Combining personalized neoantigen vaccination with chemotherapy and anti-PD-1 to treat NSCLC

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In this issue of Cancer Cell, Awad et al. report a phase 1b clinical trial combining a personalized vaccine NEO-PV-01 with chemotherapy and anti-PD-1 pembrolizumab in first-line metastatic non-squamous NSCLC. They demonstrate this treatment regimen was well tolerated and induced neoantigen-specific CD4+ T-cell responses with effector phenotype.

Mutations in tumor cells can generate neoantigens that can be presented on the cell surface by major histocompatibility complex (MHC) molecules and subsequently recognized by T cells. Neoantigens are very immunogenic as they are present only in tumors but not in normal tissues, hence circumvent the central thymic tolerance. However, most neoantigens are unique and different from patient to patient, so neoantigen vaccines need to be specifically produced for each patient as personalized vaccines, limiting the clinical use. Recent progresses in sequencing and bioinformatics technology allow rapid identification of candidate neoantigens in individual patients using ad-hoc algorithms. Nevertheless, the key challenges are to select appropriate candidates able to trigger tumor-killing T cell responses, and to generate the vaccine swiftly with an efficient platform that is easy to manufacture. Two groups overcame these challenges and pioneered an initial set of clinical trials on melanoma patients, demonstrating that personalized neoantigen vaccines are clinically feasible and safe (Ott et al., 2017; Sahin et al., 2017), and have the potential to synergize with immune checkpoint inhibitors (ICIs) such as pembrolizumab (Ott *et al.*, 2017; Ott et al., 2020). However, the impact of systemic chemotherapy on neoantigen vaccine-induced anti-tumor immunity is unclear. Given that the combination of pembrolizumab and chemotherapy is emerging as a new standard of care for different cancers, such as non-small-cell lung cancer (NSCLC), incorporating neoantigen vaccine in a triple combination therapy may add clinical benefit to the patients.

Awad et al. report data from a phase 1b clinical trial combining the personalized vaccine NEO-PV-01 with chemotherapy and pembrolizumab in first-line metastatic non-squamous NSCLC (Awad et al., 2022). NEO-PV-01 is a personalized vaccine comprising up to 20 unique neoantigens per patient, in the form of synthetic long peptides formulated with the TLR3 agonist poly-ICLC as an immune adjuvant. The trial was designed to allow patients to receive standard-of-care treatment, i.e. 4 cycles of chemotherapy combined with pembrolizumab, during the vaccine production (Figure 1). This single arm study enrolled 38 patients with no prior systemic treatment for metastatic disease and no prior immunotherapy. Enrollment

was not restricted based on tumor PD-L1 status. Patients received at least one dose of pembrolizumab and were defined as the intention-to-treat (ITT) set. Seventeen patients (45%) in the ITT set were not vaccinated due to early study termination for various reasons, mostly the inability to manufacture the vaccine because of low tumor content in the biopsy and/or insufficient number of candidate neoantigens. Only 16 patients completed the vaccination with minor related adverse events reported, showing that NEO-PV-01 in combination with chemotherapy and pembrolizumab was safe and well-tolerated.

Molecular and immune analyses of both the tumor microenvironment (TME) and peripheral blood revealed several factors that were correlated with clinical response. T-cell infiltration and MHC II expression in the TME at the pre-treatment timepoint correlated positively with clinical response, in line with previous findings (Cabrita et al., 2020; Ganesan et al., 2017; Ulloa-Montoya et al., 2013). Also, TCR diversity in the pre-treatment TME had a positive correlation with progression-free survival (PFS), with increased or stable diversity over the course of the study in patients with better PFS. The immune responses elicited by NEO-PV-01 vaccination were assessed in 13 patients with available samples at the pre-vaccine timepoint compared to the post-vaccine timepoint (8 weeks after the first vaccine dose). Although immune reactivity to the vaccine epitopes was higher at the post-vaccine timepoint, only 55% of vaccine peptides elicited a T-cell response. Among these responses, 49% were detectable *ex vivo* in an overnight assay, the rest were detected after a 5-day stimulation with peptides. Hence, only 27% of the candidate epitopes generated a strong immune response. However, all patients responded against at least one epitope with a strong response measurable *ex vivo*. As expected, responses were usually stronger against the neoepitope as compared to the corresponding wild-type peptide. CD4+ and CD8+ T-cell responses were observed against 39% and 31% of the vaccine peptides, respectively. Long-term persistence of the vaccine-induced immune responses was observed after 40 weeks for 85 % of the 34 epitopes tested. In 12 patients, the cytotoxic potential of the T-cell responses was evaluated by measuring IFNγ and CD107a co-expression on CD4+ and CD8+ T cells recognizing 92 peptides. Co-expression was observed for 61% of the peptides tested, with 11 out of 12 patients showing at least one such response. Surprisingly, these responses were mostly CD4 only responses (93%), while 7% involved both CD4+ and CD8+ T cells, and none involved solely CD8+ T cells.

Importantly, epitope spread (Corbiere et al., 2011; Hu et al., 2021) to non-vaccinating epitopes was also investigated and validated. Nine out of 13 (69%) patients had T-cell responses against highly ranked neoepitopes that were not included in the vaccine, only at the post-vaccine timepoint. Interestingly, epitope spread responses were observed toward common driver mutations *KRAS* G12C and *KRAS* G12V across multiple patients, with 3 out of 4 patients who had epitope spread to a KRAS mutation achieving PFS of at least 9 months after initiation of therapy. A fraction (42%) of the responses to spread epitopes were potentially cytotoxic ($CD107a^+$ $IFN\gamma^+$), and all of them were CD4 responses. Single-cell sequencing of neoantigen-specific CD4+ T cells suggested an effector cytotoxic phenotype, in line with a potential role of vaccine-induced CD4+ T cells in mediating tumor-cell killing. Nevertheless, a direct proof of the T-cell cytotoxic activity on tumor cells is required to consolidate this.

Although this study demonstrated the safety and feasibility of NEO-PV-01 in combination with pembrolizumab and chemotherapy as first-line treatment in NSCLC, clinical validation of vaccine efficacy remains to be determined in a randomized trial involving a control arm. Even though clinical responses were observed, most of them started before vaccination and likely resulted from the chemotherapy combined with pembrolizumab. While the combination of NEO-PV-01 with anti-PD-1 was previously shown to be immunogenic (Ott *et al.*, 2020), the current study indicates that addition of chemotherapy does not hamper immunogenicity of NEO-PV-01.

This promising approach faces several challenges. One is the inability to manufacture the vaccine in nearly half of the enrolled patients, because of low amounts of tumor cells in the biopsy or insufficient high-quality neoantigens predicted. Also, some predicted neoantigens were too hydrophobic to be synthesized as long peptides. Another challenge is the difficulty to predict the processing of the candidate epitopes, which may not be processed and presented at the cell surface. This may contribute to the high percentage (45%) of candidate epitopes that failed to generate a T-cell response. Of note, this limitation does not apply to antigens targeted by epitope spreading, which are naturally processed and presented *in vivo*. Last but not least, the poor induction of CD8+ T cells against neoepitopes is likely limiting the anti-tumor efficacy of the vaccine-induced immune responses. Even though neoantigen-prediction algorithms largely focused on MHC I epitopes, most of the vaccine-induced T-cell responses are skewed towards CD4 responses. This was also observed in previous studies (Ott *et al.*, 2017; Ott *et al.*, 2020; Sahin *et al.*, 2017). The reason for this is unclear, but may be attributed to the vaccine platforms used in these studies: synthetic long peptides and mRNA. These vaccine platforms may not be able to induce robust CD8+ T-cell responses as compared to viral vector vaccines (McAuliffe et al., 2021), which however take longer to manufacture. Different vaccine platforms have their pros and cons, and head-to-head comparisons will yield useful information to further advance cancer vaccine development.

In summary, the combination of personalized neoantigen vaccine NEO-PV-01 with pembrolizumab and chemotherapy is safe, practical, and immunogenic, but the anti-tumor efficacy of the vaccine remains to be determined.

DECLARATION OF INTERESTS

C.S.L. and B.J.V.D.E. are inventors on a patent that covers viral vectors and methods for the prevention and treatment of cancer. B.J.V.D.E. is member of the Scientific Advisory Board of Vaccitech.

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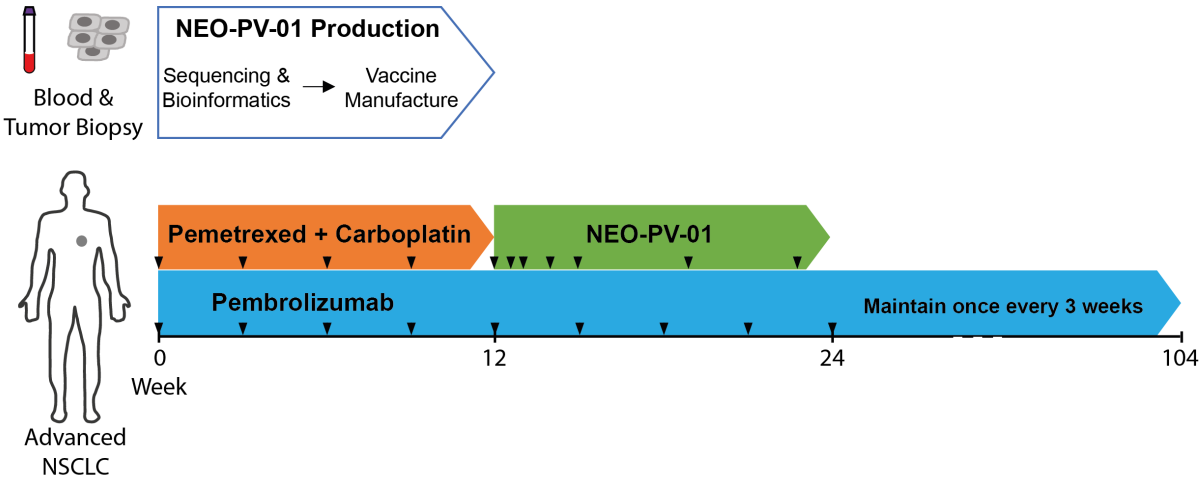

Figure 1. Clinical study design of the personalized neoantigen vaccine NEO-PV-01. Patients with advanced non-squamous NSCLC received the first-line standard-of-care treatment of pembrolizumab and pemetrexed with carboplatin at week 0. The neoantigen vaccine NEO-PV-01 was produced in the first 12 weeks. Patient's tumors and matched normal cells from blood were sequenced, followed by neoepitope prediction and synthesis of the vaccine. Arrow heads indicate time points of the treatments, with pemetrexed and carboplatin administered every 3 weeks for 4 cycles. NEO-PV-01 was administered between weeks 12-24, pembrolizumab was given once every 3 weeks and continued for up to 2 years.