Development of a targeted lipid nanoparticle platform for in vivo RNA delivery to hematopoietic stem and progenitor cells

by

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Hematopoietic stem cells (HSCs) are rare cells residing in the bone marrow that are responsible for the generation and maintenance of the body's immune system through a process known as hematopoiesis. Because of their self-renewal capability and crucial role in producing immune cells, HSCs have garnered a lot of therapeutic interest for the treatment of genetic blood disorders. However, current HSC gene therapies are autologous *ex vivo* transplantations which consists of three major steps: 1) mobilization and harvest of the patient's own stem cells, 2) *ex vivo* editing of those cells, and 3) re-infusion of the edited cells back into the patient. While the results of these *ex vivo* therapies are quite promising, there are many limitations associated with the current process. First, the manufacturing process is logistically complex which results in high costs and lengthy times. Second, prior to re-infusion of the edited cells, patients must undergo a conditioning regimen (done with a chemotherapeutic) to deplete the existing cells from the bone marrow and make space for the edited cells to graft. This conditioning has many deleterious side effects including organ damage, increased risk of infection, and infertility. One strategy to bypass the existing limitations of *ex vivo* HSC therapy is to directly edit the HSCs *in vivo*.

Here, we describe the development of a targeted non-viral lipid nanoparticle (LNP) delivery system that can deliver RNA to hematopoietic stem and progenitor cells (HSPCs) *in vivo* following a single intravenous injection. We targeted CD117, a receptor that is expressed on HSCs, and conjugated an antibody against CD117 to our LNPs for receptor-mediated delivery of RNA. We demonstrated that modulation of certain LNP parameters such as circulation time and ligand density increase delivery to the bone marrow. Using this targeted platform, we demonstrated LNP uptake and delivery of both siRNA and Cre mRNA into HSPCs. In addition, in the Ai14 mouse model, we showed that HSCs transfected with our targeted LNPs maintain their stemness and functionality to produce mature immune cells. We also evaluated the overall biodistribution of our anti-CD117 LNP and investigated the downstream effects of delivery to organs other than the bone marrow. Finally, we explored *in vivo* gene editing using a variety of approaches. We optimized our LNP formulation to increase protein expression in bone marrow HSCs and used our optimized formulation for *in vivo* base editing.

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