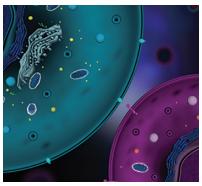
## Immunotherapy

**Major Finding:** Chimeric antigen receptors (CAR) comprised of LAT and SLP-76 enable logic-gated T-cell activation.

**Concept:** Upon dual-antigen ligation, LAT- and SLP-76–based CARs cooperate to induce T-cell effector functions.

**Impact:** This platform may improve the antitumor efficacy and reduce on-target, off-tumor toxicity of CAR T therapy.

### LOGIC-GATED CAR T CELLS ENHANCE SURVIVAL AND REDUCE ON-TARGET TOXICITY



Chimeric antigen receptor (CAR) T cells target cancer cells that express specific tumor-associated antigens, but CAR therapies that recognize a single antigen are ineffective in solid tumors and have the potential to cause severe on-target, off-tumor toxicity, as normal tissues express most tumor-associated antigens to some degree. Efforts to better distinguish tumor cells have motivated the search for highly tumor-specific expression patterns to utilize in Boolean logic–gated CAR strategies, such as AND-gated CARs, which only recognize cells concurrently expressing two distinct antigens. While promising antigen pairs have been proposed, there remains a need to design CAR molecules that enable logic-gated control, prompting Tousley and colleagues to study the intracellular components important for proximal CAR signaling. Engineered T cells expressing a conventional CD3ζ-based CAR required the tyrosine kinase ZAP-70 to induce degranulation and cytokine production upon stimulation with cognate antigen–expressing tumor cells, and a CAR comprising a ZAP-70, rather than a conventional CD3ζ endodomain, was sufficient to initiate T-cell activity after antigen encounter. Compared with CD3ζ-based CARs, which only elicited transient tumor control, ZAP-70 CARs induced potent tumor regression in a murine xenograft model of metastatic neuroblastoma, and single-cell RNA sequencing analysis suggested that superior ZAP-70 CAR performance was due, in part, to decreased antigen-independent tonic signaling. Given that endogenous ZAP-70 phosphorylates the adapter proteins LAT and SLP-76 to propagate T-cell signaling, a logic-gated intracellular network (LINK) CAR was generated, in which T cells coexpressed a LAT-based CAR and an SLP-76–based CAR, each specific for one of two concurrently expressed tumor antigens. Following modifications to the transmembrane domains as well as mutations in LAT and SLP-76 to avoid adapter-mediated leakiness in the absence of dual-antigen ligation, the AND-gated LINK CAR potently enhanced survival while preventing on-target, off-tumor toxicity in a murine xenograft model. In summary, this study introduces a robust and specific platform for logic-gated control of CAR T therapy that outperforms similar agents. ■

*Tousley AM, Rotiroti MC, Labanieh L, Rysavy LW, Kim WJ, Lareau C, et al. Co-opting signalling molecules enables logic-gated control of CAR T cells. Nature 2023;615:507–16.*

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## Gene Expression

**Major Finding:** The histone acetyltransferase KAT8 transcriptionally upregulates PD-L1 through cocondensation with IRF1.

**Concept:** KAT8 acetylates IRF1, enhancing IRF1 binding to the PD-L1 promoter and subsequent transcription.

**Impact:** These results suggest that blocking this axis can lead to enhanced antitumor immune responses.

### DISRUPTION OF KAT8–IRF1 CONDENSATE FORMATION REDUCES PD-L1 EXPRESSION

Immunotherapies that target the PD-1/PD-L1 axis have revolutionized cancer treatment, but only a portion of patients demonstrate lasting durable responses to these therapies. A better understanding of the mechanisms that regulate PD-1/PD-L1 could promote the design and outcomes of these therapies; therefore, to investigate these regulatory mechanisms, Wu, Zhou, and colleagues conducted a whole-genome CRISPR–Cas9 gene knockout screen in tumor cells after IFNγ exposure. The most significantly enriched gene from this screen encoded the histone acetyltransferase KAT8. Loss of KAT8 significantly reduced PD-L1 levels at the mRNA and total protein levels, suggesting transcriptional regulation, and its loss also enhanced T-cell killing and inhibition of tumor growth. Investigation into how KAT8 regulates PD-L1 mRNA transcription revealed colocalization of KAT8 with IRF1 in condensates both *in vitro* and *in vivo*. Multivalency from both specific and promiscuous interactions are needed for KAT8–IRF1 condensation. Moreover, formation of these condensates occurred at promoters of PD-L1. Mechanistically, KAT8 was found to acetylate IRF1 at K78, which is promoted by cocondensation, with this K78 acetylation promoting IRF1 binding to the PD-L1 promoter and subsequent transcription of PD-L1 mRNA. Additionally, acetylated IRF1 recruits KAT8 to the PD-L1 promoter, leading to enhanced H4K16 acetylation in the PD-L1 promoter, suggesting positive feedback. Disruption of KAT8–IRF1 condensates using the KAT8–IRF1 interaction–blocking peptide 2142-R8 inhibited the acetylation of both IRF1 and H4K16 in the PD-L1 promoter as well as subsequently suppressed PD-L1 mRNA and protein expression in IFNγ-treated cells. Further evaluation of 2142-R8 and its effects on antitumor immunity revealed its ability to reduce tumor volumes in murine tumor models as well as to enhance T-cell killing *in vitro* and $CD8^+$ T-cell infiltration *in vivo*. In summary, this study reveals condensate formation as a regulatory mechanism of PD-L1 expression in tumor cells and suggests that targeting this axis could be a potential therapeutic strategy to enhance therapeutic outcomes. ■

*Wu Y, Zhou L, Zou Y, Zhang Y, Zhang M, Xu L, et al. Disrupting the phase separation of KAT8–IRF1 diminishes PD-L1 expression and promotes antitumor immunity. Nat Cancer 2023;4:382–400.*

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