Site-Specific Gene Editing of Human Hematopoietic Stem Cells for X-Linked Hyper-IgM Syndrome.

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Authors: Caroline Y Kuo, Joseph D Long, Beatriz Campo-Fernandez, Satiro de Oliveira, Aaron R Cooper, Zulema Romero, Megan D Hoban, Alok V Joglekar, Georgia R Lill, Michael L Kaufman, Sorel Fitz-Gibbon, Xiaoyan Wang, Roger P Hollis, Donald B Kohn

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Public Summary:
X-linked Hyper IgM (XHIM) Syndrome is a deadly immune deficiency due to a defect in a gene encoding CD40 Ligand, which is expressed on the surface of activated T cells. This protein allows communication between different immune cells so that antibodies (or immunoglobulins) can be produced to fight infections. Without proper interaction between B- and T- cells of the immune system, immunoglobulins produced cannot fight bacteria, causing XHIM patients to suffer from serious infections of many organs, cancers, and autoimmune diseases. To date, the only known cure for X-Linked Hyper-IgM Syndrome is a bone marrow transplant using stem cells from a healthy donor, termed allogeneic stem cell transplant. However, this is limited by graft-versus-host disease, reactivation of pre-existing lung and gastrointestinal infections common in XHIM patients, and is not an option for those without a HLA matched stem cell donors. Since CD40L is tightly regulated and requires expression in its normal genetic context, traditional forms of gene therapy with viral vectors that are being used in current clinical trials are not viable options. Therefore, site-specific stem cell gene therapy followed by transplant of the patient's own, gene-corrected stem cells, termed autologous transplant, provides the possibility of a cure while avoiding the pitfalls of available standard and experimental therapies. Here we show the ability of both transcription activator-like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9) to efficiently integrate a curative copy of the CD40L gene at the correct location to allow regulated expression of the corrective gene. High levels of gene editing were achieved in primary human hematopoietic stem cells (HSCs) and XHIM-patient-derived T cells. Notably, gene-corrected HSCs engrafted in immunodeficient mice at clinically relevant frequencies. These studies provide the foundation for a permanent curative therapy in XHIM.

Scientific Abstract:
X-linked hyper-immunoglobulin M (hyper-IgM) syndrome (XHIM) is a primary immunodeficiency due to mutations in CD40 ligand that affect immunoglobulin class-switch recombination and somatic hypermutation. The disease is amenable to gene therapy using retroviral vectors, but dysregulated gene expression results in abnormal lymphoproliferation in mouse models, highlighting the need for alternative strategies. Here, we demonstrate the ability of both the transcription activator-like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9) platforms to efficiently drive integration of a normal copy of the CD40L cDNA delivered by Adeno-Associated Virus. Site-specific insertion of the donor sequence downstream of the endogenous CD40L promoter maintained physiologic expression of CD40L while overriding all reported downstream mutations. High levels of gene modification were achieved in primary human hematopoietic stem cells (HSCs), as well as in cell lines and XHIM-patient-derived T cells. Notably, gene-corrected HSCs engrafted in immunodeficient mice at clinically relevant frequencies. These studies provide the foundation for a permanent curative therapy in XHIM.