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GMP production and characterization of a fusion protein comprised of the chemokine CCL2-ligand genetically fused to a mutated and truncated form of the shiga A1 subunit.

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First generation chemokine ligand-Shiga A1 (SA1) fusion proteins (Leukocyte Population Modulators; LPMs) were previously only obtained in small quantities due to the ribosomal inactivating protein properties of the SA1 moiety which inhibits protein synthesis in host cells. We therefore developed a scalable manufacturing process capable of producing sufficient quantity of these proteins for preclinical testing. Scale up allowed the GMP production of clinical grade material of the lead candidate, OPL-CCL2-LPM, in gram quantities for clinical trials. Briefly, *E. coli* host expression cells were grown in the presence of 4-aminopyrazolo [3, 4-d]-pyrimidine, an inhibitor of Shiga A1, in order to isolate clones that could express the LPM. Clones containing mutated SA1 genes were selected and used to construct a pET9c OPL-CCL2-LPM expression system. This plasmid was subsequently transformed into the *E. coli* HMS174 (DE3) strain. A cell bank was established and used to produce OPL-CCL2-LPM in a fed-batch fermentation process. Induction of the expression of OPL-CCL2-LPM led to the production of ~ 22.2 mg/Liter per OD_{600} unit contained in inclusion bodies. The LPM was purified from inclusion bodies using solubilization, refolding and chromatography steps. The identity and purity of the OPL-CCL2-LPM was determined using several analytical techniques. The product retained the ability of the SA1 moiety to inhibit protein synthesis as measured in a cell-free system. Binding, internalization and cytotoxicity studies verified that the CCL2 chemokine moiety was responsible for the interaction of the protein via the CCL2 cognate chemokine receptor, CCR2 and the intracellular delivery of OPL-CCL2-LPM. The LPM will enter clinical trials in the near future.