Correcting RNA dysregulation to reverse epitope loss and resistance to CD22-directed chemotherapy

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Background: Antigen loss involving lineage markers such as CD22 is a major cause of treatment failures in B-cell acute lymphoblastic leukemia (B-ALL). A case in point is the CD22 antibody-drug conjugate inotuzumab ozogamicin (InO). InO has clinical efficacy against B-ALL, but the emergence of untargetable CD22LOW variants is often associated with resistance and relapse. Our previous work uncovered the prevalence of non-coding CD22 transcripts lacking the AUG codon-containing exon 2 as a major barrier to CD22 expression and InO sensitivity. Methods: To understand CD22 mRNA productive splicing and translation, we performed a flow-cytometry guided CRISPR/Cas9 screen in B-ALL cell lines. One of its top hits was METTL3. Using meRIP-qPCRs and direct RNA sequencing on the Oxford Nanopore platform, we identified high-confidence m6A marks on CD22 transcripts. Results: METTL3-depleted cells exhibited a ~3.5-fold increase in CD22 surface expression without any detectable changes in CD22 mRNA abundance or association with polysomes. However, there was an increase in CD22 exon 2 inclusion, suggesting that splicing normalization is one underlying mechanism. Mechanistically, it is likely to involve improved PI3K signaling via down-regulation of the PTEN lipid phosphatase, another direct target of METTL3. Regardless of the exact molecular mechanism, B-ALL cell lines treated with METTL3 inhibitors showed increased CD22 surface density and improved sensitivity to InO, with up to 10-fold reduction in IC50 values. In vivo, combining METTL3i with Ino reduced leukemia burden and extended survival of mice bearing patient-derived B-ALL xenografts. Conclusions:

Given that the clinical-stage METTL3 inhibitor STC-15 has a favorable safety profile in ongoing clinical trials, it could be readily repurposed as a chemosensitizer in pediatric and adults B-ALL patients, to improve response rates and mitigate severe InO-associated toxicities seen in the clinic.