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Receptor Binding, Internalization and Cytotoxicity of a CCR2 Targeting Fusion Protein.

John R. McDonald¹, Laura M. McIntosh¹, Martin Roberge², Hongsheng Su¹, Barbara K. Finck³.

Osprey Pharmaceuticals Limited, St. Laurent, QC, Canada¹, ImmuniT RIMOUSKILAB Inc., Montreal, QC, Canada², Osprey Pharmaceuticals USA Incorporated, San Francisco, CA, USA³

Modulation of pathological monocytes and macrophages via the CCL2/CCR2 chemokine ligand/receptor axis is pivotal to the pathology of a wide range of inflammatory, autoimmune and fibrotic diseases. Our therapeutic approach is to eliminate CCR2 expressing leukocyte subpopulations using a chemokine fusion protein that specifically targets the CCR2 chemokine receptor. The fusion protein, which we refer to as a Leukocyte Population Modulator (LPM), is comprised of the human CCL2 ligand genetically fused to a mutated and truncated version of the SA1 subunit from *Shigella dysenteriae* holotoxin (CCL2-LPM). Binding and internalization studies demonstrate that the chemokine moiety is responsible for targeting and binding CCR2 thereby facilitating internalization. Specifically, we have shown that CCL2-LPM binds to CCR2 expressing human, rat and monkey PBMCs, the THP-1 monocytic cell line and CCR2 transfected Chem-1 cells. CCL2-LPM was also shown to compete for binding with ¹²⁵I-human recombinant CCL2. In addition, CCL2-LPM is rapidly internalized (~15 min) by THP-1 cells consistent with the published kinetics of uptake of CCL2. The SA1 moiety is a ribosome inactivating protein (RIP) and is responsible for the depurination of ribosomes which results in the inhibition of protein synthesis and subsequent cell death. The RIP activity was characterized using an *in vitro* protein synthesis assay and showed an IC₅₀ in the 12-30 pM range. The LPM was shown to be selectively cytotoxic to only these cells shown to express CCR2. CCL2-LPM has demonstrated safety and efficacy in several animal models and will enter a phase 1b clinical trial in 2009.