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INSIGHTS

MHC one–two punch knocks out cancer

Amir Ghorbani[1] and Jonathan W. Yewdell[1]

In this issue of *JEM*, Zhang et al. (https://doi.org/10.1084/jem.20250025) describe complete T cell antigens (CTAs), comprised of class I and class II immunogenic peptides fused in a single polypeptide that greatly increase the effectiveness of cancer immunotherapy, even when the CTA antigens are not expressed in tumor cells.

The complete T cell antigen (CTA) phenomenon traces back to William Coley, who in 1891 injected sarcoma patients with bacterial cultures after noting tumor regression following serious infections (Coley, 1891). We now understand, thanks to decades of publicly funded immunology research, that Coley's bacteria acted as potent adjuvants, enhancing T cell–mediated cancer immunosurveillance. His approach presaged mycobacterial (BCG) treatment for bladder cancer—the first Food and Drug Administration–approved immunotherapy (1990)—and, more broadly, the concept that underlies checkpoint inhibitor (CPI) therapy. While a Nobel prize–worthy breakthrough (Ledford et al., 2018), CPI effectiveness remains limited to only a few tumor types and is curative in only a minority of patients. Zhang et al. (2025)'s findings offer the possibility of greatly enhancing CPI-based immunotherapy by both adjuvant targeted– and epitope targeted–effects.

A new logic for T cell collaboration

CPIs target molecular "brakes" that limit T cell activation, yet most tumors remain resistant—even those with heavy mutation burdens that generate abundant neoantigens. Most tumors resist CPI therapy, even those with high mutation burdens that generate numerous modified peptides that should escape self-T cell tolerance. Paradoxically, some tumors with fewer mutations can be effectively treated by CPI therapy (Chae et al., 2019).

CD4$^+$ T cells are increasingly recognized as key orchestrators of effective anti-tumor immunity. They license dendritic cells (DCs), potentiate CD8$^+$ T cell activation, and remodel the tumor microenvironment through cytokine secretion and costimulatory signals (Ahrends et al., 2017; Ferris et al., 2020). Without CD4$^+$ help, CD8$^+$ T cells enter an exhausted state—expressing PD-1 and other inhibitory receptors—and lose proliferative and cytotoxic capacity (Miller et al., 2019). A central challenge in cancer immunotherapy has, therefore, been to coordinate these two T cell arms. Zhang et al.'s elegant solution is to fuse their target peptides to ensure they are presented by a single DC.

A molecular epitope marriage

Zhang et al. (2025) engineered tumor cells to express a complete T cell antigen (CTA): a single polypeptide with immunogenic class I – and II–restricted epitopes. When mice were inoculated with these CTA-expressing cells, even contralateral tumors lacking the antigen were rejected following CPI treatment—a striking, target antigen-independent adjuvant effect.

This potentiation vanished when the class I and II peptides were expressed as separate proteins or when given as exogenous protein immunogens or peptide-pulsed splenocytes, despite these also activating CD8$^+$ T cells. The data imply that antigen linkage within the same expressed protein is required to drive optimal CD8$^+$ T cell responses.

Amir Ghorbani and Jonathan W. Yewdell.

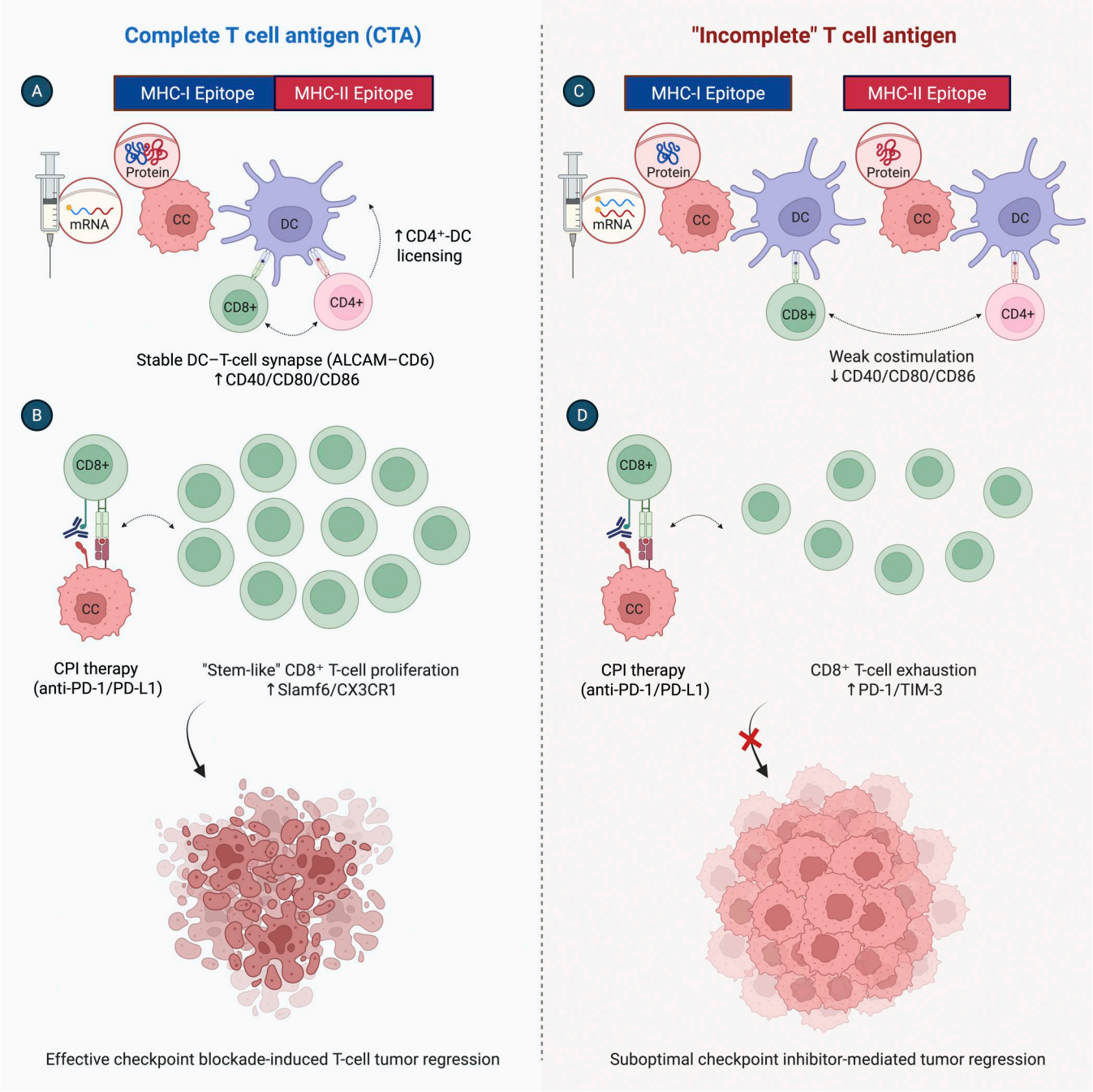
CTA immunization correlated with an influx of tumor-specific CD8$^+$ T cells and a marked shift toward proliferative "stem-like" Slamf6$^+$ CX3CR1$^+$ subsets—cells long associated with durable CPI responses (Baden et al., 2020; Im et al., 2016). Expression of PD-1, TIM-3, and CD39 exhaustion markers decreased, while cytolytic mediators perforin and granzyme rose. Depletion of CD4$^+$ T cells abolished these effects, underscoring the dependency of CD8$^+$ responses on CD4$^+$ help within this chimeric antigen framework.

Draining LN (DLN) DCs orchestrate CTA activity

CTA expression within tumor cell vaccines profoundly reprogrammed DCs in DLNs. Single-DC transcriptomics revealed upregulation of costimulatory molecules (CD40, CD80, and CD86) and activation of the ALCAM–CD6 signaling axis—a pathway that promotes stable DC–T cell synapses (Ibáñez et al., 2006)—accompanied by

[1]Cellular Biology Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA.

Correspondence to Jonathan W. Yewdell: jyewdell@nih.gov.



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Physical linkage of MHC class I and II epitopes enables DC licensing and coordinated CD4+–CD8+ T cell responses. Schematic comparing immune responses elicited by CTAs versus "incomplete" T cell antigens. Left (A and B): A CTA, in which immunogenic MHC class I– and class II–restricted epitopes are encoded within a single endogenous protein expressed by a cancer cell (CC) or delivered as an mRNA vaccine, is acquired by DCs and processed for presentation on both MHC classes by the same cell (A). Concurrent engagement of CD4+ and CD8+ T cells promotes CD4+ T cell–dependent DC licensing, characterized by increased expression of costimulatory molecules (CD40, CD80, and CD86) and stabilization of DC–T cell synapses via the ALCAM–CD6 axis. In the presence of CPI therapy, CTA priming supports the expansion of tumor-specific CD8+ T cells with a proliferative, stem-like phenotype (Slamf6+, CX3CR1+), reduced expression of exhaustion markers, and effective tumor regression (B). Right (C and D): When MHC class I and class II epitopes are expressed as separate proteins ("incomplete" T cell antigens), antigen presentation occurs on distinct DCs, limiting CD4+ T cell–mediated licensing (C). This leads to weaker costimulatory signaling, impaired CD8+ T cell differentiation, increased expression of exhaustion markers (PD-1 and TIM-3), and suboptimal tumor regression despite CPI therapy (D). Figure created using BioRender.com.

suppression of TIGIT-associated inhibitory genes. Flow cytometry validated these transcriptional shifts, confirming the emergence of a hyper-activating DC phenotype.

Functionally, CTA-primed DCs became highly efficient at activating both CD4⁺ and CD8⁺ T cells in DLNs, leading to robust expansion of tumor-infiltrating lymphocytes (TILs). These data define DCs as the central integrators of CTA-induced synergy, bridging dual-epitope presentation with checkpoint blockade responsiveness.

How are CTAs processed and presented?

The striking feature of CTAs is the requirement that MHC class I and II peptides be expressed within a single endogenous protein. This configuration likely enables a single DC to simultaneously engage CD8⁺ and CD4⁺ T cells, providing optimal costimulation for CD8⁺ differentiation.

This dependency was revealed using dual- versus single-epitope constructs, both predicted to be rapidly degraded in the cytosol due to their small size and lack of stable structure. Such rapidly degraded immunogens are inefficient for classical exogenous cross-priming (Norbury et al., 2004; Wolkers et al., 2004). If such cross-priming dominates in this system, antigen availability should be limiting and require epitopes to be physically linked to increase the chances of a single DC co-expressing class I and class II peptides. Determining whether co-expression of two stable proteins, each containing a single immunogenic class I or class II peptide, will help clarify the underlying mechanism.

Another unresolved question is why cell expression of the CTA is required at all, since DCs efficiently generate and present class I and II peptides from exogenous proteins. One possibility is that tumor cells act as adjuvants, providing innate immune cues that license DCs. Testing whether exogenous protein mixed with tumor cell lysates (or subcellular fractions) can reproduce CTA effects could reveal the adjuvant signals necessary for optimal T cell priming. This would also enable CTA vaccination using defined antigens and molecular adjuvants, avoiding the complexity of cell-based immunogens.

Nature's chimeras

Perhaps the study's most intriguing insight is that tumors may already express natural CTAs. Frameshift mutations, arising from insertions or deletions, create aberrant open reading frames that generate relatively long nonself proteins containing both MHC-I and MHC-II epitopes. Whole-exome sequencing of MC38 tumor cells revealed 25 such frameshifts; one in Notch2 (Notch2^{MUT}) encodes a 216–amino acid neoprotein harboring 11 predicted class I and 14 predicted class II binders.

Expression of Notch2^{MUT} in diverse tumor lines triggered spontaneous rejection and durable memory. When combined with anti–PD-L1 therapy, it eradicated otherwise resistant tumors. Both CD4⁺ and CD8⁺ T cells recognized Notch2^{MUT}-derived peptides, confirming its dual MHC restriction.

This discovery may explain why cancers with high long frameshift burdens—such as microsatellite instability–high tumors—respond disproportionately well to checkpoint blockade (Maby et al., 2016). Zhang et al. posit that such tumors naturally generate CTA neoantigens, enabling intrinsic dual T cell activation.

Yet caveats remain. MC38 cells, derived by methylcholanthrene mutagenesis, contain mutation loads far exceeding most human tumors. Notch2^{MUT} is unusually rich in predicted MHC-binding peptides (25 total), which, given an overall accuracy of ~70%, predicts 18 MHC-binding peptides, with further filters (levels of CTA expression, proteolytic liberation, TAP transport, and TCR recognition) predicted to greatly limit immunogenicity (Yewdell, 2006). Moreover, MC38 cells—despite harboring at least one endogenous CTA—still form tumors unless engineered to express a novel CTA. These observations highlight that endogenous CTAs may not universally induce tumor rejection.

A predictive twist: Size matters in human tumors

Mining the Cancer Genome Atlas, Zhang et al. (2025) found a remarkable correlation: colorectal cancer patients whose tumors contained long frameshift-derived peptides (>120 amino acids) had nearly double the 10-year survival (66 vs. 38%) compared with patients lacking such mutations. Importantly, total mutational burden alone did not predict survival.

This suggests that the structure of mutations—specifically, the generation of extended, multi-epitope frameshifts—may determine the efficacy of anti-tumor immunity. If validated in other malignancies, frameshift length could serve as a biomarker to predict CPI responsiveness and guide personalized vaccine design.

CTA vaccines: Back to the future

Building on this concept, Zhang et al. (2025) demonstrated that DNA vaccines encoding chimeric CTA constructs could induce potent anti-tumor immunity. Mice immunized intramuscularly with DNA encoding a dual MHC-I/II immunogenic antigen rejected established MC38 tumors, whereas immunization with a class I–only construct had minimal impact. Similarly, DNA encoding the endogenous Notch2^{MUT} protein synergized with anti–PD-L1 therapy, quadrupling TIL infiltration, expanding self-renewing CD8⁺ T cells, and shrinking tumor mass by ~30%.

The CTA effect can be viewed as a "DC checkpoint" inhibitor—a mechanism that unleashes DCs to prime balanced CD4⁺ and CD8⁺ responses alongside CPI therapy. Translating this approach to humans will require tailoring constructs to individual HLA haplotypes, though there are a number of class II epitopes known to be broadly immunogenic (Alexander et al., 1994). More simply, viral proteins such as SARS-CoV-2 Spike are natural "Super-CTAs" with dozens of epitopes that bind multiple class I and II molecules.

Indeed, in the early 1900s, reprising Coley, there were anecdotal reports of cancer regression following respiratory infection, tuberculosis, and rabies vaccination (Dock, 1904). By the 1970s, studies had shown that RNA virus–infected tumor lysates could induce tumor-specific immunity (Lindemann, 1974). More recently, case reports described lymphoma regression following SARS-CoV-2 infection (Challenor and Tucker, 2021). Strikingly, mRNA-based SARS-CoV-2 vaccination within 100 days of CPI initiation nearly doubles 3-year survival in lung cancer and melanoma (Grippin et al., 2025).

Looking ahead

Zhang et al. provide a mechanistic foundation for harnessing dual MHC-I and MHC-II peptide expression to strengthen anti-tumor

T cell cooperation. Their findings open multiple experimental and translational avenues:

- Breadth: How broadly will CTAs function across HLA haplotypes and tumor types?
- Specificity: Should vaccines incorporate epitopes that prime naïve responses, recall memory responses, or exploit persistent immunity to CMV or other herpesviruses?
- Safety: To what extent do CTA responses provoke autoimmunity?
- Delivery: Will optimal platforms be mRNA, DNA, or cell-based formulations?

Ultimately, CTAs demonstrate that a deceptively simple structural innovation—linking class I and II epitopes within one continuous sequence—can reshape the landscape of tumor immunotherapy. In immunology as in life, the whole can indeed be far greater than the sum of its parts.

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