Background The histone methyltransferase enhancer of zeste homolog 2 (EZH2)-mediated epigenetic regulation of T cell differentiation in acute infection has been extensively investigated. However, the role of EZH2 in T cell exhaustion remains under-explored.

Methods We developed an in vitro exhaustion model with OT-I cells activated with a high concentration of OVA257–264 peptide. Using this model, we performed EZH2 knockout (EZH2-KO) or treated cells with an EZH2 inhibitor, tazemetostat (clinically approved at 2020, Taz), during the in vitro expansion, and compared their phenotypes, proliferation, in vitro and in vivo anti-tumor activity. We further transiently treated human anti-GD2 CAR-T cells with Taz for phenotype, proliferation and in vivo anti-tumor analysis.

Results Compared to vehicle treated T cells, T cells transiently treated with EZH2 inhibitor expressed a higher level of memory-like markers, including CD62L and TCF-1 but lower level of inhibitory receptors, e.g. PD-1, LAG-3, Tim-3. With an in vitro exhaustion model of OT-I cells, EZH2-KO OT-I cells exhibited similar phenotype as OT-I cells treated with Taz. However, OT-I cells transiently treated with Taz made better tumor control than both vehicle treated and EZH2-KO OT-I cells. Anti-GD2 CAR-T cells that will develop profound exhaustion features during the ex vivo expansion expanded with Taz also provided improved tumor control and mice survival with a human leukemia model in NCG mice (figure 1).

Conclusions Transient inhibition of EZH2 in T cells inhibits the deposition of H3K27Me3 at memory associated loci, alleviates exhaustion, and produces T cells with stem-like or progenitor-exhausted properties. Upon adoptive transfer, EZH2 activity was restored in the absence of the inhibitor, allowing the transferred T cells to differentiate into cells with effector-like functions for better tumor control.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0811