Long-term Remission of CLL Sustained by Pauciclonal anti-CD19 Chimeric Antigen Receptor T Cells

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We recently demonstrated that sustained remission in chronic lymphocytic leukemia (CLL) patients treated with the CD19-specific, 4-1BB/CD3zeta-signaling chimeric antigen receptor T-cells (CTL019) correlated strongly with the expansion and persistence of the engineered T cells (Fraietta et al., 2018, Nat Med 24:563). We here report two advanced, chemotherapy-resistant CLL patients with the longest (8+ years) follow-up on any trial of CART19 cells. Both patients had received five therapies before being treated at the University of Pennsylvania with autologous CART19 cells (tisagenlecleucel) cells in 2010. Both patients have persistence of CAR-engineered T cells and both patients are still in remission as determined by flow cytometry and deep sequencing of IgH rearrangements for over eight years.

To understand the fate of the infused CAR-T cells we determined the phenotype, function, and clonal nature of the persisting CTL019 cells. Flow cytometric CART19 cell analyses demonstrated that early during the anti-leukemia response, activated, HLA-DR-expressing CD8+ CAR-T cells rapidly expanded, followed by similarly activated CD4+ CAR-T cells. With tumor clearance the CAR-T cell population contracted and persisted at 0.1-1% of all T cells. Next we performed deep immunophenotyping of CAR-T cells by mass cytometry, employing a custom-conjugated panel of 40 markers. Phenograph was used to identify 27 CD4+ and CD8 T cell clusters in a concatenation of all post-infusion samples. This analysis revealed that while distinct phenotypic clusters dominated the CAR T cell repertoire in either patient early after infusion, in both individuals the CAR T cell repertoire converged on persistently activated, PD1 and TIGIT-expressing CD4 T cells. These data suggest that the initial tumor clearance was mediated by CD8+ CAR-T cells, but sustained by a CD4+ CAR-T cell population that still actively engages with target cells. TCR-seq analysis of early post-infusion time points showed a) that the circulating CAR-T cell populations consisted of hundreds to thousands of distinct clones which in patient 1 and 2 displayed clonal focusing by 21 and 1 month post-infusion, respectively, with some clones making up as much as 12% (patient 1) and 48% (patient 2) of the CAR-T cell repertoire and b) that the CAR T cell repertoire stabilized early (1 month; patient 2) and late (21 months; patient 1) after infusion. Second, clonal analysis of the CAR T cell repertoire via vector integration site sequencing of the infusion products for both patients identified a very diverse, non-clonal make-up, containing over 8,000 and 3,700 integration sites in patients 1 and 2, respectively. The higher degree of clonality in patient 2 but not 1 CAR-T cells as seen by TCR-seq was confirmed by integration site analysis, as was the sharing of CAR-T cell clones over time. Importantly, whereas the CAR integration site repertoire in patient 1 was diverse in the first two years, it stabilized and trended towards oligoclonality 21 months after infusion. Lastly, CAR integration site analysis revealed a high degree of clonal persistence, suggesting that tumor control and B cell aplasia were maintained by few, highly functional CD4+ CAR-T cell clones.