The use of resonance energy transfer of nano-bioluminescence (nanoBRET) from Promega in the determination of our target in the development of a treatment for hepatic ischemia-reperfusion injury

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The last few years, a growing need in liver transplant is observed, leading to a shortage of grafts. In order to overcome this issue, the selection's criteria have been expanded which leads to a risk of ischemia-reperfusion injury even more important than usual. The ischemia-reperfusion (I-R) injury is the process starting from the moment when the organ is deprived from oxygen in the donor patient's body and ending when blood enters the organ once transplanted in the receiving patient's body. During this period, tissues suffer from several lesions which increases the risk of reject or post-surgery complications. The Inserm INFINITE (U1286) team has identified NOD1 as a potential target in the treatment of liver I-R injuries that occur after organ transplant and recently brought proof of concept on a murine model of liver I-R injury. The cytoplasmic receptor NOD1 is a receptor that recognizes Pathogen Associated Molecular Patterns (PAMP). It's known for increasing pro-inflammatory cytokines production *via* recruitment of the RIP2 kinase. In order to treat I-R injuries, our group develops NOD1 inhibitors and we recently discover compounds with an activity at the nanomolar range on the NOD1 inflammatory pathway.

In order to verify if our molecules target RIP2, we used a test of resonance energy transfer of nano-bioluminescence (nanoBRET) from Promega. This test allowed us to observe our molecules capacity to bind to RIP2 directly in the cell, as described by Hrdinka et al.[1].

Briefly, HEK-Blue™-*h*NOD1 cells are transfected by the RIP2 kinase fused with a luciferase: NanoLuc-RIP2. The cells are then incubated with with different concentrations of our molecules and a nanoBRET tracer (Promega). NanoLuc substrate (Promega) is added and the BRET ratios are determined using a plate reader. This test gives us an evaluation of RIP2

kinase inhibition in the cell and allows us to confirm that our molecules do interact with RIP2 in order to inhibit NOD1 pathway. This confirmation is essential for a therapeutic chemistry project, because knowing our real target is primordial for making the best decisions in the development and evaluation of our compounds.

1. Hrdinka M.; *et al*. Small Molecule Inhibitors Reveal an Indispensable Scaffolding Role of RIPK 2 in NOD 2 Signaling. *The EMBO Journal*, 37(17), **2018**