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Loss of *TET2* Uncouples Proliferative and Effector Functions in CAR T Cells

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Chimeric antigen receptor (CAR) T cells have opened a new paradigm for the treatment of leukemia and lymphoma. Their production, however, is laborious, requiring tens of millions of CAR T cells per infusion. This constraint could be significantly alleviated if safe and more efficacious T cells could be generated. In a patient with chronic lymphocytic leukemia, treated with anti-CD19 CAR T cells, a recent report described the emergence of a single T cell clone that at its expansion peak accounted for 94% of circulating CAR T cells, coinciding with the development of cytokine release syndrome and tumor regression (Fraietta et. al. Nature 2018). Insertional mutagenesis in this T cell had disrupted an allele of *TET2*, an epigenetic regulator mediating the oxidation of 5-methylcytosine. The other allele appeared to bear an inherited hypomorphic variant, resulting in the near complete loss of *TET2* function in this clone. To understand the mechanisms accounting for this chance clinical finding, we investigated the effect of *TET2* loss in human T cells engineered to express different chimeric receptors.

Using CRISPR/Cas9, we edited *TET2* in T cells engineered to express a CD19-specific second-generation CAR encompassing the costimulatory domain of either CD28 or 4-1BB (Rv-1928z and Rv-19BBz). *TET2* disruption enhanced the *in vivo* anti-tumor activity of Rv-19BBz but not Rv-1928z CAR T cells tested under stress test conditions using limiting CAR T cell doses (as previously described in a human B cell acute lymphoblastic leukemia (B-ALL) NALM6 model, Zhao et. al. Cancer Cell 2015). Since Rv-1928z induces potent effector differentiation but limited persistence compared to Rv-19BBz, we hypothesized that loss of *TET2* could amplify the expansion and persistence of 4-1BB-costimulated T cells but not override the differentiation program imparted by Rv-1928z.

To test this hypothesis, we utilized two orthogonal approaches known to limit exhaustion and increase persistence of CD28-costimulated CAR T cells, Rv-1928z co-expressed with 4-1BB ligand (Rv-1928z-41BBL) and 1928z driven by the *TRAC* promoter (*TRAC*-1928z). Disruption of *TET2* enhanced the anti-tumor efficacy of both these CAR T cells and promoted acquisition of a central memory phenotype. However, over time (50-200 days), *TET2*-edited *TRAC*-1928z and Rv-1928z-41BBL attained a hyper-proliferative phenotype ultimately requiring euthanasia due to splenomegaly and extensive CAR T cell accumulation in various organs. Post-mortem analysis found no evidence of NALM6 in these mice. This was in contrast to stress test studies with Rv-1928z and Rv-19BBz where most mice succumbed to NALM6 progression. These observations established an essential role for CAR signaling in determining the phenotypic outcome of *TET2* loss in T cells.

To examine the long-term effects of *TET2* disruption in the context of all 4 receptors, we treated human B-ALL bearing mice with curative doses of all 4 CAR T cells and followed them for up to 200 days. We found that all 4 CAR expressing *TET2*-edited T cells could eventually attain a hyper-proliferative phenotype, but with varying frequency depending on the CAR design (Rv-1928z-41BBL and TRAC-1928z > Rv-19BBz > Rv-1928z). To assess their effector function, NALM6-bearing mice were infused with adoptively transferred hyper-proliferative *TET2*-edited CAR T cells. Strikingly, these T cells were unable to elicit any tumor control, despite their maintaining a central memory phenotype as assessed by flow cytometry. This loss of effector function was observed for all 4 CAR T cell types, suggesting a discrepancy between function and flow cytometric phenotype.

Transcriptional, methylation and genome accessibility studies revealed a unique T cell state wherein the proliferative program is uncoupled from effector response. We identified a unique transcriptional and epigenetic signature that is manifested in a loss of effector function while maintaining robust proliferation. This state stands in contrast to the classically described T cell exhaustion state where loss of effector function is preceded by loss of proliferative ability. *TET2* disruption thus promotes a CAR T cell proliferative program that depends on the CAR design but does not in itself enhance anti-tumor activity.

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