"A non-oxidable HMGB1 mutant for wound healing"

Background and Description of Invention. High Mobility Group Box 1 (HMGB1) is a nuclear protein that signals tissue damage when released into the extracellular medium, thus working as a Damage Associated Molecular Pattern (DAMP) (Bianchi, 2007). Extracellular HMGB1 can act as a chemoattractant for leukocytes as well as a proinflammatory mediator to induce both recruited leukocytes and resident immune cells to release TNF, IL-1, IL-6 and other cytokines. Notably, immune cells secrete HMGB1 when activated by infection or tissue damage (Andersson and Tracey, 2012) while mesothelioma and other cancer cells secrete HMGB1 constitutively (Jube et al., 2012). Recently, our scientists' results indicated that different molecular forms of HMGB1 orchestrate such key events in sterile inflammation, leukocyte recruitment and activation of cytokine release. The key question to be addressed at this stage was: which HMGB1 variant could maintain chemoattractant properties without inducing cytokine/chemokine production? Our scientists performed studies on the involvement of individual cysteines in the cytokine-stimulating and chemotactic activities of HMGB1 by generating HMGB1 mutants. The activity of these HMGB1 mutants was tested on macrophages, monocytes and fibroblasts: on the one hand they all failed to induce cytokines/chemokines expression by macrophages, on the other they all induced fibroblast and monocyte migration. Namely, it was shown that by generating a non-oxidizable HMGB1 mutant, in which serines replace all cysteines (*i.e.* 3S-HMGB1), the latter did not promote cytokine production, but recruited leukocytes *in vivo* more effectively than wild-type HMGB1 (Venereau et al., *JEM* 2012) .

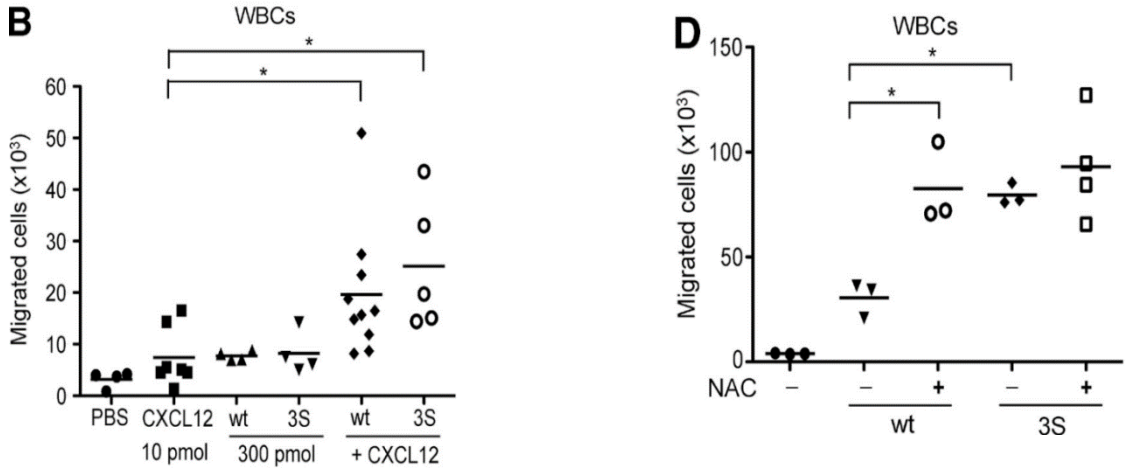

Figure 1. 3S-HMGB1 induces leukocyte recruitment *in vivo*. Our scientists previously showed that the HMGB1–CXCL12 heterocomplex induces a massive influx of leukocytes into air pouches created by the injection of air in the dorsal derma of mice; such air pouches provide a cavity into which drugs can be administered and from which recruited cells can be recovered (Schiraldi et al., 2012) . Panel B: At day 6, scientists injected into air-pouches WT or 3S-HMGB1 (300 pmol) together with CXCL12 (10 pmol). HMGB1 (WT or 3S) or CXCL12 alone failed to induce leukocyte recruitment, but both WT and 3S-HMGB1 in association with CXCL12 induced a massive influx of leukocytes. Notably, the number of recruited leukocytes was increased in response to 3S-HMGB1–CXCL12 when compared to WT HMGB1–CXCL12. Panel D: same experiment was performed in the presence or absence of an antioxidant such as *N-acetylcysteine (NAC)*. After 6 h, cells were collected from air pouches, stained with anti-Ly6C and anti-CD11b antibodies, and analyzed by flow cytometry (WBCs, white blood cells; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, ANOVA plus Dunnett's posttest).

Overall, the inventors' work demonstrates how different redox states of HMGB1 impact on its chemotactic properties, revealing its therapeutic potential for the treatment of pathologies requiring tissue regeneration, such as wounds, fractures and physical trauma recovery, ischemia and recovery thereof of various tissues and organs.

Patent information. An international patent application was published as WO2014016417. Patent applications pending in Europe and US. The patented technology is available for licensing worldwide.

Stage of Development. Our scientists investigated the redox state of HMGB1 *in vivo* during muscle injury and its subsequent sterile inflammation, using electrophoretic mobility assay. Tibialis anterior muscles of mice were damaged by cardiotoxin (CTX) injection, which causes muscle cell necrosis (Ownby et al., 1993). Muscles were harvested 2, 6, 24, or 72 h after CTX injection. HMGB1 was abundant in the medium bathing CTX-injured muscles, while it was barely detectable in the medium bathing healthy muscles of untreated control mice. In the model of muscle injury, all-thiol-HMGB1 was prevalent in the extracellular environment immediately after damage, and disulfide-HMGB1 appeared a few hours later. Researchers suggest that all-thiol-HMGB1 was released first for the recruitment of leukocytes, which in turn produced disulfide-HMGB1, directly by secretion and/or indirectly by partially oxidizing extracellular HMGB1 through ROS (reactive oxygen species). Finally, sustained ROS production eventually induced terminal oxidation of HMGB1, which was inactivated during the resolution of inflammation. Thus, the HMGB1 variant disulfide-HMGB1 can be considered as a marker of tissue damage.
Inventors are now testing the molecule in the process of recovery from muscle damage, with highly encouraging results, and are discussing the models currently available for testing its healing potential in bone fractures, which they want to address with high priority.

Potential Applications and Competitive Advantages. The invention is a valuable tool to be used for therapeutic applications promoting cell recruitment for damaged tissue repair.

We seek a potential commercial partner focused on treatment of pathologies requiring tissue regeneration and interested in further developing selected HMGB1 variants as therapeutic tools.

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