

1516 ADVANCING IMMUNOTHERAPY THROUGH PATIENT-IDENTIFIED REGULATORS OF T CELL STEMNESS

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Background Epigenetic reinforcement of T cell exhaustion is a well-established barrier limiting multiple modalities of T cell-based immunotherapies for cancer. Disruption of the epigenetic regulators DNMT3A and TET2 has been previously used to preserve the proliferative capacity of chronically stimulated CD8 T cells in the setting of both chimeric antigen receptor (CAR) T cells and PD-1 blockade-mediated therapy. These epigenetic regulators, in addition to ASXL1, are commonly mutated in clonal hematopoiesis, providing a survival advantage to stem cells and suggesting a universal role of these factors in controlling stemness. This concept was recently supported by clinical data showing that ASXL1 mutations in the T cell compartment of myelodysplastic syndrome (MDS) patients are associated with extended survival after immune checkpoint blockade (ICB). Given the established role of DNMT3A and TET2 in controlling cellular multipotency, we investigated the impact of ASXL1 in the development of functional and exhausted CD8 T cells.

Methods ASXL1 and AAVS1 (WT) knock-out P14 T cells were generated using CRISPR-Cas9 technology. Acute lymphocytic choriomeningitis virus (LCMV)-specific CD8 T cells were generated by adoptive transfer of ~3000 congenically distinct naive ASXL1 KO and WT P14 CD8 T cells. One day later, mice were infected with chronic LCMV by i.v. injection of 2×10^6 PFU LCMV per mouse. Mice were treated with PD-L1 on days 31, 34, 37, 40 and 42 post infection. Longitudinal phenotypic and epigenetic analysis were performed. Tumor experiments were performed using LLC1-GP33 and tracking tumor size and survival in mice who received WT versus ASXL1 KO P14 T cells.

Results Disruption of *Asxl1* in murine LCMV-specific CD8 T cells enabled the stem-like subset to persist in chronically infected mice for one year. These cells retained the capacity to give rise to potent effector T cells in adoptive transfer settings. Additionally, the *Asxl1* KO LCMV-specific CD8 T cells retained a heightened ability to undergo a proliferative burst in response to ICB and exhibited significant efficacy against historically aggressive tumors. These data document clonal hematopoiesis-associated epigenetic checkpoints control the transition of stem-like progenitor T cells into terminally exhausted T cells.

Conclusions Collectively, these data demonstrate that ASXL1 is a novel epigenetic regulator controlling the durability of the stem-like T cell population that is responsible for sustaining clinical response during T cell-based cancer immunotherapy.

Acknowledgements This work was supported by the National Institutes of Health (R01CA237311 to BY; K08CA279926 to CZ), the National Comprehensive Cancer Network Young Investigator Award (to CZ), the Alex's Lemonade Stand Young Investigator Award (to CZ), SU2C (to BY) and the American Lebanese Syrian Associated Charities (ALSAC to CZ and BY).

<http://dx.doi.org/10.1136/jitc-2023-SITC2023.1516>