UJEMI PEARLS

Oncolytic Newcastle disease virus can enhance anti-PD-1 checkpoint blockade for the treatment of muscle-invasive bladder cancer

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SUMMARY Urothelial carcinoma (UC) is the most frequent malignancy of the urinary tract and the tenth most common cancer worldwide, resulting in almost 200,000 deaths every year. Muscle-invasive bladder cancer (MIBC) is an advanced stage of bladder cancer that affects 1 in 4 people diagnosed with UC. The standard treatment for MIBC is radical cystectomy, resulting in removal of the entire bladder and often the prostate in men and the ovaries in women. Only a small fraction of MIBC patients are eligible for bladder preservation therapy, consisting of a trimodal treatment with tumor resection, chemotherapy, and radiotherapy, but the efficacy remains low. There are several immunotherapy clinical trials for the treatment of MIBC, including checkpoint inhibitors as a monotherapy and in combination with chemotherapy, but durable response rates remain low. Despite preclinical data showing the efficacy of anti-programmed cell death protein-1 (anti-PD-1) and anti-programmed death-ligand 1 (anti-PD-L1) antibodies in inducing antitumor immune responses, 70-80% of patients remain unresponsive to immune checkpoint inhibition due to the immunosuppressive tumor microenvironment (TME). Currently, oncolytic virotherapy is being explored as a therapeutic for MIBC, specifically using Newcastle disease to mediate tumor cell lysis and activate tumor-specific immune responses. This article will explore the synergistic combination of oncolytic virotherapy with immune checkpoint blockade for the treatment of MIBC. Preclinical and clinical data have shown that increased immune cell infiltration and cytokine influx as a result of oncolytic virotherapy primes the TME for subsequent immune inhibitory checkpoint blockade. Other studies have shown that extensive tumor cell lysis and virus replication associated with oncolytic virotherapy is not necessary for immunotherapeutic efficacy when used in combination with checkpoint blockade. Therefore, understanding how the local TME changes after oncolytic virotherapy will allow us to exploit its immunomodulating effects and develop combination therapies for MIBC regardless of pre-existing immunity to oncolytic viruses.

INTRODUCTION

Bladder cancer, or urothelial carcinoma (UC), is the most frequent malignancy of the urinary tract and the tenth most common cancer worldwide [1-3]. In 2018, there were over 549,000 new cases of UC, resulting in almost 200,000 deaths worldwide [1-3]. Bladder cancer is four times more common in men than in women, making it the sixth most common cancer in men [1]. The main risk factors for bladder cancer are age, smoking, and occupational exposure to chemical and water contaminants [1]. The incidence rate of UC increases with age, and the median age at the time of diagnosis is 73 years [2].

Bladder cancers are classified into three groups: non-muscle invasive, muscle invasive, and metastatic [2]. Non-muscle invasive bladder cancer (NMIBC) is early stage UC, where the tumor has not grown beyond the cells lining the bladder or urinary tract [2]. NMIBC includes carcinoma *in situ* (CIS), tumors in the mucosa (Ta), and tumors in the submucosa or lamina propria (T1) [2]. One in four UC patients present with muscle invasive bladder

Published Online: September 2020

Citation: Faith Cheung. 2020. Oncolytic Newcastle disease virus can enhance anti-PD-1 checkpoint blockade for the treatment of muscle-invasive bladder cancer. UJEMI PEARLS 4:1-8

Editor: François Jean, University of British Columbia

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cancer (MIBC), ranging from stages T2 to T4 with increasing invasion of the tumor into the muscle layer of the bladder wall [2]. Stage T4 UC is metastatic bladder cancer, in which the tumor cells have spread from the bladder to other parts of the body, commonly the lymph nodes, liver, lungs, and bone [2]. The survival rate of patients with NMIBC is notably higher compared to that of MIBC [2]. The relative survival at 5 years after diagnosis is 55% for stage T2 UC compared to 16% for stage T4 [2]. Thus, early diagnosis and treatment are essential for preventing metastasis and cancer-related mortality.

Currently, there are no routine screening procedures for UC, and most patients are not diagnosed until symptoms appear such as blood in the urine [2]. The current standard treatment for UC is radical cystectomy (RC), which is the surgical removal of the entire bladder [2, 3]. As a consequence, the urinary stream is diverted into a conduit that connects to the outside of the abdominal wall, necessitating the use of a draining pouch or a urinary reservoir [4]. In males, RC also involves removal of the prostate and seminal vesicles, and in females the ovaries, uterus, and parts of the vagina [4]. Therefore, while the prognostic outcomes following RC are positive, the procedure involves significant physical and psychological burdens, such as loss of functional independence, sexual dysfunction, and body image issues [4].

For a small subset of UC patients, a bladder-preserving treatment known as trimodal therapy (TMT) is a viable alternative option [2, 3]. TMT involves maximal transurethral resection of the bladder tumor, followed by aggressive chemotherapy and radiotherapy [3]. Patients with multifocal tumors, hydronephrosis, palpable mass upon examination, or insufficient tumor margins are ineligible for TMT [3]. However, while TMT is able to preserve the bladder, the local recurrence-free survival of patients is 35%, compared to 74% of RC patients [5]. The recurrence rate after TMT is as high as 40%, with a median time to recurrence of only two years [5]. Therefore, there is an unmet need to broaden bladder preserving treatment options for UC patients, especially for those with advanced MIBC associated with high relapse rate and metastatic potential.

This article will explore emerging therapies for MIBC, namely immune checkpoint blockade and oncolytic virotherapy. It will discuss the current state of research and the limitations, and how combination therapy can potentially overcome the drawbacks of each monotherapy. There are several immunotherapy agents in the clinical trial phase for the treatment of MIBC, as both monotherapies and in combination with chemotherapy or radiotherapy [2, 6]. However, durable response rates remain low and 70-80% of patients remain unresponsive to immune checkpoint inhibition despite pre-clinical data showing the efficacy of anti-PD-1 and anti-PD-L1 antibodies [5, 6]. Oncolytic virotherapy is also being explored as a therapeutic for MIBC that uses replication-competent viruses to mediate tumor cell lysis and activate tumor-specific immune responses [7]. Infection by oncolytic viruses (OVs) increases immune cell infiltration and cytokine influx, priming the tumor microenvironment (TME) for subsequent therapy [7]. Understanding how the local TME changes after oncolytic virotherapy is essential for exploiting its immunomodulating effects to develop combination therapies for MIBC. Developing better bladder preservation therapies for MIBC would not only improve disease prognosis but also improve the quality of life for patients after treatment.

RESEARCH QUESTIONS

In order to understand more about the biology of MIBC and how better treatment options can be developed, a few concerns must be addressed. Firstly, immune checkpoint blockade is a relatively new concept compared to chemotherapy and radiotherapy for MIBC. It is critical to look at what has been done to develop immune checkpoint blockades for bladder cancer and use the results of past research to improve this therapy. Second, a promising new therapy for MIBC is the use of OVs to target bladder cancer cells. In the context of MIBC, there are many different viruses being tested in the pre-clinical and early clinical phases. Understanding the biology of the virus, and also the effect of the virus on the tumor and TME, is critical for engineering the right OV that is both safe and effective.

Once this is understood, oncolytic virotherapy and immune checkpoint blockade can be combined synergistically to further improve therapeutic outcomes for MIBC patients. Because there are limits to what either monotherapy can achieve, combination therapy can further enhance the antitumor efficacy. In this article, oncolytic virotherapy will be examined as a synergistic partner for immune checkpoint blockade as a treatment for MIBC.

PROJECT NARRATIVE

How is immune checkpoint blockade being used for the treatment of MIBC and what are the limitations? Immune checkpoint blockade is a type of immunotherapy that targets key regulators of the immune system, blocking inhibitory checkpoints to restore the immune response [7, 8]. One of the main types of effector immune cells that is activated during an antitumor immune response is T cells [8]. T cell-mediated immunity involves activation by interaction with antigen-presenting cells (APCs), T cell proliferation, transition, and propagation of effector functions [8]. These processes are regulated by both inhibitory and stimulatory signals [8]. Normally, inhibitory signals function to limit the natural immune response and prevent autoimmunity [8]. One way that cancer cells have evolved to avoid immune detection is by upregulating the expression of immune checkpoint molecules, such as programmed cell death protein 1 (PD-1), programmed death ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [8]. Tumor cells with upregulated PD-L1 bind to PD-1 on the immune cell surface, inhibiting its effector function (Figure 1A) [8]. Therefore, antitumor immunity can be recovered using antibodies that block the interaction of upregulated immune checkpoint molecules on the tumor cell and the inhibited immune cell [8].

Monoclonal antibodies against PD-1 have shown robust antitumor activity in pre-clinical and early clinical trials for MIBC patients. One of the most studied checkpoint inhibitors for MIBC is pembrolizumab, an anti-PD-1 antibody that is in currently being tested in a Phase III clinical trial for recurring or progressive MIBC after chemotherapy (NCT00256436) [9]. Pembrolizumab is a highly selective, humanized monoclonal IgG4 antibody that binds to PD-1, disrupting its interaction with PD-L1 and restoring effector T cell functions [9]. Pembrolizumab is very well studied in other types of cancers and has

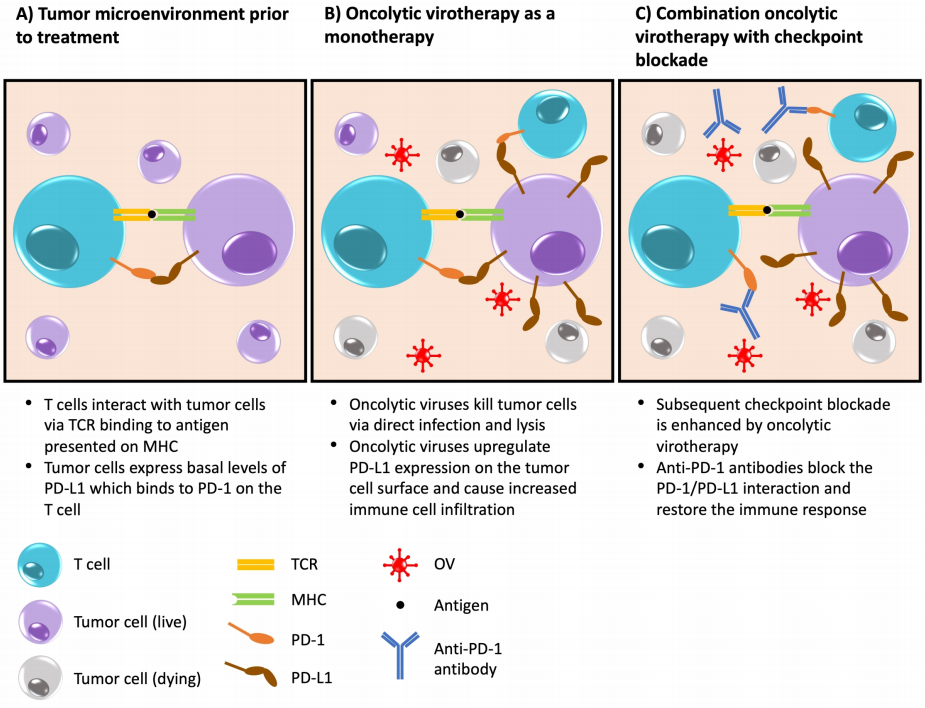

FIG. 1 Combination therapy: oncolytic virotherapy and immune checkpoint blockade. Proposed mechanism of the synergistic combination between oncolytic virotherapy and anti-PD-1 checkpoint blockade. (A) In the tumor microenvironment prior to treatment, tumor cells express basal levels of PD-L1 which binds to PD-1 on the T cell. (B) Oncolytic viruses infect and lyse tumor cells as a direct anti-viral mechanism, and also change the tumor microenvironment, resulting in increased immune cell infiltration and tumor cell PD-L1 expression. (C) Subsequent administration of anti-PD-1 antibodies block the PD-1/PD-L1 interaction, decreasing T cell suppression and restoring the anti-tumor immune response.

received approval from the U.S Food and Drug Administration (FDA) for treatment of metastatic non-small cell lung cancer [10], head and neck squamous cell carcinoma [11], melanoma [12], and Hodgkin lymphoma [13], among others. In phase I clinical trials, pembrolizumab showed anti-tumor activity and acceptable safety among MIBC patients [14], supporting subsequent phase II trials with similar successes [15]. However, the results of the phase III trial show only a 15% response rate [9]. While pembrolizumab improved the overall survival by three months, there was no significant difference in the duration of progression-free survival compared to chemotherapy treatment [9].

Consequently, in 2018, the FDA suspended the use of pembrolizumab as a monotherapy to treat MIBC due to the low expression of PD-L1 and the lack of long-term durable response rates compared to chemotherapy [16]. However, pembrolizumab remains a promising checkpoint inhibitor for MIBC because of its past success stories in other cancers and the mounting evidence for its safety and efficacy in pre-clinical, phase I, and phase II clinical trials. In hopes of increasing the effectiveness of pembrolizumab in MIBC, there is a need to upregulate intratumoral T cell infiltration and increase PD-L1 expression on the tumor cell surface.

How can oncolytic virotherapy be used for the treatment of MIBC and which virus should be selected?Oncolytic virotherapy is an emerging cancer treatment that uses oncolytic viruses (OVs) to destroy tumor cells through multiple anti-tumor mechanisms [17, 18]. As part of their lytic life cycle, OVs can infect, replicate, and lyse the tumor cell, thereby killing the tumor cell and releasing viral progeny [17, 18]. Each OV has a specific cellular tropism that determines the cell types it can infect, based on its surface glycoproteins and the corresponding receptors on the host cell [18]. Furthermore, OVs are engineered to be tumor cell-specific by deleting genes that are needed for viral replication and trans-complemented by tumor-specific genes [17, 18]. OVs promote an immunogenic response upon tumor cell lysis through the release of tumor-specific antigens such as pathogen-associated molecular patterns and damage-associated molecular patterns, which are important triggers for initiating the innate and adaptive immune responses [17, 18]. Therefore, OVs can kill tumor cells through direct virus-mediated cytotoxicity as well as through cytotoxic immune effector mechanisms (Figure 1B) [17, 18].

There are many different OVs being developed for the treatment of cancer, and one promising candidate for MIBC is the Newcastle disease virus (NDV). NDV is an avian paramyxovirus in the *Rubulavirus* genus of the family *Paramyxoviridae* [20]. NDV is a negative stranded RNA virus with a relatively simple 15.2 kb non-segmented genome organized into six genes that encode six structural and two non-structural proteins [19]. RNA viruses have been shown to be more effective than DNA viruses at triggering the immune response because of the formation of double-stranded RNA during their life cycle, which activates type I interferons and downstream cellular defense mechanisms [20]. NDV is a good candidate for oncolytic virotherapy because it is not linked to any known human disease [20]. While NDV is transmissible to humans, infection results in only mild conjunctivitis and influenza-like symptoms [20]. Thus, it is widely accepted to be nontoxic in humans and safe to use as a form of cancer therapy.

One of the potential limitations of oncolytic virotherapy is preexisting immunity to the viral vector, either due to previous exposure or through vaccination, resulting in the body eliminating the virus before virus-mediated tumor cell death can take place [17]. However, the general population is seronegative for anti-NDV antibodies and so preexisting anti-viral immunity should not be a limiting factor for NDV [20]. In addition, studies in syngeneic mouse tumor models have shown that vaccinating with NDV prior to using NDV as an OV does not compromise tumor clearance, anti-tumor immune effects, or survival rates [21]. This provides a clinical rationale for using NDV for oncolytic virotherapy.

Recently, *in vitro* studies have investigated the possibility of using non-lytic NDV strains to treat bladder cancer cell lines [7]. Using human bladder cancer cell lines that are resistant to NDV-mediated lysis, one study showed that the immunogenic effects of NDV

are independent of its lytic potential [7]. In these resistant cell lines, there was evidence of immunogenic cell death and activation of the innate and adaptive immune responses, even though NDV was not able to propagate [7]. This is an advantage that is unique to NDV and reduces the risk of off-target viral replication and spread seen in other OVs [17].

In light of this supporting evidence for NDV as an oncolytic virotherapy, *in vivo* mouse model studies have been tested to further assess the safety and efficacy. Mice were injected with NDV lysis-resistant bladder cancer cells and then subsequently treated with NDV therapy [7]. An increase in CD8 and CD4 T cell infiltration into the TME was consistently observed, as well as a trend towards improved survival [7]. However, the percent survival after tumor challenge was shy of statistical significance in the NDV-treated group compared to the PBS control group [7]. Consequently, there was been work done to increase in the efficacy of NDV virotherapy in hopes of understanding the underlying mechanisms at the molecular level in the TME.

How can oncolytic virotherapy alter the tumor microenvironment and enhance immune checkpoint blockade? The most recent developments in MIBC treatments involve combination therapy in hopes of overcoming the limitations faced by monotherapy treatments. In particular, there is evidence supporting the synergistic combination of immune checkpoint blockade and oncolytic virotherapy [22]. Tumors often alter their microenvironment, resulting in a “cold” immunosuppressive TME with poor immune cell infiltration and low expression of PD-L1 by the tumor cells [23]. Conversely, a “hot” TME is one with high immune cell infiltration, especially cytotoxic T lymphocytes, and high levels of chemokines and cytokines that mediate the trafficking of effector cells to the TME [23]. Because immunotherapies have been shown to work better in “hot” TMEs, understanding how OVs change the TME is important in designing combination therapy [22, 23]. Therapeutic administration of OVs triggers strong antiviral immune responses, strategically changing the TME from “cold” to “hot”, which primes the TME for subsequent immunotherapy (Figure 1C) [22, 23].

As previously mentioned, the limiting factor affecting the efficacy of pembrolizumab in MIBC treatment is low PD-L1 expression by the tumor cells [16]. In bladder cancer cell lines, NDV treatment caused an upregulation of PD-L1 on the tumor cell surface, using either lytic or non-lytic NDV strains [7]. This observation provides a rationale for combination therapy of intratumoral NDV with systemic pembrolizumab checkpoint blockade. *In vivo* mouse models have shown that combination therapy with NDV and anti-PD-1 antibodies result in delayed tumor growth and improved overall survival [7]. Thus, NDV virotherapy enhances PD-1 checkpoint blockade and overcomes the limitations seen with both monotherapies. NDV infection results in increased T cell infiltration and PD-L1 expression, which enhances the subsequent action of the anti-PD-1 checkpoint blockade, resulting in decreased effector T cell suppression and a stronger anti-tumor immune response.

The exact mechanism of NDV-induced upregulation of PD-L1 is not fully understood. A current hypothesis suggests a paracrine response to the innate immune stimuli induced by the virus-associated antigens released during the lytic lifecycle [7]. In addition, investigation into the specific viral protein that can trigger these effects is worthwhile for further development of combination therapy. Because of the simple genome of NDV, it may be possible to identify which protein(s) are critical for priming the TME, given that even non-lytic NDV strains are capable of upregulating PD-L1 expression [7, 19]. In conclusion, combination therapy of immune checkpoint blockade and oncolytic virotherapy is a promising field of new therapeutics with the potential to treat solid cancers such as MIBC.

CONCLUSIONS

There is an unmet medical need for better treatments for patients with advanced muscle-invasive bladder cancer. Current bladder-preserving therapies are only available for a selective cohort of patients and the efficacy remains below that of radical cystectomy [5].

Emerging therapies such as immune checkpoint blockade and oncolytic virotherapy show promising pre-clinical and early clinical results, but ultimately fall short as monotherapies [9, 17]. Pembrolizumab is a well-studied anti-PD-1 checkpoint blockade effector that has been shown to be efficacious in other cancer types [10-13]. However, phase III trials in MIBC patients show a low response rate and no improvement in progression-free survival compared to chemotherapy alone [9].

Pembrolizumab can be enhanced by increasing PD-L1 expression on the tumor cell surface. To this effect, oncolytic virotherapy using Newcastle disease virus has been shown to have a synergistic effect, resulting in increased immune cell infiltration and tumor expression of PD-L1 [17]. NDV is a promising OV because it is not linked to any known human disease, the general population is seronegative for anti-NDV antibodies, and there is evidence that non-lytic NDV strains are equally as effective in triggering the immune response as the lytic strains [7, 20]. Therapy using NDV has been tested in bladder cancer cell lines and mouse models, and the combination of both NDV and anti-PD-1 therapy is a promising treatment regime for MIBC.

Elucidating how to balance anti-tumor and anti-viral immune responses will be important in determining the staging of these two therapies, with the ultimate goal of treating MIBC at an earlier stage and prevent development of advanced cancer. This article highlights the rationale for studying patients receiving combination versus monotherapy to determine potential biomarkers that correlate with responders to each treatment type.

This new combination therapy can be thought of as ‘immunovirotherapy’, in which the immunomodulating effects of OVs are used to prime the TME for subsequent immunotherapies. This synergistic effect can be extended to other applications of oncolytic virotherapy. Because OVs can be engineered as delivery vectors, there are many possibilities for combination therapy. OVs can deliver immune-modulating cytokines, immunostimulatory ligands, or agonist antibodies including, but not limited to, checkpoint blockade effectors to the TME [17]. Bladder cancer cell lines have also been shown to upregulate expression of CTLA-4, expanding the possible targets for immune checkpoint blockade [24]. Strategies have been developed use OVs to deliver interleukins (IL) directly to the tumor in order to potentiate the adaptive immune response [25]. Cytokines such as IL-2, IL-12, IL-15, and IL-18 have been proposed to activate T cells, and chemokines like CCL5 are used to increase T cell trafficking [25]. Oncolytic virotherapy can also be combined with epigenetic treatments, namely DNA methyltransferase and histone deacetylase inhibitors, which target genetic and epigenetic alterations identified in MIBC patients [26].

Ultimately, oncolytic virotherapy is a novel and safe form of cancer therapy that can be used in combination with other immunotherapies. OVs can be engineered to be tumor cell-specific and can be used as delivery vectors for other small molecules. With new evidence that the immunomodulatory effects of OVs are not dependent on cell lysis, non-lytic OVs can be developed to reduce possible side effects without compromising efficacy. Oncolytic virotherapy continues open up new frontiers in molecular virology and has undiscovered potential as a cancer therapy.

ACKNOWLEDGEMENTS

The author would like to thank Dr. François Jean for his guidance and assistance in the construction of this article. His feedback and insight were extremely helpful for providing direction and constructive criticism for this paper. In addition, the author wishes to thank her peers for their contribution and support.

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ACRONYMS

APC	Antigen-presenting cell
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
FDA	Food and Drug Administration
MIBC	Muscle invasive bladder cancer
NDV	Newcastle disease virus
NMIBC	Non-muscle invasive bladder cancer
OV	Oncolytic virus
PD-1	Programmed cell death protein-1
PD-L1	Programmed death-ligand 1
RC	Radical cystectomy
TME	Tumor microenvironment
TMT	Trimodal therapy
UC	Urothelial carcinoma