



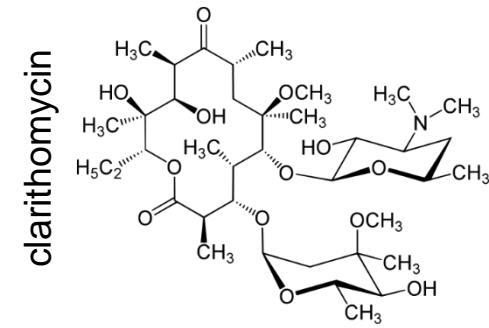
**Democritus University of Thrace**  
**Inflammation Research Group**  
**Laboratory of Molecular Hematology**  
**Alexandroupolis, Greece**

Medical School

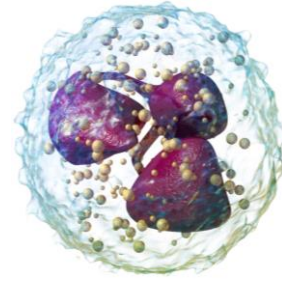
**A new mechanism of action, via formation of Neutrophil Extracellular Traps, for the widely used and established macrolide antibiotic Clarithromycin**

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**Molecular Biologist & Geneticist**

# Macrolides

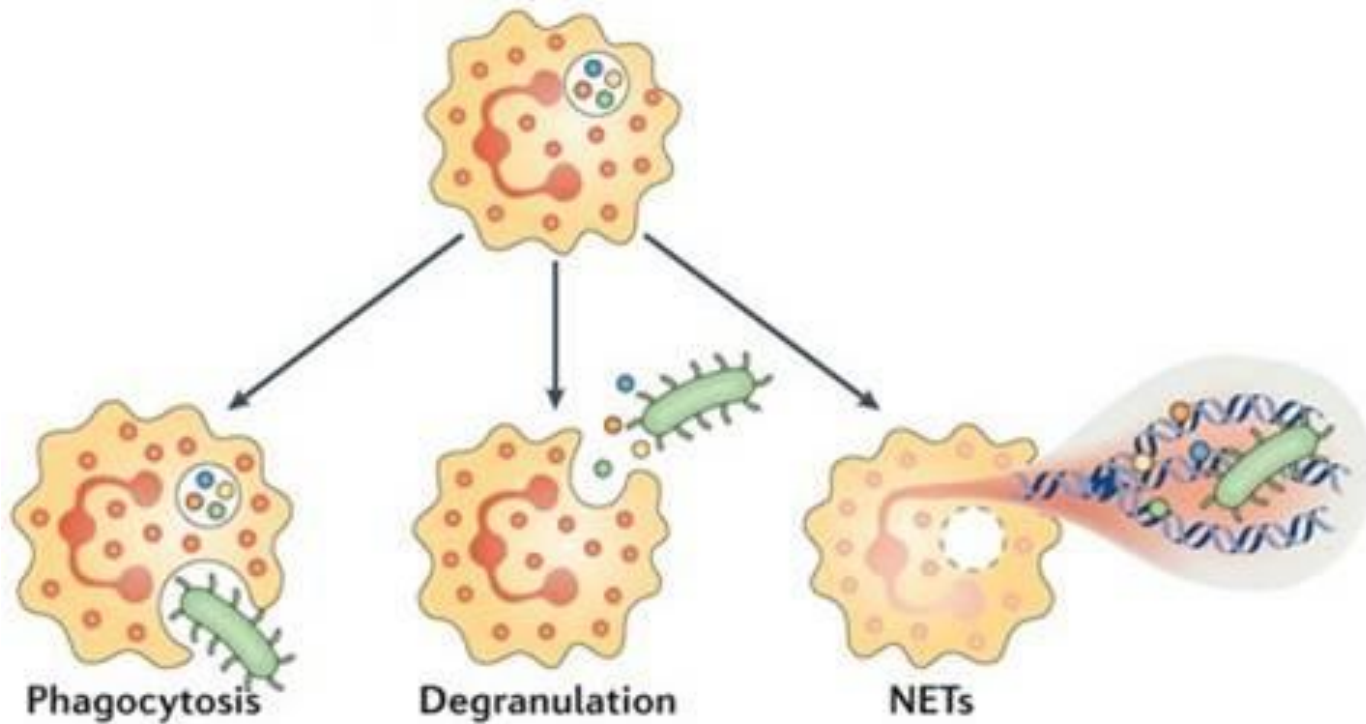


- Some macrolides have antibiotic activity by inhibiting protein synthesis.
- They also can act as immunomodulatory agents as they induce the activity of various immune cells.
- The knowledge regarding their role in neutrophils has been very limited.
- In patients with clarithromycin (macrolide antibiotic) resistant bacterial infections, treatment with clarithromycin was positively correlated with the time of resolution of the infection.

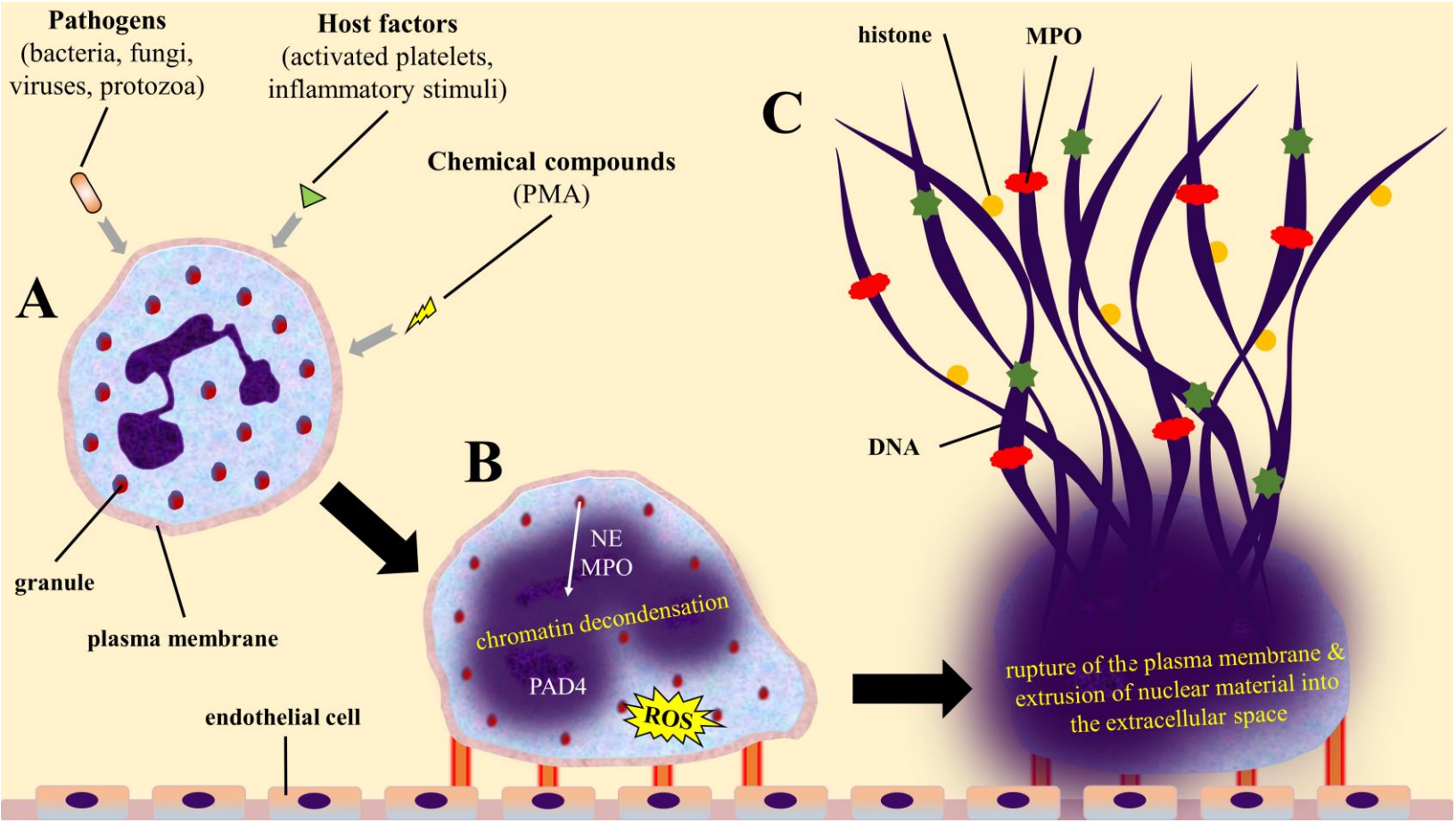


# Neutrophil

- Neutrophils are the most abundant circulating inflammatory cells and the first line of defense against pathogens.
- Neutrophils employ three major strategies to fight against microbes:

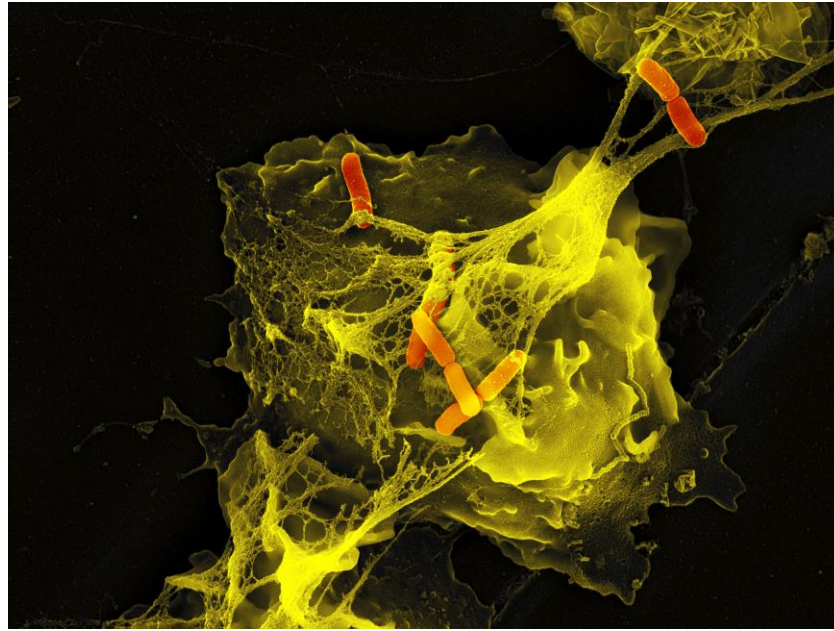


# Neutrophil extracellular traps (NETs)



NETs are composed of chromatin which is decorated with neutrophilic proteins such as myeloperoxidase (MPO), neutrophil elastase (NE) and LL-37 (antimicrobial peptide).

# Neutrophil extracellular traps (NETs)



Max Planck Institute for Infection  
Biology

- NETs not only elicit their antimicrobial effect through pathogen immobilization via entrapment but also have a direct microbicidal effect that depends on antimicrobial peptides, histones or DNA.
- It has been also reported that some microorganisms, such as *Acinetobacter baumannii*, escape NETs or possess inhibitory strategies against NET formation mechanism.

# Objective of the study

- Investigation of the role of clarithromycin in the generation of NETs.
- Investigation of how clarithromycin derived NETs could affect pathogens that naturally are not able to trigger the mechanism of NETosis and are resistant to clarithromycin, such as *Acinetobacter baumannii*.

# Methods

  
**Healthy individual**

Blood sample collection  
→



*In vitro* stimulation  
with clarithromycin  
→



**Helicobacter pylori positive gastritis patients**

Blood sample collection before & after treatment with clarithromycin  
→

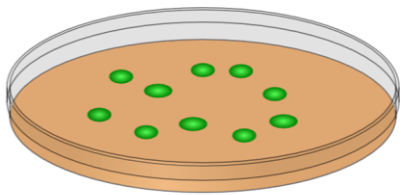
**Isolation of neutrophils**

*Ex vivo* neutrophil culture  
→



**Production of NET structures & NET proteins**

Acinetobacter co-culture with *in vitro* / *ex vivo* generated NET structures  
→

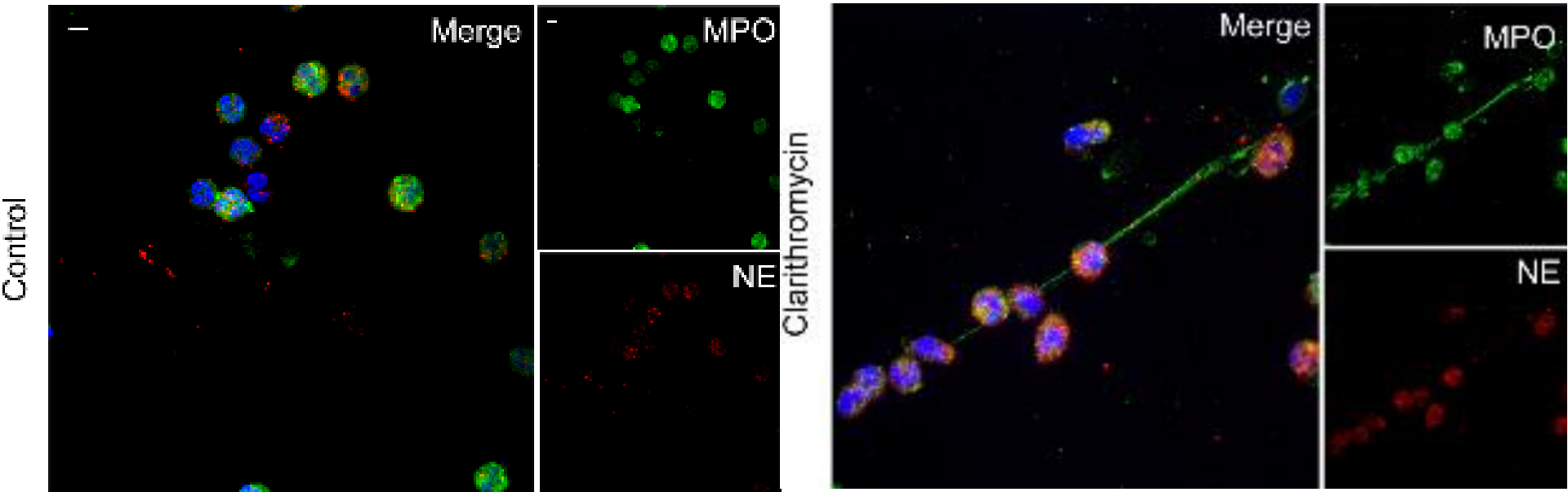


**Colony counting**



- Immunofluorescence - imaging with confocal microscopy
- Western blot

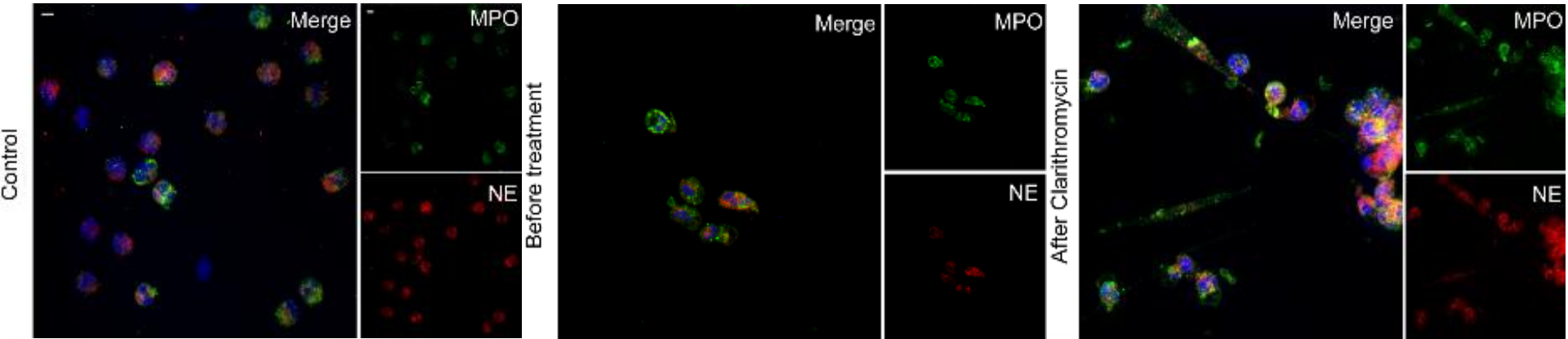
# Clarithromycin induces NETs *in vitro*



*In vitro* stimulation of neutrophils from healthy individuals with clinically relevant concentrations of clarithromycin induced NET formation.

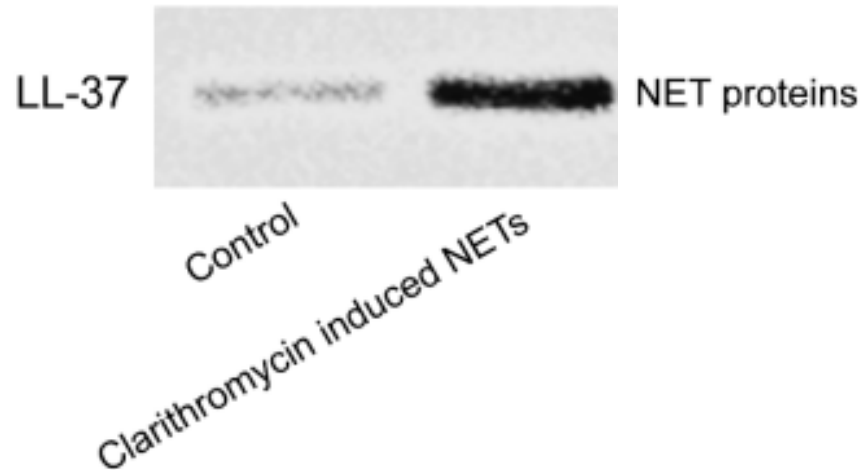
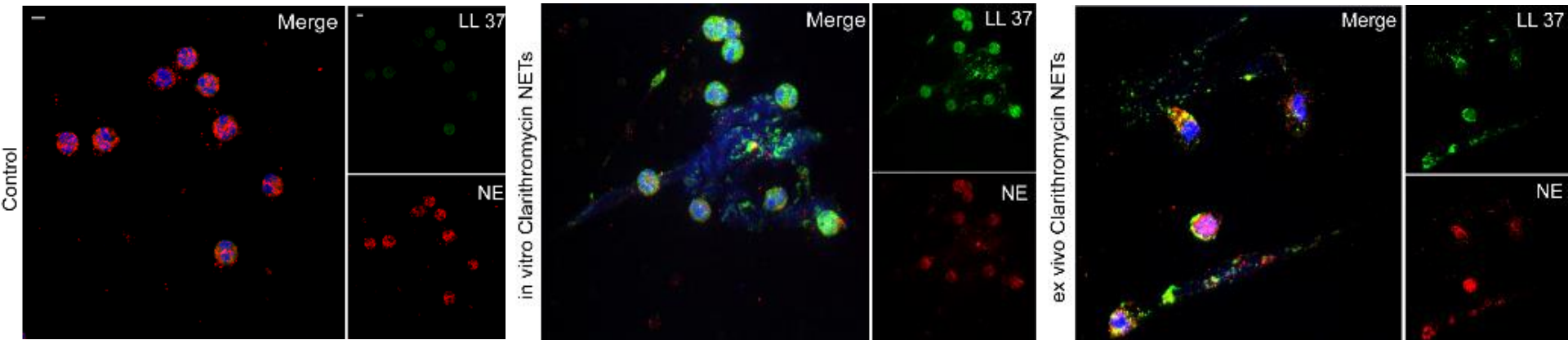


# Clarithromycin induces NETs *in vivo*



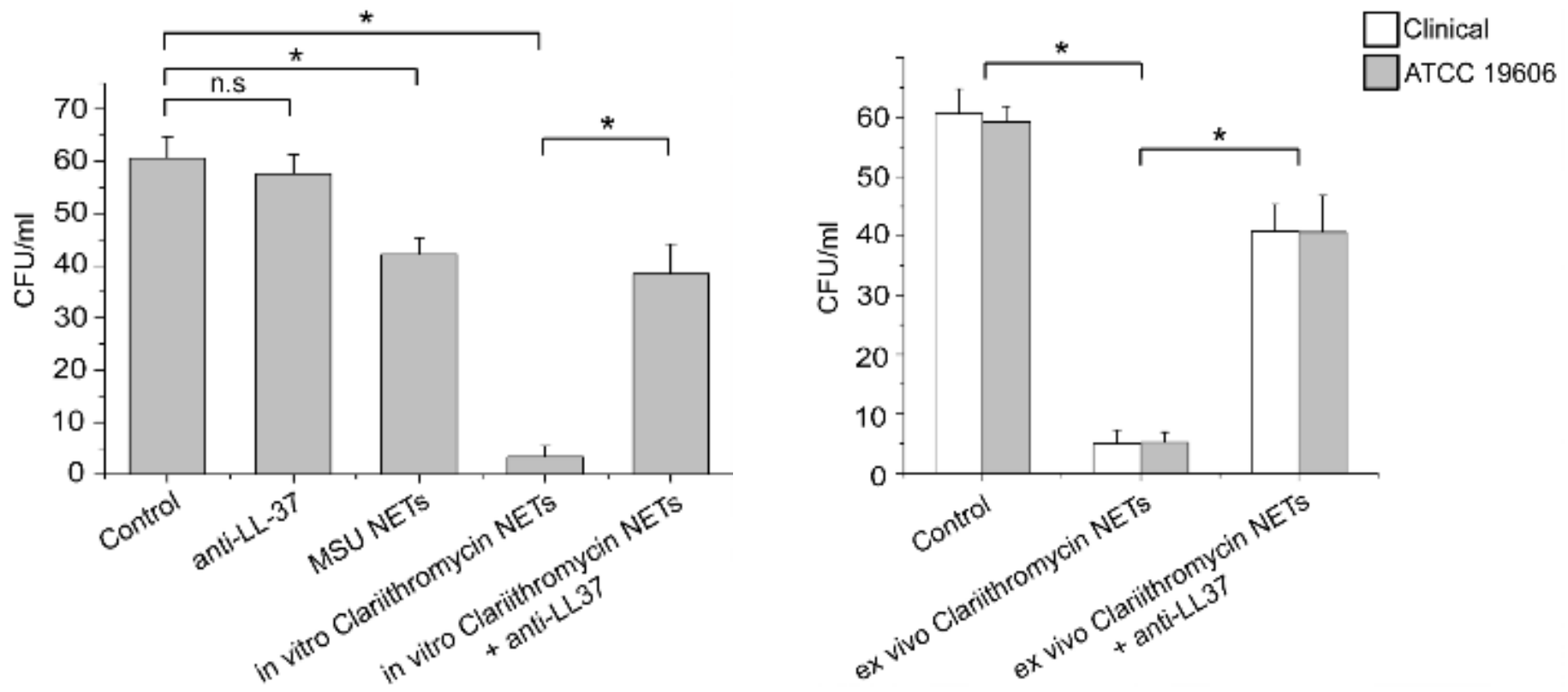
The neutrophils during clarithromycin therapy demonstrated increased *ex vivo* NETs formation compared to neutrophils before clarithromycin initiation and neutrophils from healthy individuals.

# LL-37 is present on clarithromycin NETs



NETs released by neutrophils from control subjects treated with clarithromycin were decorated with LL-37. Neutrophils derived from patients treated with clarithromycin also demonstrated presence of LL-37 on NETs.

# Clarithromycin NETs inhibit *Acinetobacter baumannii*



- Both *in vitro* and *ex vivo* clarithromycin-induced NETs significantly reduced bacterial growth of *Acinetobacter baumannii* compared to control cultures.
- LL-37 neutralization with anti-LL-37 antibody abolished the antimicrobial action of clarithromycin-induced NETs.

# Take home message

- Clarithromycin induces NET generation *in vitro* and *in vivo*.
- LL-37 is present on NETs induced by clarithromycin both *in vitro* and *in vivo*.
- Both *in vitro* and *in vivo* clarithromycin-induced NET inhibit *Acinetobacter baumannii* in a LL-37 dependent manner.
- Since bacteria are becoming more resistant to antibiotics, the knowledge of new mechanisms of action of widely used antibiotics could be proved useful.



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**Thank you.**