

 <p><b>UniSR</b> Università Vita-Salute San Raffaele</p>	<p><b>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</b></p>	<p><b>MO 20-5</b> ed. 01 del 21/02/2025 PO 20 Page 4 of 10</p>
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**PROJECT**

**Supervisor:** Nicola Clementi

**Title:** Development and characterization of *in vitro* models to evaluate bacterial cytolysin activity and its inhibition by molecules or monoclonal antibodies

**Curriculum:** Experimental and Clinical Medicine

Link to the personal page of the University or relevant hospital site website: <https://www.unisr.it/en/docenti/c/clementi-nicola>

**Description of the Project (max 3,000 characters including spaces)**

**Background/gap of knowledge**

*Streptococcus pneumoniae* (pneumococcus) is the leading cause of bacterial otitis media, pneumonia, meningitis, sepsis, and other severe infections. It is responsible for up to 45% of pneumonia cases, highlighting the high morbidity and mortality associated with pneumococcal diseases. While current vaccines based on polysaccharide capsules protect against a quarter of known serotypes, they do not prevent colonization or infection by nonencapsulated pneumococci. Pneumolysin (PLY), a pore-forming toxin produced by *S. pneumoniae*, is a key virulence factor and a potential target for protein-based vaccines. PLY plays a significant role in severe outcomes, including lung and myocardial dysfunction, by disrupting the endothelial barrier, increasing permeability, and causing pulmonary edema. The toxic effects of PLY have been demonstrated in animal models of pneumonia, suggesting that neutralizing PLY's activity could reduce pneumococcal pathogenicity.

**Rationale and hypothesis**

Beyond pore formation, PLY exerts additional pathogenic effects on host cells. At sublytic doses, it may facilitate pneumococcal invasion of alveolar macrophages and dendritic cells by suppressing proinflammatory cytokine responses, thereby evading host defenses. Additionally, sublytic PLY can trigger cytoskeletal rearrangement and proinflammatory signaling. Antibodies can neutralize its cytolytic activity by blocking receptor binding or disrupting oligomerization.

**Objectives and specific aims**



Monoclonal antibodies against pneumolysin (PLY) will be isolated using a phage display library and screened through biopanning with recombinant PLY. ELISA and Western blot will confirm PLY-specific clones, which will be expressed and purified for characterization. Functional assays will assess their ability to neutralize PLY hemolytic activity and block receptor interactions. In vitro studies using alveolar epithelial and endothelial cells will evaluate their protective effects against PLY-induced cytotoxicity and inflammation, supporting their potential as therapeutic agents against pneumococcal disease.

### **Expected outcomes**

This project is expected to yield a panel of monoclonal antibodies endowed with specificity for PLY and/or other bacterial lysins and selected by Phage Display. These antibodies will be characterized for their ability to neutralize PLY hemolytic activity and block its interactions with host receptors, providing insights into their mechanism of action. In vitro studies using alveolar epithelial and endothelial cells will demonstrate their protective effects against PLY-induced cytotoxicity and inflammation, offering potential therapeutic candidates for mitigating pneumococcal toxin-related pathogenesis. The findings will contribute to the development of antibody-based strategies for combating pneumococcal infections.

### **Skills that the student should acquire** (max. 600 characters including spaces):

The student should acquire skills in phage display technology to isolate and characterize monoclonal antibodies. The student will gain proficiency in antibody characterization using ELISA and Western blotting, and learn to perform functional assays to assess PLY neutralization. The student will develop expertise in working with primary and immortalized cell lines, using confocal microscopy, and conducting protein purification. Additionally, they will gain experience in in silico epitope prediction, cloning procedures, data analysis, and scientific communication.

### **References** (max. 15)

Cima Cabal MD, Molina F, López-Sánchez JI, Pérez-Santín E, Del Mar García-Suárez M. Pneumolysin as a target for new therapies against pneumococcal infections: A systematic review. *PLoS One*. 2023;18(3):e0282970. Published 2023 Mar 22. doi:10.1371/journal.pone.0282970

Kucinskaite-Kodze I, Simanavicius M, Dapkunas J, Pleckaityte M, Zvirbliene A. Mapping of Recognition Sites of Monoclonal Antibodies Responsible for the Inhibition of Pneumolysin Functional Activity. *Biomolecules*. 2020;10(7):1009. Published 2020 Jul 8. doi:10.3390/biom10071009



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Sheng Q, Hou X, Wang N, et al. Corilagin: A Novel Antivirulence Strategy to Alleviate *Streptococcus pneumoniae* Infection by Diminishing Pneumolysin Oligomers. *Molecules*. 2022;27(16):5063. Published 2022 Aug 9. doi:10.3390/molecules27165063

Xu L, Fang J, Ou D, et al. Therapeutic potential of kaempferol on *Streptococcus pneumoniae* infection. *Microbes Infect*. 2023;25(3):105058. doi:10.1016/j.micinf.2022.105058

Yu VL, Chiou CC, Feldman C, et al. An international prospective study of pneumococcal bacteremia: correlation with in vitro resistance, antibiotics administered, and clinical outcome. *Clin Infect Dis*. 2003;37(2):230-237. doi:10.1086/377534