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COMMENTARY

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Beyond checkpoint inhibitors: A new hope of personalized neoantigen vaccines for treatment of renal cell carcinoma

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In a recent study published in *Nature*,[1] Braun and colleagues conducted a surprisingly hilarious phase I clinical trial to evaluate the efficacy of a peptide-based neoantigen personalized cancer vaccine (PCV) in high-risk, surgically resected renal cell carcinoma (RCC) patients, alongside adjuvant ipilimumab. Remarkably, all nine participants elicited tumor-specific T-cell immune responses that targeted key driver mutations in genes like VHL, PBRM1, BAP1, KDM5C, and PIK3CA, inducing robust antitumor immunity (Figure 1), with no recurrences observed during a median follow-up of 40.2 months post-surgery and 34.7 months post-vaccination.

Early therapeutic vaccination approaches primarily target tumor-associated antigens (TAAs)—self-antigens that are aberrantly or overexpressed in tumors. However, this approach is somewhat hindered by the impact of central or peripheral tolerance on T cells specific to TAAs, as well as the risk of autoimmune toxicity due to TAA expression in non-malignant tissues.[2] A superior approach involves exploiting mutations within tumor cells to create novel self-antigen epitopes, called neoantigens. These neoantigens can stimulate de novo T cell responses against tumors while sparing healthy tissues, as they bypass central T cell tolerance due to their origin in somatic mutations.[3] Neoantigens can arise through various tumor-induced mechanisms, including transcriptomic alterations, genomic mutations, viral-encoded open reading frames, and post-translational modifications.[4]

In this study, tumor samples from nine eligible RCC patients harbored an average of 45 high-confidence coding mutations, guiding and administering the synthesis of peptide libraries comprising 8-19 neoantigens per patient. The multiepitope vaccine, consisting of four peptide libraries, induced strong vaccine-specific immunogenicity, primarily targeting multiple epitopes, with the most potent response observed against peptides featuring the prevalent VHL frameshift mutations, all of which yielded 88-1,051 spot-forming units per 10^6 peripheral blood mononuclear cells. Using a combination of single-cell TCR sequencing (scTCR-seq) and bulk TCRβ sequencing, the study precisely tracked 98 vaccine-specific T cell clones that exhibited rapid expansion, with a mean 166-fold increase, and long-term persistence exceeding three years. Next, the study established a three-tiered "prediction-function-presentation" screening system for epitope selection and immunogenicity validation. In the prediction phase, HLAthena and NetMHCpan 4.0 algorithms identified high-affinity HLA-I–binding peptides; 89% of candidate peptides were confirmed as strong or weak binders. During the functional validation, IFNγ ELISpot and flow cytometry assays showed that 65% of driver-mutation–derived peptides, such as those in VHL and PBRM1, elicited multifunctional memory T cells characterized by a $CD45RO^+$ $PD\text{-}1^+$ phenotype. In the presentation phase, epitope-binding affinity measurements and mass spectrometry directly validated eight endogenous retroviruses (ERVs)-derived peptides, thereby confirming effective peptide-HLA complexes for immune presentation. Moreover, single-cell transcriptomics and plasma proteomics revealed robust immune remodeling after vaccination. Skin biopsies revealed a marked increase in infiltrating cytotoxic $CD8^+$ T cells expressing GZMK and PRF1, accompanied by the migration of dermal dendritic cells to draining lymph nodes and sustained elevation of proinflammatory cytokines IL-12 and IL-18 in plasma. Functionally, 77.8% of patients' T cells recognized autologous tumor cells, and VHL mutation peptides mediated a 65±12% killing rate of $CA9^+$ tumor cells. These multiparametric functional assays enabled a comprehensive evaluation of vaccine-induced immunity, while an optimized intradermal-subcutaneous co-delivery with poly-ICLC adjuvant tripled antigen screening efficiency.

This approach's advantages go beyond vaccine design, reflecting a deep integration of tumor biology, particularly driver mutation dependency and immune activation strategies. Tumor-specific driver mutations confer potent immunogenicity; for instance, frameshift VHL mutations in over 90% of clear cell RCC give rise to neo-open reading frame peptides that evade central tolerance and provoke robust antitumor immunity. A multidimensional antigen screening strategy further optimized vaccine quality, with algorithms such as HLAthena and NetMHCpan prioritizing high-affinity HLA class I-binding peptides. Remarkably, vaccine-elicited T cells were predominantly $CD4^+$ (98.7%), exhibiting a memory phenotype ($CD45RO^+PD\text{-}1^+$) and multifunctional cytokine secretion ($IFN\gamma^+$ TNF^+ $IL\text{-}2^+$ accounting for 66%), which support $CD8^+$ T cell activation and dendritic cell maturation—key elements of a systemic anti-tumor immune network. This strategy effectively overcame the traditional limitations associated with low tumor mutation burden (TMB), validating a "less-is-more" approach focused on high-frequency driver mutations and distinguishing itself from melanoma vaccine strategies that rely on high TMB.

Regarding MRD clearance, although circulating tumor cells were not directly assessed, the observed 34.7-month median disease-free survival without recurrence suggests effective MRD eradication. Several mechanisms may underlie this outcome, foremost being the vaccine's ability to rapidly expand effector T cell clones within three weeks and sustain their proliferation for over three years, achieving an average 166-fold increase. $CD4^+$ clones correlated with IFNγ ELISpot responses, indicating sustained recognition and clearance capacity for residual tumor cells. Second, skin biopsies demonstrated pronounced immune remodeling at the injection site, characterized by elevated cytotoxic $CD8^+$ T cells expressing $GZMK^+$ and $PRF1^+$, along with dermal dendritic cell (DC1/DC2) migration to draining lymph nodes, thereby potentially amplifying systemic immune activation via enhanced antigen presentation. This localized immune activation likely contributed to the clearance of micro metastases in circulation or tissues. Third, the vaccine appeared to remodel the immunosuppressive microenvironment by upregulating both pro-angiogenic factors like VEGFA and co-stimulatory molecules such as CD27 and CD28, indicating a dynamic equilibrium of inflammatory signals that helped sustain effector T cell functionality.

Despite its promising implications, the study is limited by suboptimal MRD assessment, as it lacks dynamic tracking of circulating tumor DNA or quantitative analysis of circulating tumor cells. Moreover, the trial's inclusion of only nine patients with clear cell RCC limits both sample size and histological representation, underscoring the need for larger cohorts to validate therapeutic efficacy and assess relevance across non-clear cell RCC subtypes, which comprise approximately 25% of cases. Indeed, the relatively short follow-up period in the clinical component of the study is another significant limitation, as RCC is notorious for its potential to relapse years or even decades after primary resection, and the prognostic importance of the host immune status has been well-documented. Further work should also optimize treatment for unresectable tumors, which often display poor response due to immunosuppressive microenvironments. At this stage, combinatorial strategies integrating vaccines with PD-1 inhibitors, particularly to reinvigorate vaccine-induced $PD\text{-}1^+$ $LAG3^+$ exhausted T cells, or employing epigenetic modulators like DNMT1 inhibitors to augment endogenous retrovirus antigen presentation, may represent compelling therapeutic avenues. Through large-scale trials, dynamic MRD tracking, and rational combination regimens, this approach may ultimately pave the way toward curative treatment for low-TMB tumors.

Building upon the elegant work by the same research group recently published in *Cell*,[5] we have distilled key insights into the proposed therapeutic approaches, while highlighting critical challenges that must be addressed for further development and clinical translation. From a technical perspective, the current study relies on mass spectrometry for peptide identification, but its sensitivity and the sequence homology among ERVs pose challenges in accurately identifying immunodominant antigens. Single-cell sequencing

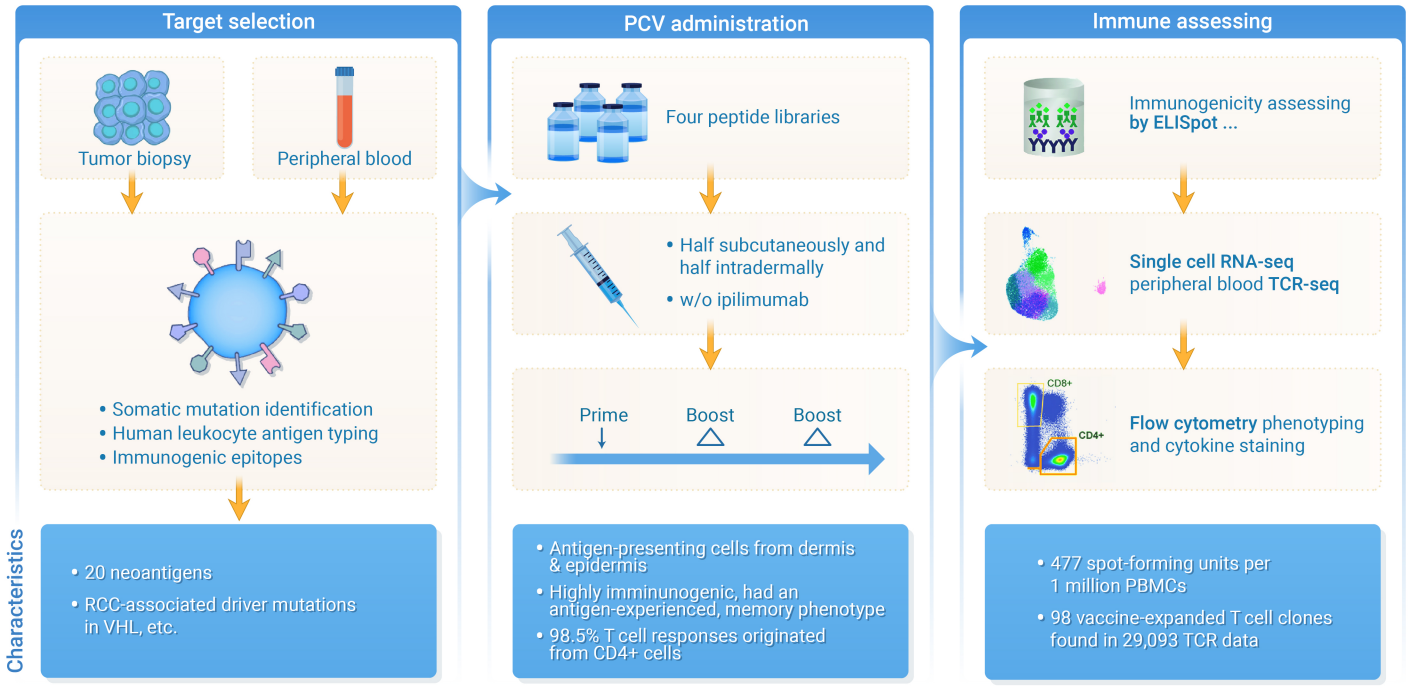

Figure 1. Overview flowchart and key features of neoantigen-based PCV manufacturing, administration, and subsequent immune response evaluation plus clinical outcomes
The vaccine manufacturing process requires careful consideration of several factors, including the number of high-quality coding mutations present in each tumor, the selection and dosage of neoantigen vaccine peptides administered to each patient, and the RCC-specific driver mutations targeted by the vaccine. To rigorously assess vaccine immunogenicity and the targetability of driver mutations, a comprehensive quantitative analysis of measurable immune responses was performed, incorporating endogenous repertoire of expanded T cell, antitumor reactivity, and vaccine-induced changes in skin-infiltrating immune cells. Representative graphic icons were adapted from the displays in Reference 1.

data reveal substantial intratumoral heterogeneity in ERV expression, yet existing analyses only cover a limited range of human leukocyte antigen (HLA) alleles, restricting our capacity to comprehensively capture the diversity within patient populations. Clinically, while T cell responses have been observed in renal cancer cell lines and preclinical models, human trial data are limited, and T cells in patients with advanced cancer are often exhausted, potentially reducing ERV peptide immunogenicity and compromising therapeutic efficacy. The interactions between ERV targets and epigenetic modifiers, such as DNA methylation, also remain incompletely understood, restricting the precise control of ERV induction conditions.

Yet their broad clinical application is constrained by key challenges, notably the time-consuming multi-omics integration required for neoantigen identification, which typically takes 3 weeks from sequencing to peptide nomination, along with further delays from the validation of low-frequency mutations and indels. Second, vaccine manufacturing is hampered by stringent GMP requirements, including prolonged timelines for peptide purity and sterility testing, high per-patient costs may exceed $0.2 million, and additional delays associated with personalized design. Third, the applicability of cancer vaccines across tumor types is limited by both mutation burden and HLA diversity, as low-mutational-load tumors such as prostate cancer rely on indel-derived antigens, while rare HLA alleles often lead to high false-negative rates in epitope prediction.

To address these challenges, emerging strategies include using single-cell RNA-seq to enrich tumor cells for quicker mutation validation, developing a "core driver mutation peptide library" to reduce manufacturing costs and accelerate production, and applying machine learning to optimize the HLAthena algorithm for enhanced prediction accuracy of rare HLA alleles. Longer-term, integrating pan-cancer mutation databases with automated production platforms may enable scalable, cost-effective PCV manufacture and facilitate its transition from experimental therapy to standard of care. Other cutting-edge technologies, such as gene editing, nanotechnology and perhaps highly anticipated future artificial intelligence progress, are revolutionizing the design, production and evaluation of PCVs. For example, given the predominance of vaccine-induced $CD4^+$ memory T cells (98.7%), subsequent administration of PD-1 inhibitors such as pembrolizumab may synergize with the primed immune response. This combination, when integrated with anti-angiogenic agents, may effectively reprogram the VEGFA-driven immunosuppressive tumor microenvironment. In approximately 30% of patients with HLA defects, antigen presentation can be enhanced by CRISPR-mediated restoration of HLA-I expression and further optimized by incorporating high-affinity peptides predicted by HLAthena, such as VHL mutant epitopes that elicit robust multifunctional T cell responses.

Future progress may also be driven by integrative multi-omics and personalized vaccine design, enabling the construction of patient-specific ERV-HLA pairing databases. Furthermore, combination therapies, such as pairing ERV-stabilizing agents with DNA methyltransferase inhibitors or immune checkpoint blockers, could boost ERV expression and enhance T cell recruitment. These epigenetic-based combination strategies could form the basis for "epigenetic reprogramming combined with ERV activation" therapies to enhance tumor-specific antigen presentation. Additionally, the development of advanced antigen screening platforms could expedite peptide discovery, with techniques like precision run-on sequencing and polysome profiling analyzing ERV transcription and translation, while machine learning models enhance HLA-binding peptide predictions. Recent findings suggest that hypoxia-inducible factor (HIF) agonists can broadly induce ERV expression across cancer types, offering a potentially universal target for these therapeutics.

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DECLARATION OF INTERESTS

The authors declare no competing interests.