

COMMENTARY

A modular protein approach to next-generation *in situ* cancer vaccines: A commentary

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Abstract

Cancer immunotherapy has revolutionized *in situ* vaccination approaches that leverage endogenous tumor antigens to initiate immune responses. This commentary examines the protease-activated PSTAGylated *in situ* tumor vaccine (PPTV), a modular metalloprotein platform engineered for precise co-delivery of mitochondria-disrupting peptides and manganese ions. Utilizing ferritin as a biodegradable chassis, PPTV achieves tumor-specific activation through matrix metalloproteinase 14 cleavage, inducing immunogenic cell death and activating the stimulator of interferon genes pathway. Preclinical models demonstrate potent antitumor immunity, with combination therapy eradicating established tumors. The integration of patient-derived organoids is proposed to personalize immunotherapy, highlighting the translational potential of biomaterial-based vaccines for overcoming present limitations in cancer treatment.

Keywords: Cancer immunotherapy; Metalloprotein; Immunogenic cell death; Stimulator of interferon genes pathway; Patient-derived organoids

1. Introduction

The landscape of cancer immunotherapy has been fundamentally reshaped by strategies that harness the host's immune system to combat malignancies. Over the past decade, immune checkpoint inhibitors have revolutionized oncology by reactivating antitumor immunity; however, their efficacy remains limited to a subset of patients, particularly those with pre-existing immune infiltration.¹ This limitation has spurred intense interest in alternative approaches that can convert immunologically "cold" tumors into "hot" microenvironments amenable to immune attack. Among these approaches, *in situ* vaccination—which utilizes tumor antigens released from dying cancer cells to initiate specific immune responses—offers a promising

avenue.² However, the clinical translation of such strategies has been hampered by challenges in achieving precise spatiotemporal control over immunogenic cell death (ICD) and immune activation while minimizing systemic toxicity.³ The ideal *in situ* vaccine platform must overcome multiple biological barriers, such as efficient tumor targeting, controlled release of immunomodulators, induction of robust ICD, and activation of sustained antitumor immunity. In a significant advance published in *Science Translational Medicine*, Liu and colleagues introduce an innovative modular metalloprotein platform, the protease-activated PSTAGylated *in situ* tumor vaccine (PPTV),⁴ which addresses these challenges through sophisticated biomaterial engineering (Figure 1). Hence,

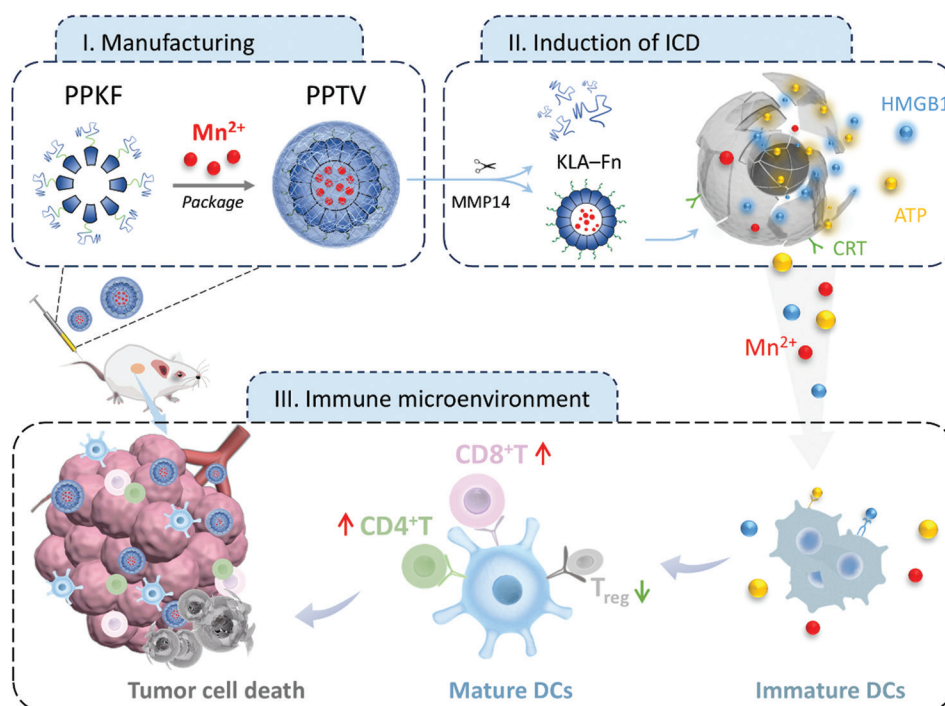


Figure 1. The schematic diagram illustrates the function of a metalloprotein-based *in situ* tumor vaccine. Image created by the authors using Microsoft Power Point-Office 365.

Abbreviations: ATP: Adenosine triphosphate; CRT: Calreticulin; DC: Dendritic cell; HMGB1: High mobility group box 1; MMP: Matrix metalloproteinase; PPKF: Plasmids encoding PSTAG-KLA-ferritin fusion protein; PPTV: Protease-activated PSTAGylated *in situ* tumor vaccine.

this commentary examines the innovative aspects of the PPTV platform, discusses its translational implications, and explores how emerging organoid technology could further accelerate the development and personalization of such immunotherapeutic approaches.

Across the DNA recombination technique, PSTAG (a masking sequence) and KLA (a mitochondria-disrupting peptide) were introduced into the N-terminal of wild-type ferritin to construct a series of plasmids encoding PSTAG-KLA-ferritin fusion proteins containing different lengths of PSTAG masking sequences. After prokaryotic expression and purification, PPTV was obtained by loading manganese ions, a potent enhancer of the stimulator of interferon genes (STING) pathway. PPTV exerts potent anti-tumor efficacy after intravenous injection. Tumor cells undergo ICD and STING pathways activation, thus mediating dendritic cell maturation and T cell activation for systemic immune responses.

2. Technical description

Developing effective *in situ* vaccines requires careful consideration of both ICD inducers and their delivery vehicles. Conventional chemotherapeutic agents, such as anthracyclines and oxaliplatin can induce ICD but often cause significant off-target toxicity, limiting their therapeutic index.⁵ Similarly, while pattern recognition receptor agonists, such as STING ligands show potent immunostimulatory

activity, their poor pharmacokinetics and dose-limiting toxicities have hampered clinical development.⁶ Nanocarrier-based approaches offer potential solutions through improved biodistribution and targeted delivery, yet they face their own challenges, including premature drug leakage, non-specific uptake by the reticuloendothelial system, and potential immunogenicity.⁷

The PPTV platform exemplifies the power of rational design in overcoming these limitations through biomaterial science. By utilizing ferritin as a structural chassis, the researchers leverage its natural cage-like architecture for dual functionality: Encapsulation of manganese ions within the cavity and display of mitochondria-disrupting peptides (KLA) on the surface.⁴ This core design is further refined through the incorporation of a protease-cleavable PSTAG polypeptide shield,⁸ which enables tumor-specific activation through matrix metalloproteinase-14 (MMP14)—an enzyme frequently overexpressed in the tumor microenvironment. This masking strategy represents a critical innovation, as it simultaneously addresses multiple limitations of conventional nanocarriers. It reduces non-specific uptake by the reticuloendothelial system, minimizes liver sequestration, and prevents premature drug leakage.^{8,9}

The choice of ferritin as a delivery vehicle is particularly ingenious given its natural biological properties. As an endogenous iron-storage protein, ferritin exhibits inherent

tumor tropism through binding to transferrin receptor 1, which is frequently overexpressed on cancer cells.¹⁰ While transferrin receptor 1 expression is not exclusive to malignancies, the PPTV platform incorporates a critical additional layer of specificity. The protease-cleavable PSTAG shield ensures that the immunostimulatory activity is preferentially unleashed within the tumor microenvironment, where MMP14 is highly active, thereby mitigating potential off-target effects in normal proliferative tissues. This combination of natural targeting and engineered activation avoids the need for complex surface modifications required by synthetic nanoparticles while enhancing tumor selectivity.¹¹ Furthermore, ferritin's symmetrical cage structure provides a defined cavity for drug encapsulation while offering precise spatial control over surface modifications.¹²

3. Key findings

The PPTV platform demonstrates remarkable efficacy across multiple tumor models, including subcutaneous and orthotopic breast cancer. Administration of PPTV significantly suppressed primary tumor growth and metastatic progression.⁴ Notably, in preclinical murine models, the combination therapy with anti-programmed death-ligand 1 antibodies resulted in complete tumor eradication and established durable immune memory, protecting mice from subsequent tumor rechallenge. These results highlight the platform's ability to transform immunologically "cold" tumors into "hot" microenvironments—a crucial advancement given the limited efficacy of immune checkpoint inhibitors in non-inflamed tumors.¹³ The synergistic combination of KLA-mediated mitochondrial disruption and manganese ion-potentiated STING pathway activation creates a potent immunogenic cascade that promotes dendritic cell maturation, enhances antigen presentation, and facilitates robust cytotoxic T-cell infiltration. Mechanistic studies demonstrated that both components (KLA peptide and manganese ions) are essential for optimal therapeutic outcomes, with manganese ions alone being insufficient to elicit robust adaptive immune responses. This finding underscores the importance of coordinated ICD induction and innate immune activation for generating effective antitumor immunity.

Importantly, the platform's biocompatibility is ensured through the use of biodegradable polypeptide components, while its manufacturability is enhanced by the ability to produce the core construct efficiently in prokaryotic expression systems. The incorporation of manganese ions addresses a critical gap in present immunotherapy approaches. While STING agonists have shown promise in preclinical models, their clinical translation has been challenging due to stability issues and suboptimal pharmacokinetics.¹⁴ It is noteworthy that the utility of

manganese ions as an immunostimulant must be balanced against their potential for neurotoxicity at high systemic doses, a concern documented in other contexts (e.g., occupational exposure).¹⁵ The PPTV platform's tumor-targeting design is crucial here, as it localizes manganese ion delivery to the tumor microenvironment, thereby minimizing systemic exposure and potential off-target effects. Manganese ions represent a natural potentiator of the STING pathway that not only enhances STING activity but also promotes T-cell function and memory formation.¹⁶ It is important to emphasize that these promising results, including complete tumor regression and long-term protective immunity, were observed in murine models. While these preclinical data robustly support the therapeutic potential of the PPTV platform, their translation to human patients warrants future clinical investigation.

4. Limitations and future potential applications

While the present study provides robust proof-of-concept, the integration of organoid technology could substantially enhance the translational pathway of the PPTV platform and similar immunotherapeutic approaches. It is, however, critical to acknowledge the inherent limitations of personalized tumor vaccine strategies in both scientific research and clinical translation. Scientifically, a primary challenge lies in the faithful recapitulation of an individual's tumor immune microenvironment *in vitro*. The tumor immune microenvironment is a complex ecosystem comprising diverse immune cells, stromal components, and vascular networks, all of which influence immunotherapy response. Many present pre-clinical models fail to capture this heterogeneity, limiting their predictive value.¹⁷ Furthermore, the high cost and extended timeframes required to develop autologous therapies pose significant barriers to widespread application. From a clinical translational perspective, personalized vaccines face substantial hurdles in manufacturing and regulation. Establishing scalable and robust Good Manufacturing Practice-compliant processes for patient-specific biologics is immensely challenging.¹⁸ Clinical trial design is also complicated by the inherent variability of personalized products, making it challenging to define standardized endpoints and recruit large, homogeneous patient cohorts. In addition, identifying reliable biomarkers to select patients who will benefit most from a specific personalized vaccine remains an unmet need. In this context, patient-derived organoids (PDOs) have emerged as powerful tools that faithfully recapitulate the histopathological architecture, genetic diversity, and functional heterogeneity of original tumors.¹⁹ Their application in evaluating similar immunotherapeutic approaches could address several critical aspects of therapeutic development. First,

PDOs could serve as predictive biomarkers for treatment response. This concept is powerfully supported by studies, such as Neal *et al.*,²⁰ where PDOs from metastatic gastrointestinal cancer patients were used to predict clinical response to chemotherapeutics, demonstrating a striking 100% positive predictive value and 93% negative predictive value.¹⁹ Applying this paradigm to PPTV, researchers could use PDOs to identify patient subgroups most likely to benefit from protease-activated therapy based on MMP14 expression levels, tumor mutational burden, and immune cell infiltration patterns. The establishment of biobanks comprising organoids from various cancer types and molecular subtypes would enable high-throughput screening of efficacy across diverse genetic backgrounds, potentially revealing novel biomarkers of response beyond MMP14 expression.

Moreover, the development of immune-organoid co-culture systems represents a fascinating frontier for evaluating cancer immunotherapies. As pioneered by Li *et al.*,²¹ these complex models, which incorporate autologous immune cells alongside tumor organoids, can provide unprecedented insights into the dynamic immune-tumor interactions triggered by treatment.²⁰ For instance, the protease-dependent activation of PPTV can be precisely validated using genetically engineered organoids with tunable MMP14 expression. Researchers could knock down or overexpress *MMP14* in PDOs to directly correlate the extent of PSTAG shield cleavage with the subsequent induction of ICD markers and STING pathway activation. This would robustly confirm the tumor-specificity of the platform in a human-relevant context. Furthermore, the modular nature of PPTV, which combines mitochondrial disruption and innate immune activation, makes it an ideal candidate for deconstruction analysis in organoid models. By treating PDOs with the individual components or the fully assembled PPTV, one can dissect their relative contributions to the overall immunogenic cascade. This approach could quantify how KLA-mediated mitochondrial damage synergizes with manganese ion-driven cyclic guanosine monophosphate-adenosine monophosphate synthase-STING signaling to promote dendritic cell maturation and T-cell activation within the co-culture system. Such experiments would not only validate the core design principle of PPTV but also help identify biomarkers of response, such as specific patterns of cytokine release or immune cell infiltration, which could predict patient sensitivity to this therapy. The integration of air-liquid interface systems could further enhance these models by incorporating stromal components and physiological tissue barriers,²¹ and enabling real-time monitoring of PPTV penetration, activation kinetics, and the resulting tumor cell killing dynamics. Such advanced models would be especially valuable for studying combination therapies,

allowing researchers to identify optimal sequencing and dosing of immunomodulatory agents while minimizing the need for animal testing.

The potential applications of organoid technology extend beyond basic research to clinical trial design and personalized therapy development. PDOs established from patients enrolled in clinical trials could serve as platforms for drug testing, helping to decipher mechanisms of response and resistance.²² In the context of cancer immunotherapy, organoids derived from pre- and post-treatment biopsies could reveal how the tumor immune microenvironment evolves under therapeutic pressure, identifying adaptive resistance mechanisms that could be targeted in subsequent treatment lines.²³ Looking further ahead, the integration of PDOs with microfluidic systems and functional imaging technologies could enable real-time monitoring of immune cell trafficking and activation, providing dynamic readouts of therapeutic efficacy that complement traditional endpoint measurements.²⁴

Despite these promising directions, significant challenges remain in fully realizing the potential of organoid models for immunotherapy development. Present limitations include the variable representation of immune populations in standard organoid cultures, the difficulty in maintaining functional immune cells over extended time periods, and the need for standardized protocols across research centers.²⁵ However, recent advances in organoid generation—such as the incorporation of endogenous immune cells through specific culture conditions or the reconstruction of immune niches through co-culture with peripheral blood lymphocytes—are rapidly addressing these limitations.²⁶ As these technologies mature, they are poised to become indispensable tools for bridging the gap between preclinical discovery and clinical application.

5. Conclusion

The PPTV platform represents a significant advancement in biomaterial-based cancer vaccines, demonstrating how rational design can yield multifunctional systems capable of orchestrating complex immune responses. The integration of organoid technology into the development pipeline of such innovative therapies offers exciting opportunities to enhance their predictive accuracy, accelerate clinical translation, and ultimately enable more personalized approaches to cancer immunotherapy. As we stand at the intersection of biomaterial engineering, immunology, and organoid biology, the convergence of these fields promises to unlock new paradigms for therapeutic development. Future work should focus on establishing robust organoid-based screening platforms that can capture the complexity of human tumor-immune interactions, validate biomarker strategies for patient selection, and explore combination therapies

that leverage the unique strengths of the PPTV platform. Through continued innovation and interdisciplinary collaboration, we move closer to realizing the full potential of *in situ* vaccination strategies for cancer treatment.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

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Availability of data

Not applicable.

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