Background Cultured AML blasts can differentiate into antigen-presenting cells, however, stimulating leukemic cell differentiation and immunogenicity in patients has not translated into therapeutic effects. We previously demonstrated that eliminating STAT3 signaling in Cbfb/MYH11/Mpl (CMM) leukemia using siRNA or decoy DNA strategies permits TLR9-induced AML cell differentiation and thereby leads to potent adaptive immune responses and leukemia regression.1,2

Methods Here, we interrogated cellular and molecular underpinnings of leukemic cells differentiation following STAT3-inhibition and TLR9-activation (STAT3i/TLR9a).

Results Our whole genome and single-cell transcriptomic analyses combined with immunophenotyping revealed that the combined STAT3i/TLR9a had a dramatic effect on CMM cell differentiation. The treatment induced heterogenous leukemia cell clusters at different stages of myeloid cell differentiation towards macrophage and antigen-presenting cell phenotype. These were characterized by decreasing gene signatures of proliferation/survival genes with appearance of signature of leukocyte activation, antigen presentation, and in case of more differentiated AML-derived clusters, type I and type II IFNs. The transcriptional profiling of CMM cells isolated from mice treated using inducible STAT3 gene silencing or decoy STAT3 in combination with TLR9 stimulation, revealed the increased expression of genes regulating myeloid cell differentiation such as Irf8, Cebpa, and Cebpe with decreased expression of leukemia-promoting Runx1. The combination treatment also increased expression of genes regulating antigen-presentation such as Gadd45A, Citta, Il12a, and Ifng in leukemic cells. We further confirmed that IRF8 expression is required for AML cell differentiation as shown by conditional Irf8 silencing. The AML cell reprogramming was likely regulated epigenetically as indicated by the reduced gene methylation profile of crucial myeloid cell differentiation genes such as Irf8. These changes were associated with reduced expression of DNMT1 and DNMT3ab, known STAT3 target genes.

Conclusions Our studies suggest that eliminating STAT3 checkpoint allows for TLR9-mediated reprogramming of leukemic cells into antigen-presenting cells capable of stimulating adaptive T cell immune responses against AML. Furthermore, these findings support further development of CpG-STAT3 inhibitors as a new bi-functional agent for AML immunotherapy.

Acknowledgements This project was supported by the National Cancer Institute of the National Institutes of Health under award number R01CA213131 to M.K.

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