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Outcome of Hematopoietic Stem Cell Gene Therapy for Wiskott-Aldrich Syndrome

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Background

Wiskott-Aldrich syndrome (WAS) is a rare X-linked disorder characterized by combined immunodeficiency, eczema, microthrombocytopenia, infections, autoimmunity and lymphoma. Gene therapy (GT) using autologous CD34+ cells is an emerging alternative treatment with advantages over standard allogeneic hematopoietic stem cell transplant for patients who lack well matched donors, avoiding graft-versus-host-disease. An initial experience with gene therapy using a γ-retroviral vector showed correction of hematological defects in 9/10 patients, but was aggravated by development of leukemia in 7 of them. We report the outcomes of a phase I/II clinical trial in which 5 WAS patients underwent GT using a self-inactivating lentiviral (SIN-LV) vector expressing the human *WAS* cDNA under the control of a 1.6kB fragment of the human *WAS* promoter.

Subjects and Methods

Five patients with severe WAS (clinical score 3-5) were enrolled at a median age of 1.8 years (1.4 - 8 years) at a single pediatric tertiary care center. WAS protein (WASP) was absent or markedly decreased in 2 and 3 subjects, respectively. Purified CD34+ cells from mobilized peripheral blood (n = 4) or both mobilized peripheral blood and bone marrow (n = 1) were transduced ex-vivo with the SIN-LV vector and re-infused after conditioning with busulfan (target AUC of 70-80 mg*h/L) and fludarabine (120mg/m^2). The median dose of CD34+ cells infused was 9.8 x 10^6 cells/kg (6.3 - 24.9 x 10^6 cells/kg) with a mean vector copy number (VCN) of 1.7 copies/cell in CD34+ cells (0.54 - 3.37). In addition to eczema, thrombocytopenia and WAS-related infections in all patients, two subjects also had autoimmunity pre-GT, manifested as skin vasculitis and autoimmune cytopenias.

Results

All 5 subjects were alive and well at median follow-up of 4.8 years (2.5 - 5.9 years). Multi-lineage vector gene marking was sustained over time. All subjects had improvement or resolution of eczema and none have had any intercurrent severe infectious events. WASP expression measured by flow cytometry in T cells was increased over baseline in all patients, but remained below normal levels and correlated with VCN and cell dose received. Proliferation of T cells in response to anti-CD3, which was initially defective in 4/5 patients, improved post-GT. Humoral immune deficiency was also ameliorated, as evidenced by independence from Ig replacement and vaccine responses in those tested. All subjects remained platelet transfusion-free and none have had severe bleeding events. Platelet levels increased to >50 x 10^3 cells/uL in two patients with a VCN ≥2 in transduced stem cells and myeloid VCN ~1 copy/cell

in neutrophils; the other 3 subjects sustained platelet counts <50 x 10^3 cells/uL. Cytoskeleton function was highly abnormal in myeloid cells pre-GT, as shown by the near absence of podosome formation in monocyte-derived dendritic cells. At 12 months post-GT, the % of podosome-forming cells was improved in all subjects, and reached the level of healthy controls in the 2 patients with highest VCN in myeloid cells. Both subjects with pre-existing autoimmunity had post-GT autoimmunity: patient 4 had a flare of autoimmune cytopenias at 18 months post-GT, and patient 5 developed refractory autoimmune hepatitis and hemolytic anemia at 8 months post-GT. While all subjects had WASP expression in lymphocytes, those with autoimmunity had poor recovery of T cells, Tregs, and transitional B cells at the time of clinical symptoms. IL-10 producing regulatory B cells were deficient pre-GT and recovered to varying degrees in all subjects.

No severe GT-related adverse events have occurred to date. Replication-competent lentivirus was not detected. Analysis of integration site distributions in five subjects showed reconstitution to be highly polyclonal, with no clones expanded to >20% of the transgene-marked cell population. To date, there have been no malignancies reported, either related to GT or WAS itself.

Conclusion

In summary, our data confirm and extend the safety and efficacy of GT in correcting disease manifestations associated with WAS, as seen in other studies using SIN-LV. Higher VCN in the drug product and in transduced stem cells correlated with better reconstitution of platelets and myeloid function. In contrast to other groups, we found in our study that patients with poor lymphocyte reconstitution post-GT may be at risk of ongoing autoimmunity despite high-level gene marking.

Disclosures

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OffLabel Disclosure:

CliniMACS technology for CD34+ cell selection

Author notes

*Asterisk with author names denotes non-ASH members.