# Reprogramming the Enemy Within:

## Patient-Derived CAR-T Cells Effectively Target Inflammation-Inducing B cells

**By Isabella Press**

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies by B cells. Under normal conditions, autoreactive B and T cells are eliminated during maturation. However, in SLE, central tolerance is disrupted, resulting in widespread inflammation and tissue damage.[1] Current therapies include the use of monoclonal antibodies to aid in the depletion of autoreactive cells, although these antibodies may fail to adequately penetrate the affected tissues where cells reside.[2] In contrast to monoclonal antibodies, CAR T cells have been shown to exhibit superior tissue penetration[3] resulting in greater depletion of B cells in SLE patients. Specifically, a second generation[4] anti-CD19 fully human CAR construct that includes a CD28 costimulatory domain,[5] as well as a CD8α hinge and transmembrane domain and a CD3ζ activation domain,[6] was developed using a CAR vector Hu19-CD828Z provided by Kyverna Therapeutics. In their 2024 paper, Dingfelder *et al*. denote the potential of autologous, fully human CAR T cells in treating SLE patients.[7]

The authors first sought to determine whether CAR T cells could be generated from cryopreserved leukapheresis material obtained from SLE patients who had undergone immunosuppressive therapies. To do this they transduced cryopreserved $CD4^+$ and $CD8^+$ T-cells with a lentiviral vector to express the Hu19-CD828Z CAR fully human anti-CD19 second generation CAR from five SLE patients and five healthy donors (HD). Both SLE- and HD- transduced T cells exhibited similar expansion rates and CAR expression levels with no significant differences between the two groups. Although the authors observed comparable CAR expression and expansion between SLE-derived and HD-CAR T cells, they noted a more balanced CD4/CD8 T cell ratio in the SLE group. In both SLE and HD samples, central memory T cells ($T_{cm}$; $CD45RO^+CCR7^+$) predominated. However, SLE-derived CAR T cells exhibited a modest increase in the proportion of effector memory T cells ($T_{em}$; $CD4^+CD45RO^+CCR7^-$) relative to HD-derived cells. The authors also noted that the exhaustion markers (TIM-3, LAG-3, and PD-1) increased minimally in CD4+ SLE and HD CAR T-cells.

In order verify CD19 dependent proliferation of CAR T-cells, the authors first characterized CD19 expression in B cells enriched from the leukapheresis of both SLE and HDs. B cells enriched from SLE patients were found to have 1.8-fold lower CD19 expression when compared to B cells enriched from HDs. The CD19+ control cell line NALM-6 was found to express significantly higher CD19 expression than primary B cells isolated from SLE patients and HD, while the $CD19^-$ control cell line U937 exhibited no expression at all.

To demonstrate CD19-dependent proliferation, researchers co-cultured both CAR T cells and non-transduced T cells with each of the B Cell lines characterized above. SLE and HD CAR T cells exhibited notably higher proliferation rates than non-transduced T cells when co-cultured with $CD19^+$ autologous B cells and NALM-6 cells. SLE-derived CAR T cells exhibited reduced proliferation in response to autologous B cells relative to HD-derived CAR T cells, consistent with lower CD19 expression levels in SLE B cells. However, both SLE and HD CAR T cells exhibited similar proliferation when cultured with $CD19^+$ NALM-6 cells indicating that CD19 expression has a direct effect on the proliferation of the T Cells. Minimal proliferation was seen when co-cultured with $CD19^-$ U937 cells confirming CD19-dependent proliferation of Hu19-CD828Z CAR T cells.

In addition to characterizing CD19-dependent proliferation, the authors tested the in vitro cytotoxic activity of SLE- and HD-CAR T cells. SLE CAR T cells demonstrated CAR-mediated cytolytic activity against $CD19^+$ autologous B cells as well as NALM-6 cells. Once again confirming the CD19-specific targeting of the Hu19-CD828Z CAR T cells.

Finally, the authors characterized the in vitro functionality of the SLE CAR T cells. SLE CAR T cells co-cultured with autologous B cells exhibited elevated production of IFN-γ and TNF-α relative to non-transduced controls, while IL-2, IL-6, and IL-1β remained at comparably low levels. When co-cultured with the $CD19^+$ NALM-6 cells however SLE CAR T cells produced significant amounts of IFN-γ, TNF-α, IL-2, IL-6 and IL-1B over control cells, however HD CAR T cells notably produced significantly more of these cytokines. The authors noted that the lower cytokine release seen in SLE CAR T cells may be attributed to the low dose steroid treatments SLE patients received at the point of apheresis. Importantly, SLE CAR T cells still exhibited therapeutic efficacy despite reduced cytokine production.

These findings support the feasibility of generating autologous CAR T cells from cryopreserved leukapheresis material of SLE patients, even after immunosuppressive treatment. The resulting T cells demonstrate CD19-specific cytotoxic activity with reduced proinflammatory cytokine release, highlighting their potential as a therapeutic option for B cell depletion in autoimmune disease.

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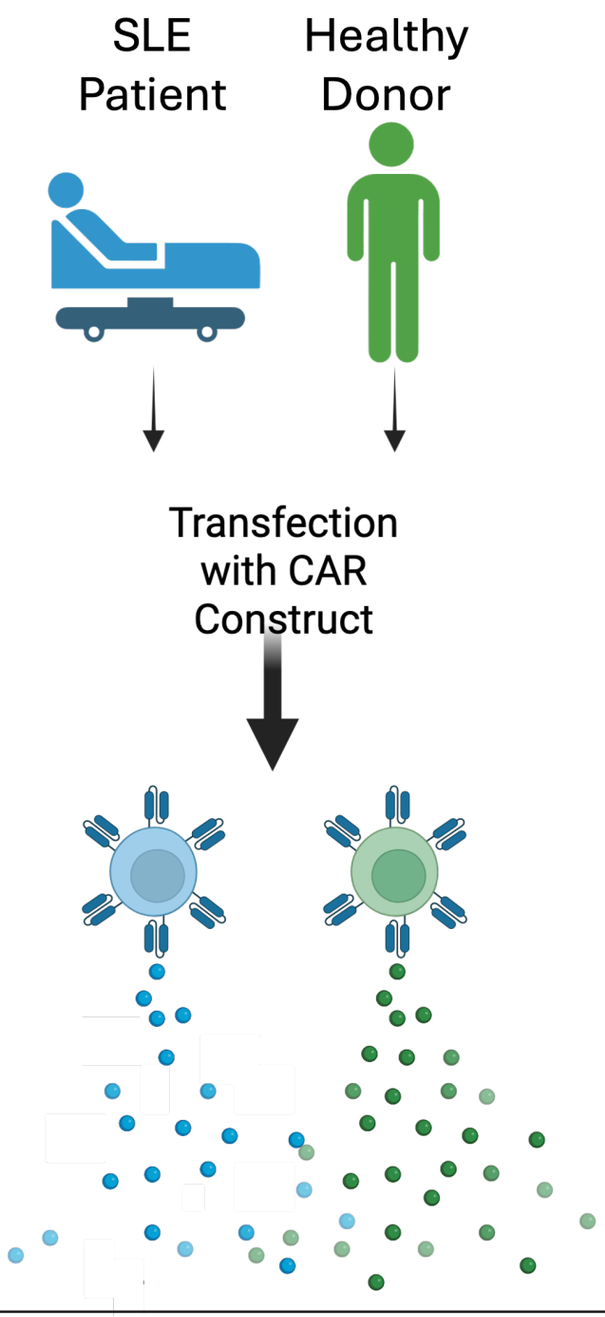


**Figure 1| SLE Patient-Derived Cells produce Effective CAR T Cells.** Patient-derived cells proliferated similarly to those derived from healthy donors and also produced similar, but lower, amounts of pro-inflammatory cytokines.