Pharmacophore-based drug optimization targeting human CD73 enzyme

Braka A.[2], Peyrottes S.[3], Jordheim L.P.[4], Ménétrier-Caux C.[4], Chaloin L.[*,1,2]

*lead presenter ; [1]laurent.chaloin@irim.cnrs.fr

[2] Institut de Recherche en Infectiologie de Montpellier (IRIM), Univ. de Montpellier, CNRS, Montpellier, France
[3] Institut des Biomolécules Max-Mousseron (IBMM), CNRS, Univ. Montpellier, ENSCM, Montpellier, France.
[4] Centre de recherche en cancérologie de Lyon, Univ. Claude Bernard Lyon 1, INSERM 1052, CNRS 5286, Centre Léon Bérard, Lyon, France.

CD73 or ecto-5'-nucleotidase is a dimeric membrane-bound enzyme which controls the extracellular adenosine (ADO) concentration. CD73 catalyzes the hydrolysis of AMP in adenosine and acts in concert with the upstream CD39 enzyme that catalyzes ATP conversion in AMP. CD73 is primarily expressed on both immune and cancer cells with overexpression largely observed in tumor cells. The resulting increase in ADO induces a strong immunosuppressive response which favors in fine tumor growth and metastasis. To stop this abusive production of ADO and to restore the immune response, we have developed a new family of small molecule inhibitors able to block CD73 activity. The strategy consisted in altering the enzyme dynamic and therefore the large conformational changes occurring during the reaction leading to the complete inhibition of enzymatic activity.

By combining molecular dynamics simulations and virtual screening, we first explored the conformational space used by CD73 during domains closure (impressive rigid body motions of about 120°) in order to extract several transient conformations and use them for virtual screening. The MolPort chemical library was used to screen the dimerization interface as target binding site. Best-ranked hit compounds were further evaluated by inhibition kinetics assays using the purified recombinant enzyme. This experimental study was required to evidence the inhibitory activity as well as the mechanism of action. Since we targeted a site different to that of substrate binding site, we expected an allosteric inhibition mechanism. Then, lead molecules were optimized by using chemoinformatics approaches such as 3D-pharmacophore models based on the most active compounds, the final goal being to end up with more potent drugs showing high selectivity and bioavailability. Few optimized leads have been synthetized and evaluated *in vitro* and finally using cancer cell lines to determine their efficacy in restoring the anticancer immune response.

Bibliography:

Rahimova R., Fontanel S., Lionne C., Jordheim L.P., Peyrottes S. and **Chaloin L.** "Identification of allosteric inhibitors of the ecto-5'-nucleotidase (CD73) targeting the dimer interface". *PLoS Comp. Biol.* **(2018)** 14(1): e1005943. *doi: 10.1371/journal.pcbi.1005943*