DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

Proposta di assegnazione di una borsa di Dottorato

Titolo della ricerca: Inhibition of colistin resistance in gram-negative pathogens

Docente guida proposto: Ascenzioni Fiorentina (PA, SSD BIO/19)

DESCRIZIONE DELLA RICERCA

Summary

At present polymyxins, comprising polymyxin B and colistin (polymyxin E), are the last-resort therapeutic option in infections by multi-drug resistant pathogens (1-2). However, as colistin resumption started, colistin resistant bacteria were identified (2). In Cystic Fibrosis (CF), colistin has been used for decades suggesting that resistance to this antibiotic might soon become critical for CF patients. Indeed, an increase of colistin-resistant *Pseudomonas aeruginosa* isolates in CF has been recently reported.

The antibacterial activity of colistin relies on its interaction with LPS and aminoarabinosylation of the lipid A moiety of LPS reduces such interaction making bacteria resistant to colistin (2, 3). Therefore, the main objective of this project is to develop small molecules that revert colistin resistance by targeting lipid A aminoarabinosylation.

Taking advantage of the resolved structure of the aminoarabinose transferase (4), which catalyzes the last step of lipid A aminoarabinosylation, and using a structure-guided in silico approach, we have identified a short list of candidate hits from the screening of a library of natural compounds. Growth-inhibition assays of a colistin-resistant tester strain led to the identification of one lead compound which reverts colistin resistance to sensitivity (5).

This PhD project comprises the following aims:

- optimization of potency and specificity of the lead compound. This aim comprises the screening of derivatives of the lead compound produced by our collaborators (Prof. B. Botta, Department of Chemistry and Technology of Drug, Sapienza University) through combined computational and medicinal chemistry. The screening will be done in *P. aeruginosa* tester strains.
- analysis of the efficacy of lead inhibitors of colistin resistance on different pathogens. Those compounds that show an increased activity will be analyzed in different colistin resistant bacteria including *P. aeruginosa*, *Klebsiella pneumoniae* and *Burkholderia cenocepacia* using planktonic coltures and biofilms.
- Development of nano-delivery systems for the colistin inhibitors.
- *in vitro* cytoxicity analysis on cellular models

Pertinent Publications of the proponent (last 5 years)

Ghirga F, Stefanelli R, Cavinato L, Lo Sciuto A, Corradi S, Quaglio D, Calcaterra A, Casciaro B, Loffredo MR, Cappiello F, Morelli P, Antonelli A, Rossolini GM, Mangoni M, Mancone C, Botta B, Mori M, **Ascenzioni F**, Imperi F. A novel colistin adjuvant identified by virtual screening for ArnT inhibitors. J. Antimicrob. Chemother. 2020, Jun 8:dkaa200. doi: 10.1093/jac/dkaa200.

Quaglio D, Corradi S, Erazo S, Vergine V, Berardozzi S, Sciubba F, Cappiello F, Crestoni ME, **Ascenzioni F**, Imperi F, Delle Monache F, Mori M, Loffredo MR, Ghirga F, Casciaro B, Botta B,

Mangoni ML. Structural Elucidation and Antimicrobial Characterization of Novel Diterpenoids from Fabiana densa var. ramulosa. ACS Med Chem Lett. 2020 Jan 30;11(5):760-765. doi: 10.1021/acsmedchemlett.9b00605. eCollection 2020 May 14.

Cavinato L, Genise E, Luly FR, Di Domenico EG, Del Porto P, **Ascenzioni F.** Escaping the Phagocytic Oxidative Burst: The Role of SODB in the Survival of Pseudomonas aeruginosa Within Macrophages. Front Microbiol. 2020 Mar 10;11:326. doi: 10.3389/fmicb.2020.00326. eCollection 2020.

Luly FR, Lévêque M, Licursi V, Cimino G, Martin-Chouly C, Théret N, Negri R, Cavinato L, **Ascenzioni F**, Del Porto P. MiR-146a is over-expressed and controls IL-6 production in cystic fibrosis macrophages. Sci Rep. 2019; 9(1):16259. doi: 10.1038/s41598-019-52770-w.

Di Domenico EG, Cavallo I, Capitanio B, **Ascenzioni F**, Pimpinelli F, Morrone A, Ensoli F. Staphylococcus aureus and the Cutaneous Microbiota Biofilms in the Pathogenesis of Atopic Dermatitis. Microorganisms. 2019; 7(9). pii: E301. doi: 10.3390/microorganisms7090301.

Sardo C, Di Domenico EG, Porsio B, De Rocco D, Santucci R, **Ascenzioni F***, Giammona G, Cavallaro G. Nanometric ion pair complexes of tobramycin forming microparticles for the treatment of Pseudomonas aeruginosa infections in cystic fibrosis. Int J Pharm. 2019 May 30;563:347-357. doi:10.1016/j.ijpharm.2019.03.060.

De Rocco D, Pompili B, Castellani S, Morini E, Cavinato L, Cimino G, Mariggiò MA, Guarnieri S, Conese M, Del Porto P, **Ascenzioni F***. Assembly and Functional Analysis of an S/MAR Based Episome with the Cystic Fibrosis Transmembrane Conductance Regulator Gene. Int J Mol Sci. 2018;19(4). pii: E1220. doi:10.3390/ijms19041220.

Abrami M, **Ascenzioni F**, Di Domenico EG, Maschio M, Ventura A, Confalonieri M, Di Gioia S, Conese M, Dapas B, Grassi G, Grassi M. A novel approach based on low-field NMR for the detection of the pathological components of sputum in cystic fibrosis patients. Magn Reson Med. 2018: 79(4):2323-2331. doi:10.1002/mrm.26876.

Bragonzi A, Paroni M, Pirone L, Coladarci I, **Ascenzioni F**, Bevivino A. Environmental Burkholderia cenocepacia Strain Enhances Fitness by Serial Passages during Long-Term Chronic Airways Infection in Mice. Int J Mol Sci. 2017: 18(11). doi: 10.3390/ijms18112417.

Chronopoulou L, Di Domenico EG, **Ascenzioni F**, Palocci C. Positively charged biopolymeric nanoparticles for the inhibition of Pseudomonas aeruginosa biofilms. Manuscript accepted for publication in J of Nanoparticle Research, 2017

Di Domenico EG, Toma L, Provot C, **Ascenzioni F**, Sperduti I, Prignano G, Gallo MT, Pimpinelli F, Bordignon V, Bernardi T, Ensoli F. Development of an in vitro Assay, Based on the BioFilm Ring Test, for Rapid Profiling of Biofilm-Growing Bacteria. Front Microbiol. 2016 Sep 21;7:1429.

Gallo MT, Di Domenico EG, Toma L, Marchesi F, Pelagalli L, Manghisi N, **Ascenzioni F**, Prignano G, Mengarelli A, Ensoli F. Campylobacter jejuni Fatal Sepsis in a Patient with Non- Hodgkin's Lymphoma: Case Report and Literature Review of a Difficult Diagnosis. Int J Mol Sci. 2016 Apr 12;17(4):544. doi: 10.3390/ijms17040544

References (other citations, if appropriate)

1. Giamarellou H. Epidemiology of infections caused by polymyxin-resistant pathogens. International Journal of Antimicrobial Agents 2016; 48: 614–621
2. Poirel L, *et al*. Polymixins: antibacterial activity, susceptibility testing and resistant mechanismis encoded by plasmids or chromosomes. Clin Microbiol Rev 2017; 30: 557-596
3. Lo Sciuto A and F Imperi. Aminoarabinosylation of lipid A is critical for the development of colistin resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 2017, pii: AAC.01820-17.
4. Petrou VI, *et al*. Structures of aminoarabinose transferase ArnT suggest a molecular basis for lipid A glycosylation. Science 2016; **351**: 608-12.
5. F. Ghirga, et al., A novel colistin adjuvant identified by virtual screening for ArnT inhibitors. J. Antimicrob. Chemother. 2020. Doi: 10.1093/jac/dkaa200