Project Title: Design, production, characterization and delivery of novel CCL5 derivatives as potent therapeutic CCR5 modulators

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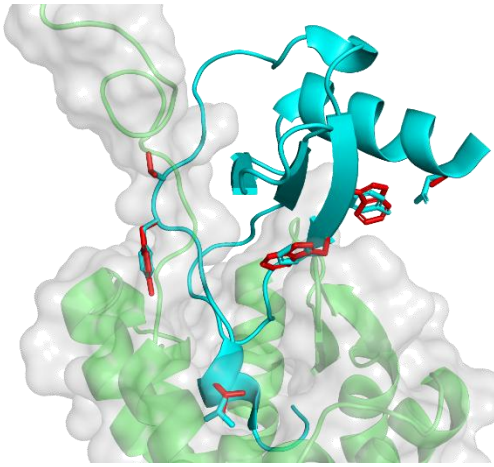
Background: CCR5 is a chemokine receptor expressed on various cell types and plays a vital role in the inflammatory response by directing cells to sites of inflammation. Massive efforts have been devoted to combat HIV-1 entry by targeting CCR5. The production of chemokine ligand variants and other molecular entities and strategies set the therapeutic standards for a wealth of different pathologies related to CCR5. Indeed, CCR5 is implicated in several infectious and inflammatory diseases. Caution is needed when acting on CCR5; a pharmacological approach (e.g., CCR5 antagonists) is possibly preferable to a definitive gene editing knock out, considering that drug discontinuation is likely to restore normal CCR5 expression and function. Among natural CCR5 ligands, CCL5 has been the most characterized and modified to attain HIV-1 entry inhibitors. We developed several CCL5-based extremely potent HIV-1 entry inhibitors, both CCR5 antagonists and CCR5 agonists. Tested in vitro, these CCL5 derivatives proved to be 1000 fold more potent than maraviroc (a CCR5 antagonist small chemical drug). In addition, we set the proof-of-principle for Lactobacillus spp.-based anti-HIV-1 live microbicides, engineered to produce the CCL5 derivatives. Here, we plan to deliver CCL5 variants by devising mRNA nanoparticle and commensal microorganism systems to be tested in vitro for HIV-1 and cancer inhibition. This project is instrumental for the perspective translation into preclinical and possibly clinical studies.

Aims: In a collaborative effort (Kazakhstan, Spain and Pavia), we are testing the engineering of different commensal microorganisms for the secretion of CCL5 variants in order to attain live CCR5 blockade. In parallel, we plan to devise a de novo approach using mRNA nanoparticles in different formulation composition and functionalization.



Rational Design of CCL5 mutants. A) The CCR5 (transparent grey surface and green ribbon):CCL5 (cyan ribbon) Complex is analyzed by different biocomputing methods and interaction hotspots are mutated and tested in the wet lab (in red, amino acid hotspots successfully tested and reported in peer-reviewed publications).

Techniques: Protein design (structure-guided design of novel CCL5 variants, *in silico*), engineering and production (different expression systems, wet lab). Design and characterization of CCL5 variants-encoding mRNA nanoparticles.