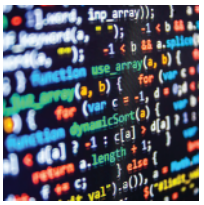
Techniques

Major Finding: The single-cell approach Perturb-CITE-seq identified CD58 as influencing immune checkpoint blockade.

Concept: Perturb-CITE-seq combines genetic screening with transcriptomic and cell-surface protein analysis.

Impact: This work highlights this method's use and suggests a role for CD58 in ICI response and resistance.

NEW SINGLE-CELL SCREENING METHOD FINDS IMMUNOTHERAPY RESPONSE MODULATOR



Understanding mechanisms of intrinsic resistance to immune checkpoint inhibitors (ICI) is critical to the development of methods to circumvent this obstacle. To address this, Frangieh, Melms, Thakore, Geiger-Schuller, and colleagues developed a method dubbed Perturb-CITE-seq, which combines the systematic CRISPR–Cas9-based single-cell transcriptomic genetic screening technique Perturb-seq with the single-cell approach CITE-seq, which enables transcriptomic profiling alongside measurement of cell-surface proteins. Using Perturb-CITE-seq, 248 genes associated with intrinsic ICI resistance were systematically targeted with 744 CRISPR–Cas9-based knockouts (KO), and then single-cell transcriptomic profiling and measurement of 20 relevant cell-surface proteins, with the final number of cells included in the analysis being approximately 218,000. In coculture experiments combining patient-derived melanoma cells and *ex vivo* expanded tumor-infiltrating lymphocytes, a viability screen (to assess the knockouts' effects on T-cell mediated antitumor immunity) was combined with Perturb-CITE-seq (to ascertain the mechanisms underlying effects seen in the viability screen). In addition to identifying genes anticipated to be involved in immune evasion based on clinical and preclinical data, *B2M, HLA-A, JAK1, JAK2, STAT1, IFNGR1*, and *IFNGR2* conferred resistance to T cell–mediated killing. Interestingly, genetic perturbation of *CD58* (also known as *LFA3*)—encoding a cell-surface protein typically expressed on antigen-presenting cells that acts as a ligand for T-cell and natural killer–cell CD2—was also a hit in the Perturb-CITE-seq screen. Mechanistically, IFNγ signaling did not affect CD58 protein expression, and MHC expression was not altered by *CD58* perturbation, implying that *CD58* loss-of-function mutations do not promote immune escape via the same mechanisms as other known mutations. Notably, patient data showed that levels of *CD58* mRNA were lower in ICI-resistant melanomas than in ICI-naïve melanomas. Collectively, this study demonstrates the utility of Perturb-CITE-seq for biological research, including on cancer, and identifies CD58 as a potential modulator of immunotherapy response. ■

Frangieh CJ, Melms JC, Thakore PI, Geiger-Schuller KR, Ho P, Luoma AM, et al. Multimodal pooled Perturb-CITE-seq screens in patient models define mechanisms of cancer immune evasion. Nat Genet 2021;53:332–41.

Clinical Trial

Major Finding: Umbralisib showed efficacy and in relapsed or refractory (R/R) indolent non-Hodgkin lymphoma (NHL).

Concept: In this phase IIb trial, the overall response rate ranged from 45% to 50% depending on NHL subtype.

Impact: The efficacy data combined with a favorable tolerability profile support continued investigation.

PI3Kδ–CK1ε INHIBITOR UMBRALISIB SHOWS PROMISE IN R/R INDOLENT NHL

Despite the fact that PI3K inhibitors have shown promise in relapsed or refractory (R/R) B-cell malignancies, their long-term use is limited by toxicities, which may be mediated in part by lack of specificity of these inhibitors for the isoform of PI3K (PI3Kδ) selectively expressed by B cells. The highly selective oral PI3Kδ inhibitor umbralisib—which also inhibits casein kinase 1ε (CK1ε), a protein involved in regulation of the WNT–β-catenin pathway and translation of some oncoproteins—has demonstrated signs of activity in R/R B-cell cancers. Fowler and colleagues conducted an open-label phase IIb trial of umbralisib in 208 patients with indolent non-Hodgkin lymphoma (NHL) that was resistant to or relapsed following rituximab and at least one line of anti-CD20 therapy. Sixty-nine patients (33%) had marginal zone lymphoma (MZL), 117 patients (56%) had follicular lymphoma, and 22 patients (11%) had small lymphocytic lymphoma (SLL). In an intention-to-treat analysis, the overall response rate was 49% for patients with MZL, 45% for patients with follicular lymphoma, and 50% for patients with SLL, with the complete response rates being 16%, 5%, and 5%, respectively. Additionally, 33% of patients with MZL, 34% of patients with follicular lymphoma, and 36% of patients with SLL experienced stable disease. After a median follow-up period of 21.4 months, among patients whose disease responded to umbralisib, the median duration of response was not reached for MZL, 11.1 months for follicular lymphoma, and 18.3 months for SLL. Safety data were consistent with those from the prior phase I dose-escalation study, with the most common treatment-emergent adverse events (TEAE) being diarrhea, nausea, fatigue, and vomiting; also of note, ALT or AST increased in 20% and 19% of patients, respectively. Ultimately, 15% of patients discontinued umbralisib treatment due to TEAEs. Although cross-trial comparisons should be made with great caution, these results suggest that umbralisib may have greater tolerability than other PI3K inhibitors for R/R B-cell malignancies, and this trial supports umbralisib's efficacy in indolent NHL. ■

Fowler NH, Samaniego F, Jurczak W, Ghosh N, Derenzini E, Reeves JA, et al. Umbralisib, a dual PI3Kδ/CK1ε inhibitor in patients with relapsed or refractory indolent lymphoma. J Clin Oncol 2021 Mar 8 [Epub ahead of print].