surface receptor for α-hemolysin



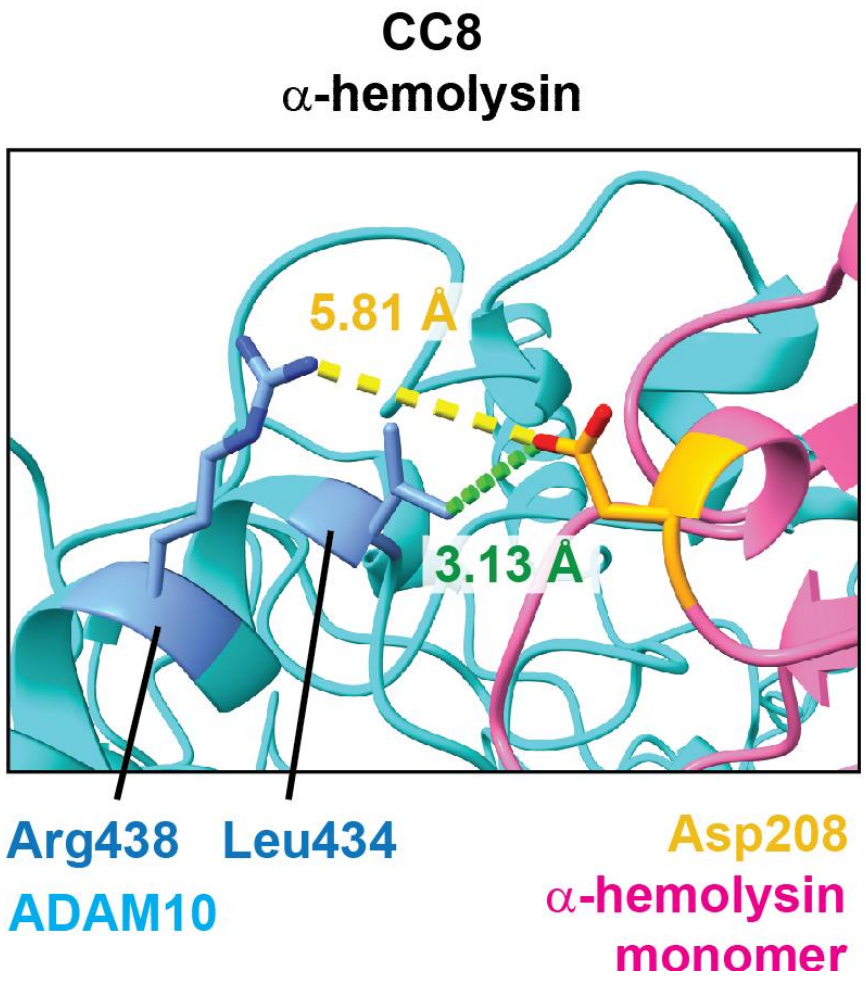
Created with BioRender.com

ADAM10: A Disintegrin and Metalloprotease 10

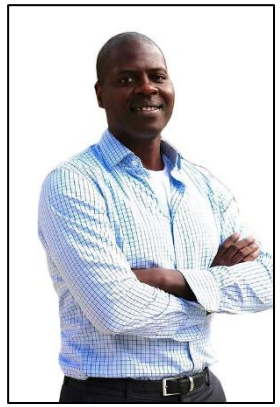
- Cell surface protease
- Involved in many cellular functions
- Embryonic lethal

Where α-hemolysin binds to ADAM10 is still unknown

CC5 Glu208 is predicted to more tightly interact with ADAM10



Angela Lackner



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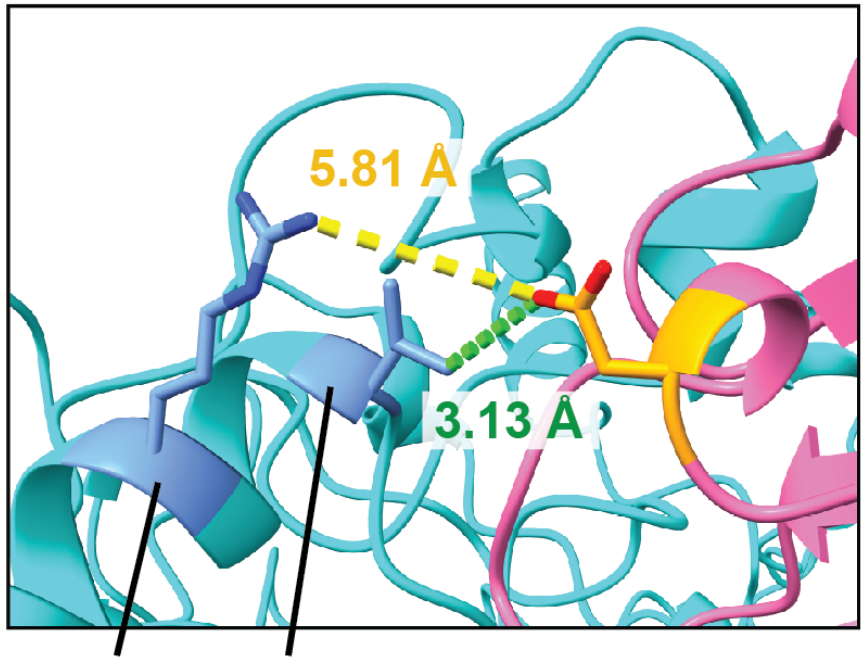
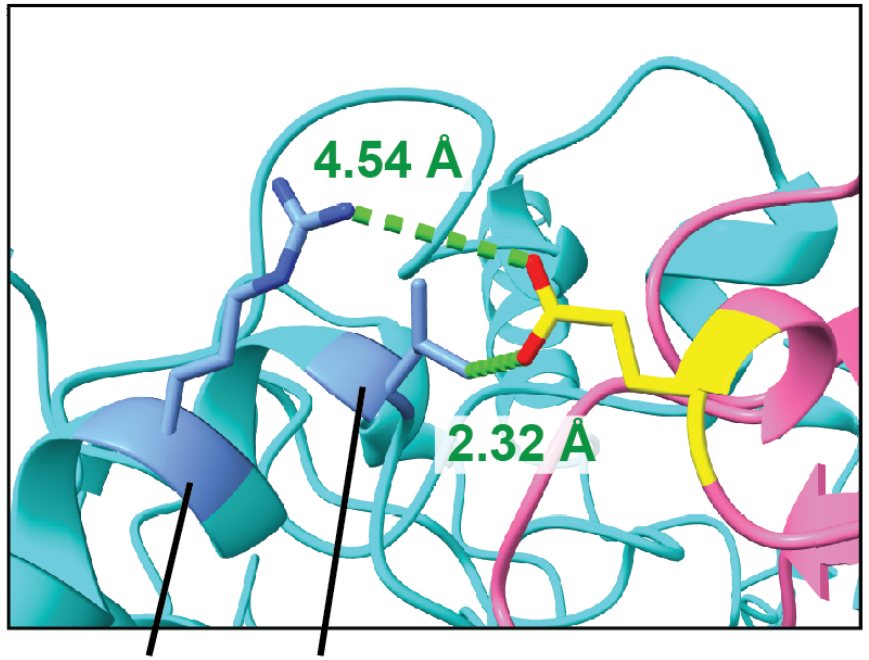
Green dotted lines: ChimeraX predicted interactions

CC5 Glu208 is predicted to more tightly interact with ADAM10

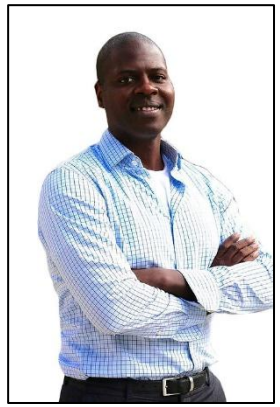
CC8
α-hemolysin



CC5
α-hemolysin



Angela Lackner



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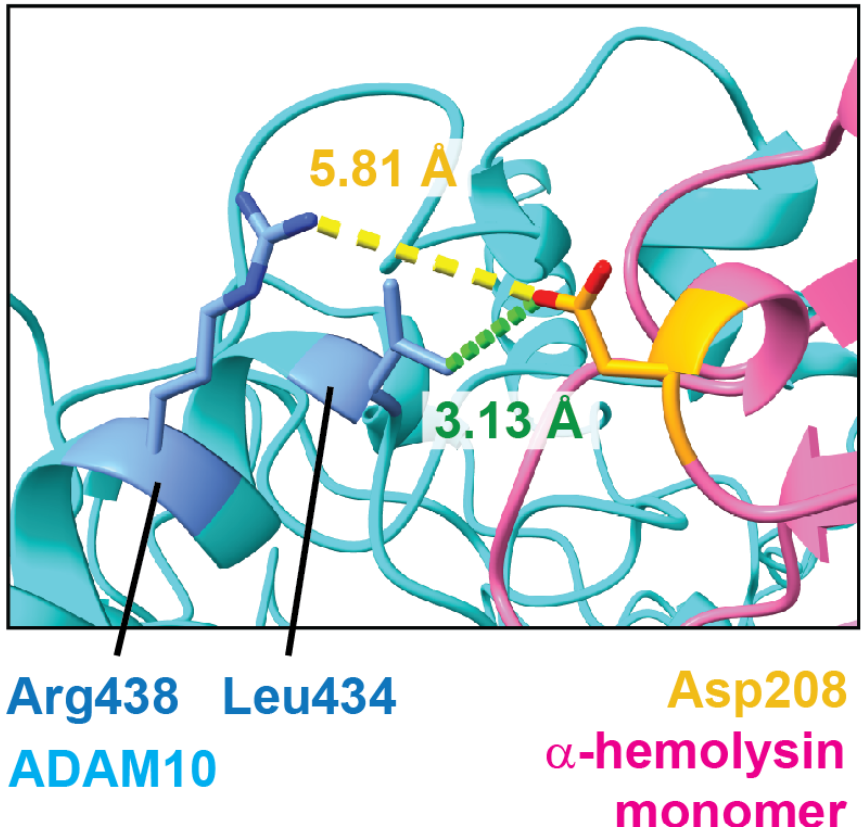
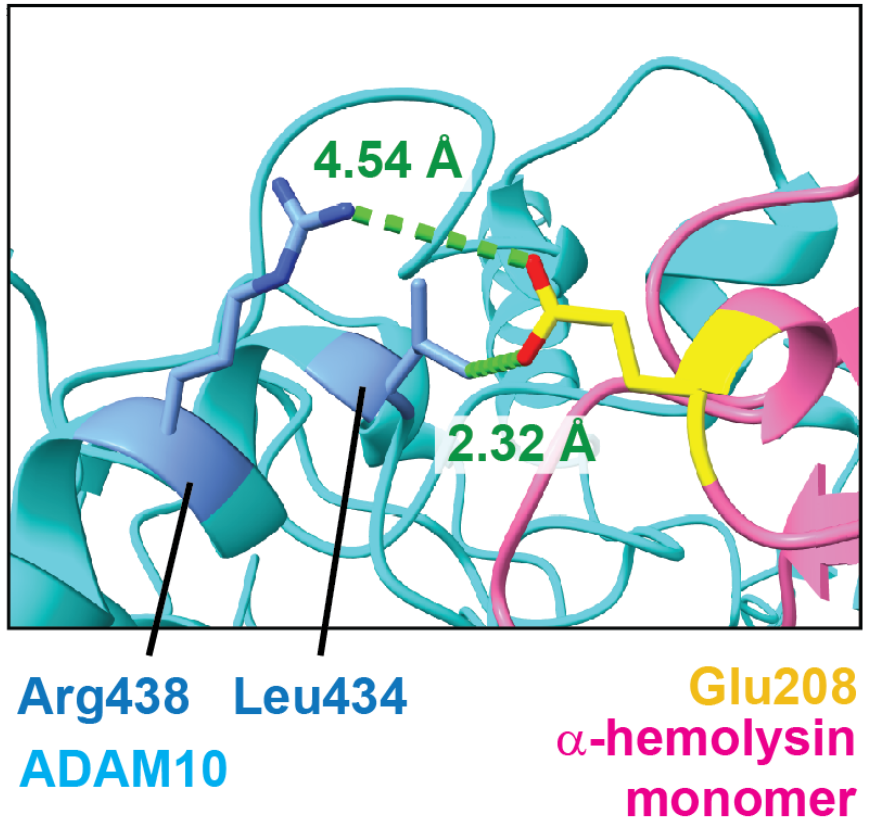


CC5
α-hemolysin



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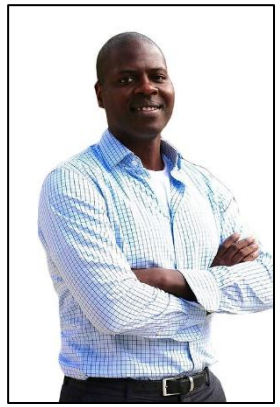

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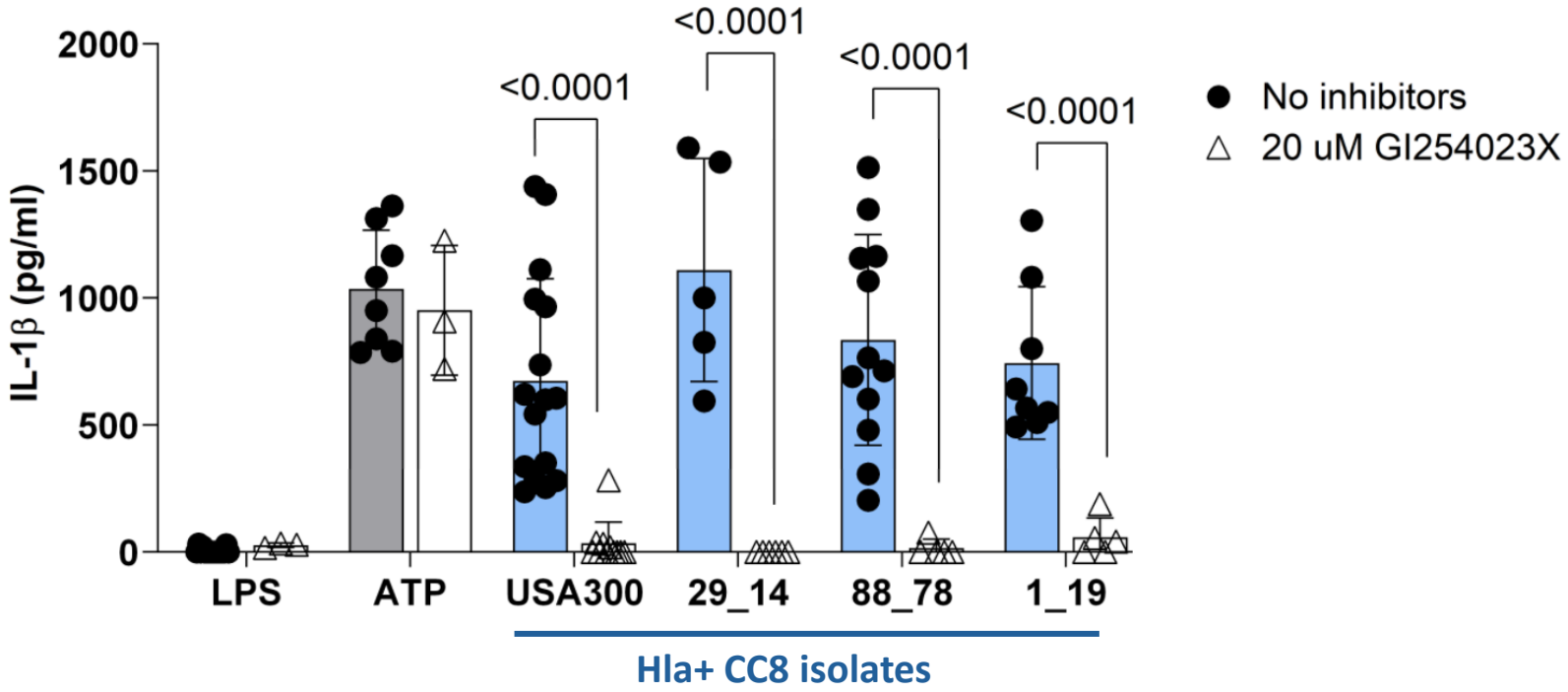
Novel binding residue?

Green dotted lines: ChimeraX predicted interactions

ADAM10 inhibitor impairs IL-1β secretion induced by CC8 but not CC5 isolates

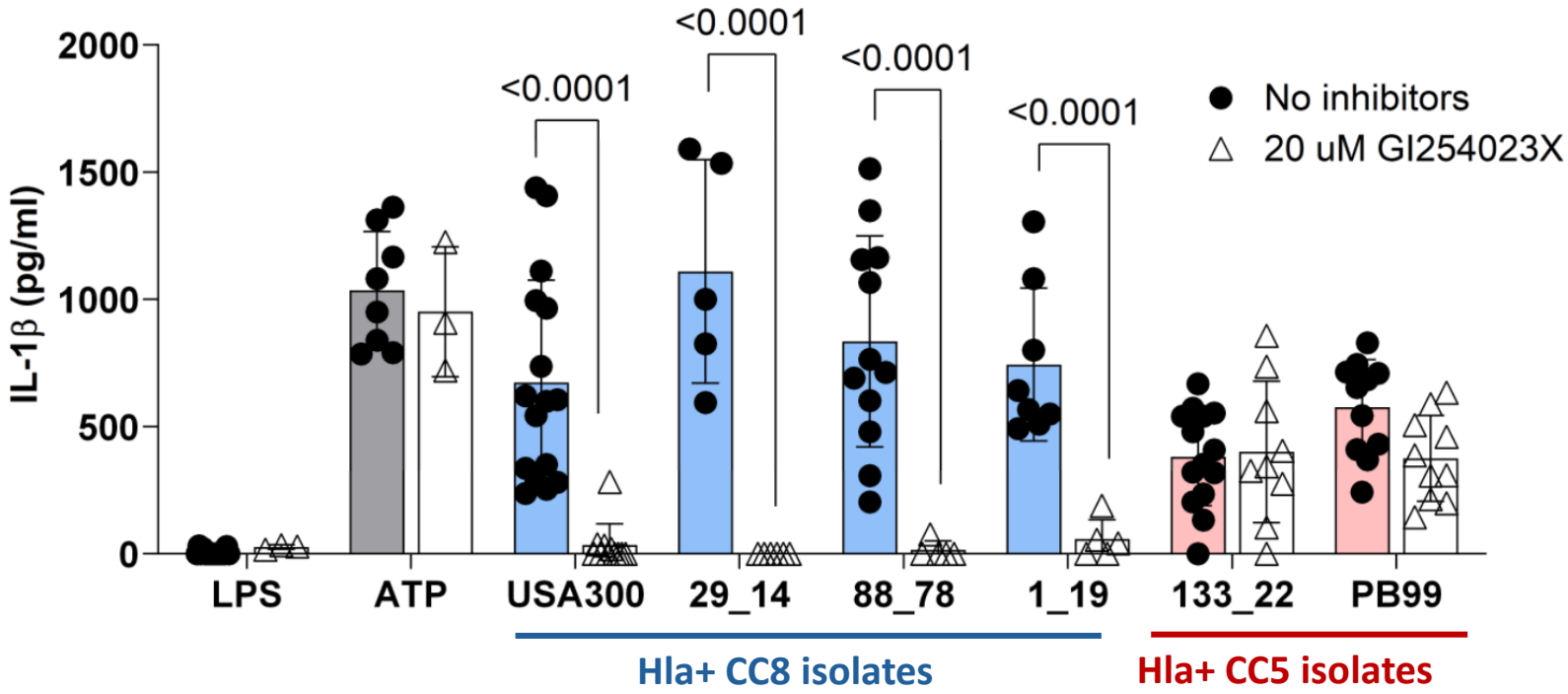
Jolynn Tran-Chau



Neutrophils incubated with **GI254023X (ADAM10 inhibitor)** for 3.5 h prior to addition of conditioned media

ADAM10 inhibitor impairs IL-1β secretion induced by CC8 but not CC5 isolates

Jolynn Tran-Chau



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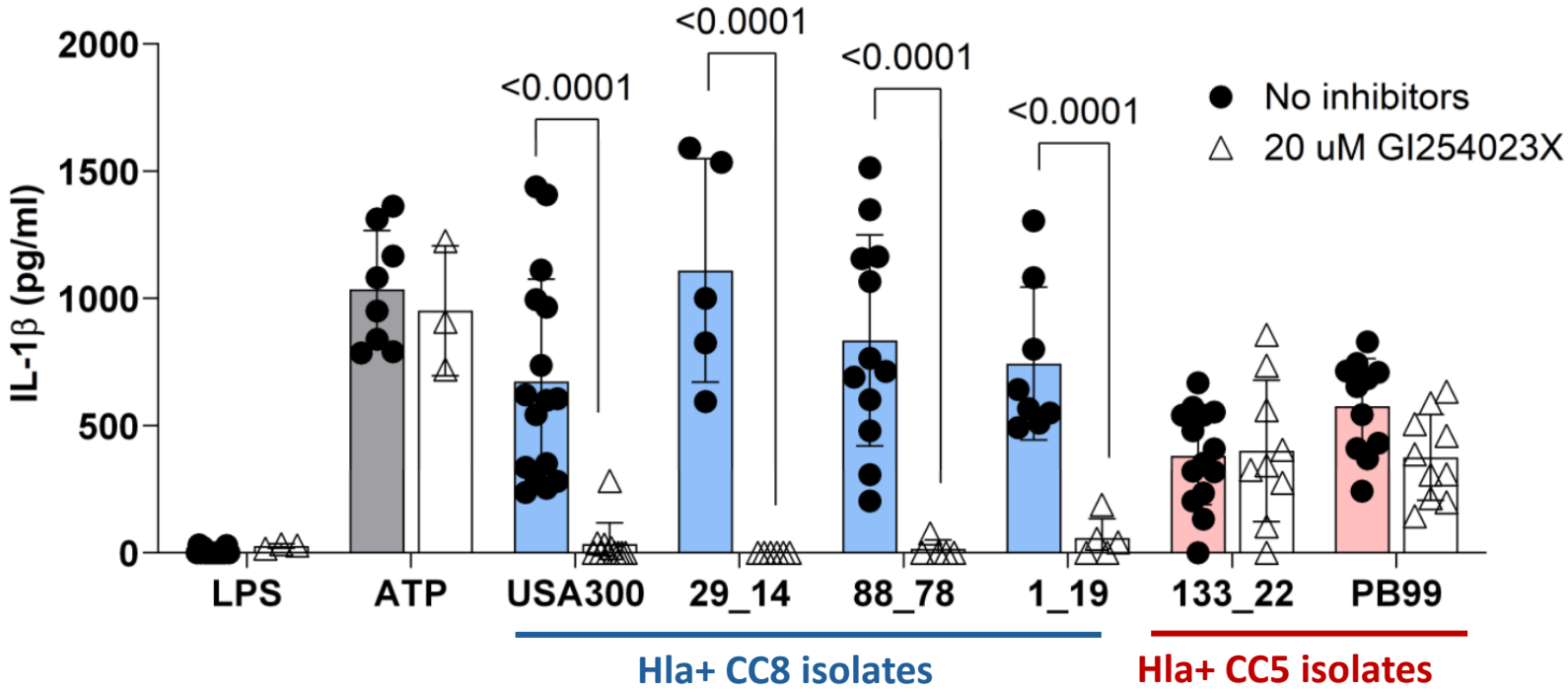


Neutrophils incubated with **GI254023X (ADAM10 inhibitor)** for 3.5 h prior to addition of conditioned media

Future binding studies of α-hemolysin with ADAM10 needed

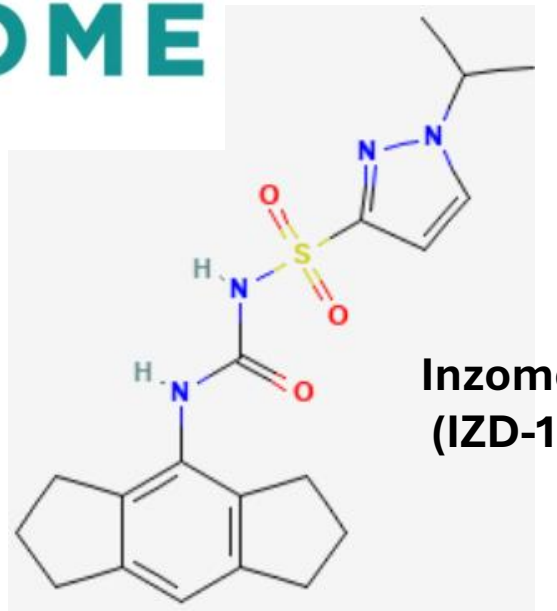
Summary

Secretion of IL-1β is critical to disease severity in MRSA keratitis and represents a druggable target for inhibiting inflammation and tissue damage

- MCC950 – inhibitor used extensively in preclinical animal models
- Inzomelid (IZD-174) – inhibitor had successful PH1 clinical trials
- GDC-2394 – inhibitor halted in PH1 clinical trials

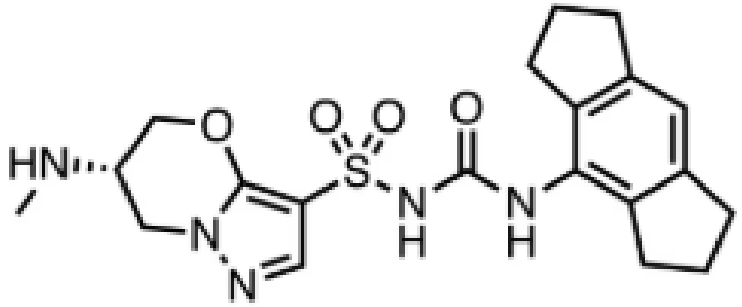
INFLAZOME

Roche



Inzomelid
(IZD-174)

Genentech
A Member of the Roche Group



GDC-2394

Summary

Secretion of IL-1β is critical to disease severity in MRSA keratitis and represents a druggable target for inhibiting inflammation and tissue damage

- MCC950 – inhibitor used extensively in preclinical animal models
- Inzomelid (IZD-174) – inhibitor had successful PH1 clinical trials
- GDC-2394 – inhibitor halted in PH1 clinical trials

α-hemolysin is a critical virulence factor for MRSA keratitis and polymorphism in CC5 MRSA isolates are suspected to impact ADAM10 binding

- Potential drug target or vaccine candidate for some MRSA isolates, but not all
- A better understanding of α-hemolysin-ADAM10 binding is relevant for biotechnologies utilizing α-hemolysin

UC Irvine

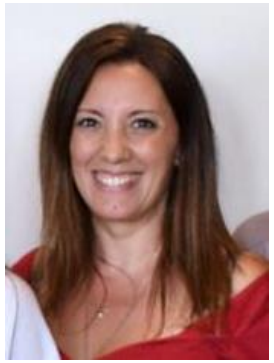
Massachusetts Eye and Ear

Eric Pearlman

James Begando

Jolynn Tran-Chau

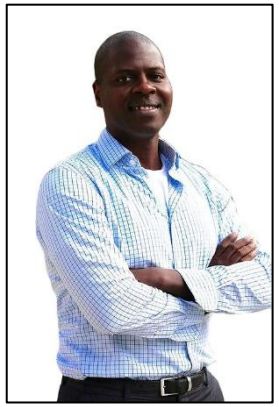
Angela Lackner



Serena Abbondante

Victor Johnson

Michaela Marshall



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George Dubyak

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T32 AI177324 (KL)

Case Western Reserve University Est. 1826

Pathogenesis of blinding corneal infections with methicillin-resistant *Staphylococcus aureus* (MRSA)

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LinkedIn

2025-09-17
Eric Pearlman Lab
University of California, Irvine

Future Directions

- Perform microbial keratitis treatment experiments comparing corticosteroids with IL-1β pathway inhibitors

- Conditioned media contains other secreted factors besides α-hemolysin
  - Site-directed mutagenesis
  - Knock-in and knock-out studies
  - Purified a-hemolysin

- Expand study to other lineages of *S. aureus*
  - Are there other lineages that harbor the same polymorphisms?

- Binding assay of CC5 α-hemolysin with cells or ADAM10