Modulation of CD19 surface expression in B cell acute lymphoblastic leukemia

Immunotherapeutic targeting of the surface glycoprotein CD19 has markedly improved outcomes in patients with relapsed and refractory B cell progenitor acute lymphoblastic leukemia. Genome-wide CRISPR–Cas9 screening identifies modulators of *CD19* mRNA processing that affect the abundance of the surface protein in human B cell leukemia cells, with the potential to improve antigen-directed immunotherapy efficacy.

This is a summary of:
Witkowski, M. T. et al. NUDT21 limits CD19 levels through alternative mRNA polyadenylation in B cell acute lymphoblastic leukemia. *Nat. Immunol.* https://doi.org/10.1038/10.1038/s41590-022-01314-y (2022).

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The problem

The teatment of chemotherapy-resistant B cell progenitor acute lymphoblastic leukemia (BCP-ALL) has been revolutionized by antigen-directed T cell-based immunotherapies, such as chimeric antigen receptor (CAR)-T cell therapy and the bi-specific T cell engager antibody blinatumomab. Unfortunately, immunotherapy is not effective in many patients with BCP-ALL owing to 'antigen escape' — the loss or absence of membrane glycoprotein CD19 required for targeting by CD19-directed, anti-leukemic T cells[1]. Several mechanisms have been shown to mediate antigen escape by rendering $CD19^{dim}$ or $CD19^{-}$ leukemic blasts unrecognizable to CD19-targeting T cells[2,3], including alternative *CD19* mRNA splicing and selection of leukemic clones that contain *CD19* genomic deletions. As such, understanding the gene regulatory mechanisms that control CD19 abundance on the surface of BCP-ALL blasts is essential when attempting to understand mechanisms of antigen escape, and may have therapeutic implications.

The observation

To identify gene regulatory mechanisms that control the abundance of BCP-ALL surface CD19, we used genome-wide CRISPR–Cas9 screening of several CD19-expressing human BCP-ALL cell lines, as well as mature B cell lines. We then separated each cell line into $CD19^{low}$- and $CD19^{high}$-expressing cells by flow cytometry (Fig. 1a). Using single-guide RNA (sgRNA) deep sequencing, we identified the gene-editing events that favor high (CD19 repressors) or low (CD19 activator) expression of the CD19 surface protein.

When focusing on putative CD19 activators common to BCP-ALL cell lines (Fig. 1b), we identified the DNA-binding protein ZNF143, which has been shown to regulate three-dimensional DNA interactions required for cell type-specific gene activation. Loss of ZNF143 in human BCP-ALL lines resulted in significant reductions in *CD19* mRNA production and, ultimately, loss of surface CD19 expression. In combination with previous studies that highlight a specific role for the B cell master regulator PAX5 in the activation of the *CD19* promoter[4], we believe that ZNF143 acts as a crucial promoter-bound *CD19* gene activator in human BCP-ALL cells.

The top-ranked CD19 repressor in our screen was RNA-binding protein and regulator of mRNA alternative polyadenylation NUDT21 (Fig. 1b). In primary healthy and leukemic human B cell progenitors, *NUDT21* mRNA expression correlated strongly with levels of *PAX5* and *CD19* mRNA. Notably, NUDT21 loss resulted in shortening of the *CD19* mRNA 3′ untranslated region, increased mRNA stability and increased levels of CD19 on transformed B cell. NUDT21-deficient BCP-ALL cells showed increased sensitivity to CAR-T cell therapy and blinatumomab treatment in vitro. Moreover, using publicly available gene expression data of patients with BCP-ALL treated with CAR-T cells and blinatumomab, we show upregulation of *NUDT21* mRNA levels coincide with CD19 loss at disease relapse.

Future directions

Our analysis of CD19 modulators highlights the importance of promoter-bound factors, such as ZNF143, in promoting *CD19* mRNA output. In addition, although many studies have highlighted the crucial importance of alternative splicing in *CD19* mRNA processing, our characterization of NUDT21 highlights a novel function for alternative polyadenylation in *CD19* mRNA stability and surface protein accumulation.

Our study has several limitations. First, the identification of CD19 modulators was performed using human BCP-ALL cell lines; however, the in vivo clinical implications remain unclear. In addition, we were limited to two cases of CD19-directed immunotherapy samples from patients with BCP-ALL, with blast-enriched or purified biospecimens subjected to bulk or single-cell RNA sequencing, as well as matched pre- and post-immunotherapy (CAR-T and blinatumomab) samples. Therefore, although we observe an inverse trend of reduced *CD19* and increased *NUDT21* mRNA expression at disease relapse when compared to pre-treatment samples, this observation requires further clinical validation.

This highlights a major challenge for the study of disease relapse in BCP-ALL after CD19 CAR-T infusion: sample availability. Although early CD19 CAR-T clinical trials boast excellent early responses in relapse and refractory BCP-ALL, many patients will relapse with CAR-T-resistant disease over time. So far, however, the dissemination of primary patient biospecimens across academic institutes has been extremely limited. Therefore, we hope that the commercialization of CAR-T products, such as Kymriah, will enable a wider breadth of research groups to explore unappreciated mechanisms of disease relapse after CAR-T therapy.

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EXPERT OPINION

"This manuscript provides important insights into the role of CD19 in the pathophysiology of B cell malignancies and will lay the base for exciting novel therapeutic agents." **Catriona Jamieson, UC San Diego Moores Cancer Center, La Jolla, CA, USA.**

FIGURE

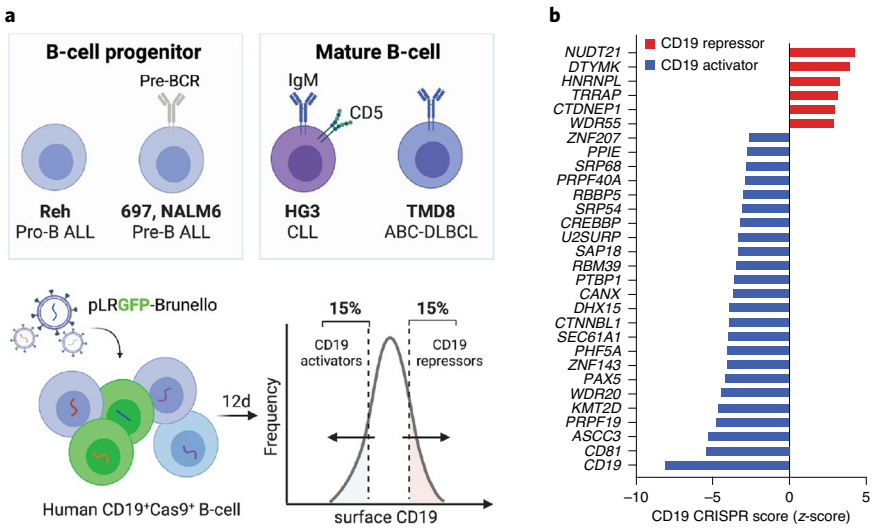

Fig. 1 | Identification of CD19 regulators in human B cell malignancies. a, Schematic of CD19-directed pooled genome-wide CRISPR screens (Brunello sgRNA library) across human Cas9-positive CD19^{+} B-cell lines — including Reh, 697 and NALM6 (BCP-ALL), HG3 (chronic lymphocytic leukemia) and TMD8 (activated B cell-like diffuse large B cell lymphoma). **b**, Histogram showing CD19 CRISPR scores (*z*-score normalized average number of sgRNAs per gene within CD19^{lo} cells and CD19^{hi} cells relative to non-targeting control sgRNAs) for individual sgRNAs targeting top gene candidates for CD19 activators (blue) and repressors (red) in human BCP-ALL cell lines (Reh, 697 and NALM6).

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BEHIND THE PAPER

I recall the difficulties advancing this study in the midst of the COVID-19 outbreak in New York. Navigating experiments with sporadic shutdowns and limited capacity presented a major challenge. This progress was only possible with the team we formed within the Aifantis laboratory and beyond, and I'm immensely proud to share first-authorship with two of the finest scientists I know — Soobeom Lee (PhD student in the Aifantis/Bonneau laboratory at New York University) and Eric Wang (Assistant Professor at Jackson Laboratories). Finally, my career has been guided by the extensive and freely available data generated by multi-institutional efforts aimed at genome-wide characterization of ALL chemotherapy failure. Personally, I hope we can explore the clinical implications of our findings in greater detail, and I am optimistic that large cohort CD19 CAR-T studies will begin to follow the outstanding example set by the pioneers of ALL genomic characterization. **M.T.W.**

FROM THE EDITOR

"Monoclonal antibody therapy or CAR-T cell treatment directed against the CD19 antigen can select leukemic blasts with reduced expression of CD19, leading to resistance to therapy. There is still limited access to primary patient samples before and after CD19-directed immunotherapy, but the current manuscript uses leukemic cell lines to identify modulators of CD19 expression that have the potential to improve therapeutic strategies". **Ioana Visan, Senior Editor, *Nature Immunology*.**