

Towards a Cure for Adenosine Deaminase 2 Deficiency Through Genetic Correction of Macrophage Polarization

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Deficiency of adenosine deaminase 2 (DADA2) is the first molecularly described monogenic vasculitis syndrome. DADA2 is caused by autosomal-recessive loss-of-function mutations in the *ADA2* gene that encodes the adenosine deaminase 2 (ADA2) protein. The ADA2 dimeric protein is secreted into the extracellular space, where it has anti-inflammatory action and functions as a deaminase to control adenosine levels at the site of inflammation. Consistently, ADA2 levels are elevated in plasma samples of patients with infectious diseases or chronic inflammation. Disease-associated mutations affect the catalytic activity, protein dimerization, and secretion of ADA2.¹⁻³ This results in a rare disease hallmarked by severe systemic and tissue inflammation. The major clinical features of DADA2 include systemic inflammation, vasculopathy, ischemic and/or hemorrhagic stroke, immunodeficiency, and hematologic alterations (eg, red cell aplasia, neutropenia, anemia, thrombocytopenia, hypogammaglobulinemia). An elevated number of neutrophils and macrophages has been observed at sites of inflammation in biopsies from patients with DADA2.¹⁻³ Although ADA2 function is still largely unknown, this protein is highly expressed in myeloid cells and plays a role in the polarization of monocytes and macrophages towards an anti-inflammatory phenotype. Thus, ADA2 deficiency has been associated to impaired anti-inflammatory M2 macrophage polarization and skewing of monocyte differentiation towards pro-inflammatory M1 macrophages. This mechanism likely contributes to the establishment of a vicious cycle of vasculopathy and inflammation.¹⁻³

DADA2 can result in highly variable, multiorgan disease with potential for high morbidity and mortality. Early identification and diagnosis of this phenotype has significant treatment implications, with potential of changing the disease course. Beyond glucocorticoids, conventional immunosuppression has been found largely ineffective in treating this relapsing and remitting disease. The first-line treatment consists of anti-tumor necrosis factor- α (TNF α) therapy, which effectively controls inflammation, preserves vascular integrity, and prevents vasculitic manifestations and stroke.⁴ Hematopoietic stem cell transplantation (HSCT) has been successful in a group of patients presenting with hematological manifestations. Although HSCT can lead to normalization of enzyme activity, as well as resolution of vasculitic, hematologic, and immunologic features, treatment-related adverse effects are not uncommon. Thus, the limited HLA-matched donor availability and risk of morbidity associated with HSCT highlight the urgent need of alternative therapeutic approaches for the cure of DADA2.^{4,5}

A recent work by Zoccolillo et al¹ published in *Blood Advances* has investigated the opportunity to cure the disease by genetic correction of autologous hematopoietic stem and progenitor cells (HSPCs) of DADA2 patients. A lentiviral vector encoding human ADA2 (LV-ADA2) under the control of the ubiquitous human phosphoglycerate kinase promoter was generated to ensure stable expression of therapeutic proteins in myeloid cells. Human CD34⁺ HSPCs isolated from the bone marrow of DADA2 patients were re-transduced with the ADA2-encoding lentiviral vector. Transduction with such lentiviral vector re-established intracellular expression as well as enzymatic activity of ADA2 in patients' CD34⁺ cells, compared to untransduced ones.

In preclinical mouse models and in vitro, ADA2 overexpression was well tolerated and did not alter the proliferation and maturation potential of myeloid and erythroid progenitors, suggesting that LV-ADA2 is an efficient tool to stably deliver ADA2 expression and restore enzymatic activity in patients' CD34⁺ cells.

Since DADA2 is associated with an exacerbated pro-inflammatory profile of macrophages, a major focus of the study was to understand whether lentiviral vector-driven ADA2 expression was able to correct macrophage skewing. Interestingly, U937 macrophage cell line deficient for ADA2 expresses and secretes elevated levels of interleukin-6 (IL-6) and TNF α , which was rescued by transduction with a lentiviral vector encoding wild-type ADA2 but not a mutant ADA2

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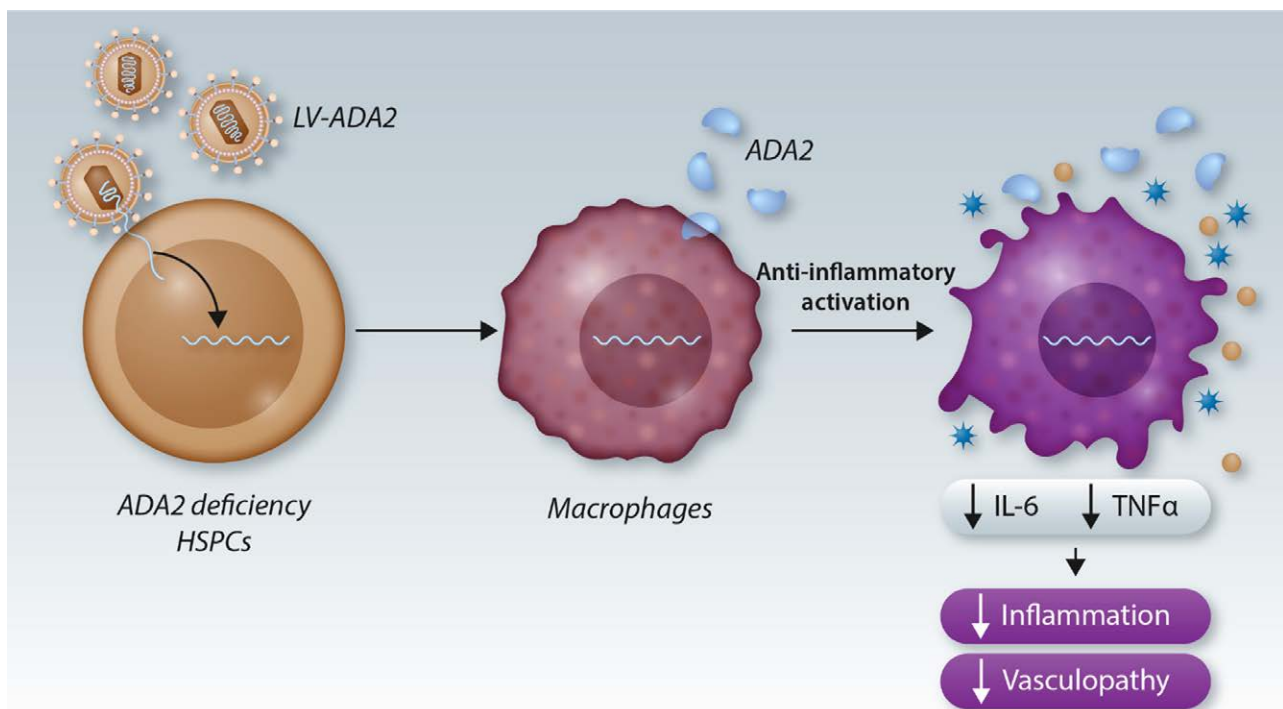


Figure 1. Macrophage reprogramming through ADA2 gene therapy. ADA2 deficiency was genetically corrected in CD34⁺ HSPCs of DADA2 patients by transduction with an LV-ADA2. Macrophages derived from transduced CD34⁺ HSPCs showed restored expression and enzymatic activity of ADA2. This resulted in anti-inflammatory macrophage reprogramming, with decreased release of IL-6 and TNF α . This is expected to have therapeutic benefit in DADA2 patients through the reduction of systemic inflammation and vasculopathy. ADA2 = adenosine deaminase 2; DADA2 = deficiency of adenosine deaminase 2; HSPC = hematopoietic stem and progenitor cell; IL-6 = interleukin-6; LV-ADA2 = lentiviral vector encoding human adenosine deaminase 2; TNF α = tumor necrosis factor- α .

lacking enzymatic activity. This confirmed the ability of lentiviral vector-driven ADA2 of restoring physiological secretion of IL-6 and TNF α in macrophages (Figure 1).

Finally, monocyte-derived macrophages from 6 DADA2 patients transduced with ADA2-encoding lentiviral vector were differentiated into M1 macrophages by lipopolysaccharide and interferon gamma exposure. Lentiviral vector-mediated ADA2 reconstitution resulted in a significant reduction in IL-6 and TNF α expression in patients' monocyte-derived M1 macrophages. Thus, lentiviral vector-mediated restoration of ADA2 corrects the inflammatory macrophage defect in DADA2 (Figure 1). These data indicate that the aggravated inflammatory response of patients' macrophages is a cell-intrinsic consequence of ADA2 loss and that ADA2 enzymatic activity is essential for the control of macrophage polarization.

This study supports the therapeutic value of the reconstitution of ADA2 expression and activity in patients' HSPCs through gene therapy. Mechanistically, this is expected to rely on the correction of the exacerbated inflammatory response of patients' macrophages, which is a major trigger of several disease manifestations.

Importantly, this work highlights how genetic reprogramming of macrophages could represent a valuable therapeutic approach for modern molecular and cellular medicine.⁶ The controllable activation of macrophages towards desirable phenotypes might provide effective treatments for a number of inflammatory and proliferative diseases, beyond DADA2. The use of macrophages as a therapeutic tool is often restricted by the lack of safe and efficient approaches for their reprogramming.⁶ Here, the overexpression of ADA2 in lentiviral vector-transduced CD34⁺ HSPCs, which is maintained in differentiated macrophages,

helps to overcome this problem and achieve a stable macrophage reprogramming. While this strategy might not be applicable to a variety of diseases where the ubiquitous overexpression of certain proteins could interfere with other cell type functions, this approach offers a valuable example of how triggering anti-inflammatory macrophage rewiring provides therapeutical benefit in a highly inflammatory disease.

Disclosures

The author has no conflicts of interest to disclose.

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