IMMUNOTHERAPY

The dynamics of an immunotherapy duo

Cancer vaccines can elicit tumor-specific T cells, but sustaining their function via immune checkpoint therapy (ICT) may be required for robust anti-tumor immunity. A new study reveals that neoantigen cancer vaccines synergize with anti-PD-L1 ICT in a preclinical model and provides mechanistic insights into this synergy.

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Antibody blockade of immune checkpoints (for example, CTLA-4 and PD-1 or PD-L1) can unleash T cells to destroy tumors, but not all patients benefit from ICT[1]. Whereas ICT blocks the function of 'checkpoints' to relieve T cell suppression, increasing the number of effector T cells by vaccination has long been an attractive cancer treatment strategy. Because T cells, via their T cell receptor (TCR), recognize primarily foreign or non-self peptides presented on major histocompatibility complexes (MHC), T cells recognizing MHC-bound peptides derived from pathogens can be readily induced by vaccination. Cancer vaccines are based on the principle that cancer cells, originating from the body's own cells gone awry, can generate antigens that can be recognized as 'foreign' by T cells[2]. Therefore, cancer vaccines are typically composed of tumor proteins or peptides, or molecular templates for producing these tumor antigens (such as viral-vector- and DNA- or RNA-based vaccines), and an immune adjuvant.

In contrast to ICT, therapeutic cancer vaccine clinical trials conducted in prior decades yielded largely negative outcomes and led to the widespread abandonment of cancer vaccine development[3]. These disappointing results were likely at least partially attributable to immune checkpoints acting as a 'brake' on the T cell response, a concept that was not yet understood when many cancer vaccine trials were being conducted[1]. Furthermore, most cancer vaccines targeted tumor-associated antigens derived from self-proteins aberrantly expressed by cancer cells, which by themselves may not be optimal because of immune tolerance for non-mutant self-peptides[2,3]. The advent of immunogenomics approaches has facilitated the development of cancer vaccines based on tumor-specific neoantigens derived from somatic alterations (for example, point mutations, insertions or deletions, gene fusions)[4,5]. Therapeutic neoantigen vaccines can induce robust anti-tumor immunity in preclinical models[4,6,7], and early-phase personalized neoantigen vaccine clinical trials have indicated that they may have clinical efficacy[3,8–12]. Although these findings are encouraging, vaccine-induced tumor-specific T cells could follow the same path as non-vaccine-induced T cells, which often lose their anti-tumor effector function and become dysfunctional or exhausted, in part due to interactions between the immune checkpoint receptor PD-1 and its major ligand PD-L1[13]. Indeed, data from early-phase clinical trials suggest that the efficacy of neoantigen vaccines may be enhanced by coadministration with anti-PD-1 or anti-PD-L1[9]. In this issue of *Nature Cancer*, Liu et al. provide evidence that combined neoantigen vaccination and anti-PD-L1 ICT provide superior anti-tumor immunity compared to monotherapy and further define features of combination-therapy-induced T cells that facilitate the destruction of tumor cells[14].

The authors began by using the mouse MC-38 colon adenocarcinoma tumor model and a neoantigen vaccine composed of a 9-mer mutant Adpgk (mAdpgk) peptide formulated with two adjuvants (poly-IC and CpG 1826)[14]. The 9-mer mAdpgk neoantigen, formed by a point mutation in the ADPGK protein in the MC-38 tumor, functions as an MHC class I (MHC-I)-binding epitope and is recognized by $CD8^+$ T cells[7]. In mice bearing MC-38 tumors, a modest delay in tumor outgrowth was observed when the mAdpgk neoantigen vaccine was administered as a monotherapy. Further analysis revealed that intratumoral neoantigen-specific $CD8^+$ T cells expressed negative regulators of T cell function, including PD-1 as well as the transcription factor TOX. These features are associated with $CD8^+$ T cells that lack effector function and are designated as dysfunctional or exhausted T cells ($T_{d/ex}$)[13]. At the same time, other immune cells within the tumor microenvironment (TME) expressed high levels of PD-L1. Therefore, Liu et al.[14] assessed whether ICT antibody blockade of PD-1–PD-L1 interaction in the TME could enhance the efficacy of the neoantigen vaccine. Whereas monotherapy via either neoantigen vaccination or anti-PD-L1 treatment slowed tumor outgrowth, combining neoantigen vaccination and anti-PD-L1 provided superior efficacy and led to complete tumor rejection (Fig. 1a).

To gain insights into the synergistic effects of neoantigen vaccination and anti-PD-L1, the authors leveraged multiple approaches to scrutinize T cell dynamics during combination therapy. They performed single-cell RNA sequencing with targeted TCR capture (scTCR-seq), along with flow cytometry, on total T cells isolated from tumors, tumor-draining lymph nodes and spleens of mice undergoing different therapies. In untreated mice with progressively growing tumors, the proportion of $CD8^+$ $T_{d/ex}$ cells in the TME increased over time, whereas both neoantigen vaccination and anti-PD-L1 given as monotherapy increased the proportion of the intratumoral $CD8^+$ T cells with an effector phenotype (T_{Eff}) relative to $T_{d/ex}$ cells. Combination treatment, which produced the most beneficial outcome, was characterized by the most prominent expansion of $CD8^+$ T_{Eff} cells, along with shrinking proportions of $CD8^+$ $T_{d/ex}$ cells. These $CD8^+$ T_{Eff} cells that emerged after combination therapy expressed lower levels of inhibitory receptor genes (*Lag3* and *Havcr2*), and high levels of genes involved in cytotoxicity (*Gzma* and *Fasl*) and chemokine signaling (*Cxcr3* and *Ccl5*), and they also produced IFNγ, a crucial cytokine involved in immune-mediated tumor rejection. Although the mAdpgk vaccine antigen is an MHC-I neoantigen recognized by $CD8^+$ T cells, the combination treatment also affected $CD4^+$ regulatory T (T_{reg}) cells and $CD4^+$ helper T (T_H) cells, a population of T cells that recognize peptides presented on MHC class II (MHC-II). Specifically, tumor-bearing mice treated with the combination therapy exhibited shrinking proportions of intratumoral $CD4^+$ T_{reg} cells and an increase in T_H1-like $CD4^+$ T cells expressing *Bhlhe40*, *Icos* and the *Ifng* gene that encodes IFNγ.

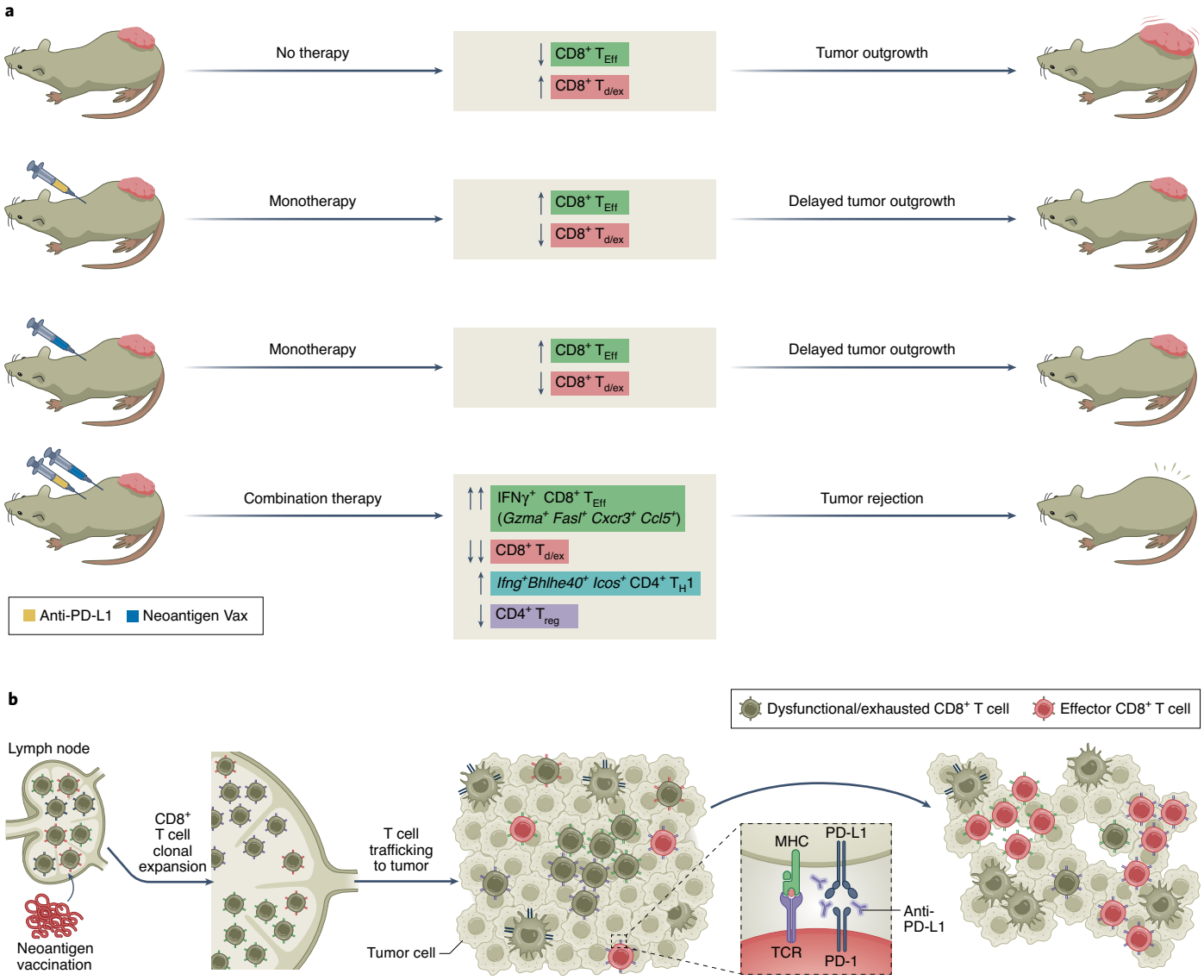

Fig. 1 | Combination of tumor-specific neoantigen vaccination and anti-PD-L1 immune checkpoint therapy synergize to enhance intratumoral effector T cells and promote tumor rejection. a, Schematic illustrating neoantigen vaccination (Vax) plus anti-PD-L1 induces robust IFN-γ$^+$ CD8$^+$ effector T cells (T_{Eff}) expressing *Gzma*, *Fasl*, *Cxcr3* and *Ccl5* transcripts as well as *Ifng*$^+$ *Bhlhe40*$^+$ *Icos*$^+$ CD4$^+$ T_H1 cells, while decreasing the abundance of dysfunctional/exhausted CD8$^+$ T cells ($T_{d/ex}$). Whereas monotherapy induces delayed tumor outgrowth, combination therapy induces tumor rejection. **b**, Neoantigen vaccination and anti-PD-L1 induce clonal expansion of CD8$^+$ T_{Eff} cells that traffic to the tumor and maintain a robust effector phenotype, likely through vaccine-induced priming of tumor-antigen-specific CD8$^+$ T cells and blockade of PD-1 signaling on T cells in the tumor microenvironment.

To gain insights into whether the combination immunotherapy reinvigorated pre-existing intratumoral T cells or whether clonal replacement of T cells from the lymphoid tissue was contributing to the therapeutic effect of combination treatment, Liu et al.[14] next tracked clonal T cells in tumors and peripheral lymphoid organs via TCR sequencing. The authors concluded that the treatment stimulated clonal CD8$^+$ T_{Eff} differentiation, expansion and migration to the tumor site (Fig. 1b). Treatment with FTY720, an S1P receptor agonist that inhibits lymphocyte egress from the lymph nodes, abrogated the therapeutic effect of the combination therapy. These findings favor the hypothesis that in this model, combination immunotherapy induces durable, functional intratumoral CD8$^+$ T_{Eff} cells via clonal replacement by T cells migrating from lymphoid tissues, rather than reinvigoration of the T cells populating the TME before therapy.

Notably, peptide–MHC-I tetramer staining revealed that combination therapy increased the percentages of not only intratumoral neoantigen-specific CD8$^+$ T cells recognizing the mAdpgk antigen that was the target of the vaccine, but also intratumoral CD8$^+$ T cells specific for p15E, an endogenous retroviral MHC-I antigen expressed in MC-38 cells. In humans, neoantigen vaccines have been shown to not only enhance the number of T cells against neoantigens that were recognized by T cells prior to treatment, but also induce de novo T cell responses against other neoantigens that had not been observed before, including distinct neoantigens not specifically targeted by the vaccine used—an occurrence known as epitope spreading[8,12]. Epitope spreading in cancer patients receiving a personalized

neoantigen vaccine concurrently with nivolumab (anti-PD-1 antibody) has been associated with progression-free survival[11].

The use of a preclinical model and a vaccine strategy targeting a single neoantigen in the MC-38 tumor by Liu et al. facilitated the interrogation of T cell dynamics, including monitoring of CD8$^+$ T cells recognizing the same neoantigen under different treatment conditions[14]. Although the use of a vaccine targeting a single neoantigen is advantageous in this context, human patient tumors are likely more heterogenous than the mouse MC-38 tumor cell line, and therefore it may be necessary to target multiple tumor antigens to decrease the likelihood that antigen-loss tumor variants may emerge. To that end, most cancer vaccine clinical trials are designed to target multiple tumor antigens[3]. In addition, the neoantigen vaccine used in the Liu et al.[14] study was composed of a 9-mer peptide that corresponds to the minimal MHC-I neoantigen epitope that is presented to CD8$^+$ T cells. However, most neoantigen vaccine clinical trials use synthetic long peptides (SLPs) or RNA-based cancer vaccines encoding long peptides, with the minimal MHC-I epitope surrounded by additional amino acids[3,9–12]. This is because MHC-II-bound CD4$^+$ T cell epitopes are typically longer than MHC-I-bound CD8$^+$ T cell epitopes, so a longer peptide product makes it possible that both CD4$^+$ and CD8$^+$ T cell epitopes will be present in the vaccine. In preclinical models and trials involving both SLPs and RNA vaccine strategies, CD4$^+$ T cell responses against MHC-II neoantigens are often elicited, even in instances when vaccines were designed to elicit CD8$^+$ T cells based on predictions of MHC-I neoantigen predictions[9,15]. In future studies, it will be of interest to extend the findings of Liu et al.[14] by modifying the vaccine protocol to include multiple SLPs containing both MHC-I neoantigens that elicit CD8$^+$ T cell responses and MHC-II neoantigens recognized by CD4$^+$ T cells, used alone or in combination with ICT.

Finally, Liu et al. administered the mAdpgk neoantigen vaccine to naive, non-tumor-bearing mice and obtained TCR sequences of the flow-sorted neoantigen-specific CD8$^+$ T cells to infer from the scTCR-seq data which CD8$^+$ T cells were likely to be mAdpgk neoantigen specific[14]. This approach enabled the authors to characterize the molecular phenotype of these neoantigen-specific T cells and identify a gene-transcription signature that was strongly associated with the TCR clonotypes linked to these cells. Analysis of human patient data indicated that this signature was associated with CD8$^+$ T cells with an effector phenotype, and correlated with the outcomes of ICT in 'hot' tumors — that is, those with strong immune infiltration. The authors suggest that this transcriptional signature and cell subsets identified could serve to distinguish tumor-antigen-specific, versus bystander, T_{Eff} cells and thereby guide the development of effective neoantigen vaccines and improved biomarkers for clinical response to immunotherapy. Overall, these findings provide important insights into the emergence, maintenance and phenotypic features of antigen-specific effector T cells that are crucial to tumor eradication and further support the rationale for combination immunotherapies incorporating neoantigen vaccines with ICT. ❐

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