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Commentary

Integrative strategies combining oncolytic adenovirus, anti-PD-1, and chemotherapy in a 3D TNBC model

Mariangela Garofalo[1,2], Lukasz Kuryk[1*]

[1]National Institute of Public Health NIH – NRI, Warsaw, Poland

[2]University of Padova, Padova, Italy

*Author for correspondence:
Email: lkuryk@pzh.gov.pl

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Commentary

Triple-negative breast cancer (TNBC) is one of the most aggressive and treatment-resistant subtypes of breast cancer and is characterized by a lack of expression of estrogen receptors, progesterone receptors, and HER2, making targeted therapies ineffective [1,2]. The limited treatment options for TNBC primarily rely on chemotherapy, and while the addition of immune checkpoint inhibitors (ICIs), such as anti-PD-1 antibodies, has shown promise in other cancers, the response in TNBC has been modest [3,4]. This publication by Kuryk *et al.* investigates the potential of an innovative combinatorial strategy using oncolytic adenovirus therapy followed by anti-PD-1 immunotherapy and paclitaxel (PTX) chemotherapy in three-dimensional (3D) models of TNBC. This study presents preclinical data that show that this sequenced approach enhances antitumor efficacy, offering significant insights into how such therapies can be integrated for maximum clinical benefit [5].

The authors used a modified oncolytic adenovirus (AdV5/3-D24-ICOSL-CD40L) that selectively infects and kills tumor cells [6,7]. In the context of this study, the virus was engineered to express immune-stimulatory molecules such as ICOSL and CD40L, both of which can activate immune responses within the tumor microenvironment (TME). The team employed both two-dimensional (2D) and 3D coculture systems, using human TNBC cell lines (MDA-MB-231, MDA-MB-468) alongside human peripheral blood mononuclear cells (PBMCs). This allowed them to examine how sequential therapy—oncolytic adenovirus first, followed by anti-PD-1 therapy (pembrolizumab), and finally PTX chemotherapy—impacts tumor growth and immune cell infiltration. The results revealed that the sequential combination produced the most significant reduction in the tumor spheroid volume compared with the monotherapies or unscheduled combinations of the treatments [5].

These findings are promising, especially in the context of TNBC, where standard chemotherapy and immunotherapy often fail to produce durable responses [4,8,9]. The authors provide evidence that priming with the oncolytic adenovirus induced substantial infiltration of immune cells, particularly CD8+ and CD4+ T cells, which are crucial for tumor recognition and elimination. Furthermore, enhanced T-cell infiltration correlated with improved tumor suppression, suggesting that the immune response plays a major role in the efficacy of the treatment, beyond the direct oncolytic effects of the virus or the cytotoxic effects of PTX. This combination appears to be synergistic, as priming the immune system with oncolytic virotherapy made the tumors more responsive to subsequent anti-PD-1 blockade and chemotherapy, resulting in greater tumor control.

One of the key strengths of this study is the sequencing of treatments. The authors demonstrated that the timing of administration is critical for optimizing therapeutic outcomes. When oncolytic adenoviruses are administered first, they are likely to "inflamed" the tumor microenvironment,

making the cancer cells more visible to the immune system. This priming step was followed by anti-PD-1 treatment, which blocked T-cell activity and promoted the expansion of tumor-specific T cells. Finally, PTX, which is known to have immunogenic properties and can alter the TME to support immune cell infiltration, was used to consolidate the antitumor effect. This sequencing approach is an important contribution to the field, as it suggests that the success of combination therapies is not solely dependent on the agents themselves but also on their order of administration.

Oncolytic Virotherapy as an Immune Primer

Oncolytic virotherapy, in which viruses are used to selectively infect and kill tumor cells, is gaining increasing attention in the oncology field. One of the significant advantages of oncolytic viruses, especially in combination with immunotherapy, is their ability to stimulate the immune system. By killing tumor cells, oncolytic viruses release tumor antigens into the TME, which can then activate immune cells such as dendritic cells and T lymphocytes. Moreover, the viral infection itself can induce an immune response, further amplifying the immune-mediated killing of cancer cells [10–12].

The addition of immune-activating transgenes, such as ICOSL and CD40L, to the oncolytic adenovirus in this study appeared to have enhanced the immune response. ICOSL is a ligand for the ICOS receptor on T cells and plays a critical role in T-cell activation and survival. CD40L is known to stimulate dendritic cells and other antigen-presenting cells, increasing their ability to present tumor antigens and activate T cells. This combination of viral oncolysis and immune priming is a novel and promising approach that could improve the efficacy of immune checkpoint inhibitors such as anti-PD-1 therapy [6,12,13] .

Immune Checkpoint Blockade in Combination

This study also highlights the role of immune checkpoint blockade in potentiating the antitumor immune response. The authors used pembrolizumab, an anti-PD-1 antibody, to release the immune system's brakes on T cells. PD-1 is an inhibitory receptor expressed on activated T cells, and its interaction with its ligand PD-L1, which is often upregulated in tumors, leads to T-cell exhaustion and immune evasion. Blocking the PD-1/PD-L1 interaction with an anti-PD-1 antibody restores T-cell function, allowing T cells to more effectively recognize and kill tumor cells.

The results from this study suggest that the timing of anti-PD-1 therapy, when given after oncolytic virus therapy, plays a pivotal role in improving therapeutic outcomes. This finding aligns with growing evidence from other studies showing that priming the immune system with viral therapies enhances the effectiveness of immune checkpoint blockade. This study also supports the idea that combination strategies involving both viral oncolysis and ICIs could be particularly effective in cancers such as TNBC, which are often resistant to monotherapies. Collectively, these observations underscore the necessity of developing novel, rationally designed combination therapies to overcome the multifaceted challenges of cancer treatment [14,15] .

Chemotherapy and Tumor Microenvironment Modulation

In addition to immune checkpoint blockade, chemotherapy plays a critical role in this sequenced approach. PTX, a taxane commonly used in TNBC treatment, is known for its ability to disrupt microtubule dynamics, leading to cell cycle arrest and apoptosis. PTX can also influence the TME by inducing immunogenic cell death (ICD), a form of programmed cell death that activates the immune system by releasing damage-associated molecular patterns (DAMPs). PTX has been shown to improve the infiltration of immune cells into tumors and enhance the effects of immunotherapies, particularly when it is administered after immune priming agents such as oncolytic viruses.

The use of PTX in this study likely serves two purposes: first, as a cytotoxic agent to kill residual tumor cells and second, as an immunomodulatory agent to enhance immune cell infiltration. The combination of PTX with both oncolytic virotherapy and anti-PD-1 therapy can potentially create a multipronged attack on the tumor—direct cytotoxicity, immune activation, and immune checkpoint modulation—that is more effective than any single therapy alone.

Strengths of the Study: *In vitro* 3D Models

The authors' use of 3D tumor models is a major strength of this study. Unlike traditional 2D monolayer cell cultures, which fail to mimic the complex architecture and microenvironment of solid tumors, 3D models better represent the physical, chemical, and immunological interactions between tumor cells and the surrounding stroma. By incorporating PBMCs into the 3D culture system, the authors were able to model immune responses more accurately, providing more clinically relevant insights into how therapies affect the tumor and its immune microenvironment.

These 3D coculture models also allowed the authors to assess not only the direct antitumor effects of the treatment but also immune cell infiltration and immune-mediated tumor suppression. Given that immune evasion is a hallmark of TNBC, studies focused on immune cell activity are particularly relevant for improving treatment strategies.

Limitations and Future Directions

While the study provides strong preclinical evidence for the effectiveness of combinatorial therapy, there are several limitations that need to be addressed in future research. First, the study relies heavily on *in vitro* models, which cannot fully replicate the complexities of an *in vivo* tumor microenvironment. *In vivo* validation in immunocompetent mouse models is essential to confirm the findings and assess the safety and efficacy of combinatorial treatment.

Second, the timing and dosing schedules of the various treatments need to be further optimized. While the study provides valuable insight into the sequencing of therapies, the ideal interval between viral infection, PD-1 blockade, and chemotherapy remains to be determined. These factors could have a significant impact on the therapeutic outcome and should be explored in future studies. Nevertheless, the presented approach has the potential to predict treatment response to the therapy since the response rates can fluctuate among cancers and within cohorts with the same malignancy.

Lastly, while the study demonstrates significant antitumor efficacy in TNBC cell lines, it is unclear whether these results will translate to clinical efficacy in patients. Although the enhanced efficacy of this multi-modal combination is encouraging, it is conceivable that combining oncolytic virotherapy, immune checkpoint blockade, and chemotherapy could introduce safety considerations. For example, the immune activation following viral priming and PD-1 blockade

might theoretically increase inflammatory or immune-related adverse effects, while subsequent chemotherapy could influence immune cell dynamics or viral persistence. These possibilities remain speculative but underscore the importance of careful evaluation of dosing, sequencing, and tolerability as such strategies are explored in more complex preclinical models and, ultimately, in clinical settings. Therefore, clinical trials will be necessary to determine the safety, tolerability, and effectiveness of this combinatorial approach in humans.

Conclusions

In conclusion, the study by Kuryk *et al.* presents a preclinical demonstration of the potential of combining oncolytic adenovirus therapy, anti-PD-1 immunotherapy, and chemotherapy in TNBC. By sequencing these therapies, the authors have shown that the combination can induce robust antitumor immunity and significantly reduce tumor growth. This work contributes to the growing body of evidence supporting the use of rationally sequenced combination therapies to overcome the immune evasion mechanisms that are often at play in solid tumors. Further validation *in vivo* and eventual clinical trials will be necessary to determine the clinical utility of this approach, but the study findings offer an exciting glimpse into the future of TNBC treatment.

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