Oncolytic Viruses and ICI

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Immuno-oncology (IO) has been an active area of oncology research. Following US FDA approval of the first immune checkpoint inhibitor (ICI), ipilimumab (human IgG1 k anti-CTLA-4 monoclonal antibody), in 2011, and of the first oncolytic virus, Imlygic (talimogene laherparepvec), in 2015, there has been renewed interest in IO. In the past decade, ICIs have changed the treatment paradigm for many cancers by enabling better therapeutic control, resuming immune surveillance, suppressing tumor immunosuppression, and restoring antitumor immune function. However, ICI therapies are effective only in a small subset of patients and show limited therapeutic potential due to their inability to demonstrate efficacy in "cold" or unresponsive tumor microenvironments (TMEs). Relatedly, oncolytic viruses (OVs) have been shown to induce antitumor immune responses, augment the efficacy of existing cancer treatments, and reform unresponsive TME to turn "cold" tumors "hot," increasing their susceptibility to checkpoint blockade immunotherapies. For this reason, OVs serve as ideal complements to ICIs, and multiple preclinical studies and clinical trials are demonstrating their combined therapeutic efficacy.

Keywords: oncolytic virus,immune checkpoint inhibitor,immuno-oncology,combination therapy

1. Introduction

In past decades, cancer treatment has made tremendous progress to include a range of therapies. One specific therapeutic area, immuno-oncology (IO), has received much renewed attention for its ability to take advantage of the body's immune system to fight cancer. Specifically, the development of antibodies (Ab) that target key immune checkpoint molecules served as a revolutionary milestone in the field of IO, as the Abs were able to disrupt the cancer cells' ability to evade the host immune response and activate anti-tumor immunity [1][2]. Such developments led to the first US FDA-approved immune checkpoint inhibitor (ICI), ipilimumab—a cytotoxic T-lymphocyte antigen-4 (CTLA-4)-targeting monoclonal Ab—for the treatment of patients with advanced melanoma [1]. After approval of ipilimumab, several other immune checkpoint blockade agents were examined, leading to numerous clinical trials that examined the efficacy of ICIs for either mono or combination therapy. To date, six other ICIs have been approved by the US FDA, comprising programmed death 1 (PD-1) inhibitors (pembrolizumab, nivolumab, and cemiplimab) and programmed death-ligand 1 (PD-L1) inhibitors (avelumab, durvalumab, and atezolizumab) [1]. Overall, the increase in ICIs is part of the growing IO trend: between 2017 and 2019, Tang et al. found a 91% increase in the number of active agents, a 78% increase in active IO targets, and a 60% increase in participating IO drug development organizations, resulting in 31 IO drug approvals by the US FDA [3].

Despite the impact of ICIs in IO and their potential in the clinical setting, accumulating evidence suggests that researchers must overcome some critical limitations: (a) some patients undergoing ICI therapy experience severe immune-related adverse events (irAE) [4], (b) only a fraction of cancer patients benefit from ICI treatment, and c) ICIs are ineffective against immunologically ‘cold' tumors characterized by a low tumor infiltrating lymphocyte (TIL) count [5]. To this end, oncolytic viruses (OV), which preferentially infect and lyse cancer cells, have been proposed as a promising modality for combination therapy to address the limitations of ICI. This is due to the unique ability of OVs to inflame a "cold" tumor microenvironment (TME) into a "hot" environment with increased immune cell and lymphocyte infiltration, making them an ideal candidate for combination with various cancer immunotherapeutics [5]. For this reason, multiple ongoing clinical trials are aiming to investigate the combined therapeutic efficacy of ICIs and OVs.

2. Immune Checkpoint Inhibitors

Immune checkpoints are regulators of the immune system that are crucial for self-tolerance and prevention of the immune system from attacking cells indiscriminately [6]. Many tumors evade the host immune system by upregulating immune checkpoints to generate immunosuppressive TME [7]. ICIs work by disrupting such tumor immunosuppressive signaling pathways, exposing the cancer cells to the host immune system. There has been overwhelming preclinical evidence

validating PD-1, PD-L1 and CTLA-4 inhibitors as viable targets for IO therapy, with demonstrated clinical efficacy against several solid and hematologic malignancies [1]. To date, six US FDA-approved drugs, comprised of three PD-1 inhibitors (nivolumab, pembrolizumab, and cemiplimab), three PD-L1 inhibitors (atezolizumab, avelumab, and durvalumab) and two CTLA-4 inhibitors, are used in the clinical setting [1][8]. In addition, new emerging checkpoint inhibitors targeting lymphocyte-activation gene 3 (LAG-3) [9][10] or T cell immunoglobulin and mucin-domain containing-3 (TIM-3) [11] have demonstrated promising preclinical results and in clinical development.

Although ICIs have had an unprecedented effect on the clinical activity of several cancers to transform IO, ICIs—both as mono and combination therapy—are not without limitations. Primarily, ICIs are known to have a broad range of side effects affecting every organ system, as well as producing irAE in a significant incidence of patients [12]. Flaherty et al. identified longer follow-ups of clinical trial population studies to accumulate evidence of late relapses, suggesting an emergence of acquired resistance to ICIs [13]. There are several interesting targets, like pattern recognition receptors, that were successfully exploited in preclinical models to circumvent resistance to T cell-targeted ICIs, but these investigations are in early stages and none have reached the clinical development stage [14]. In addition, several groups have reported that a diversity of neoantigens may be necessary to induce a sufficient clinical response from ICIs [15], with van Rooij et al. [16] and Le et al. [17] observing neoantigen-specific T cell reactivity in patients with advanced cancers. Moreover, several groups have substantiated the role of neoantigens in response to ICIs, supported by evidence of higher neoantigen load being associated with better therapeutic response to CTLA-4 and PD-1 blockade in patients with melanoma and non-small-lung cancer [18][19][20]. However, ICIs are most critically limited by their refractoriness or ineffectiveness in ‘cold' TME, as there is a lack of T cell infiltration, resulting in overall suppression of host antitumor immune response [5]. Furthermore, as many types of cancers present with ‘cold' tumors, ICIs are critically limited in therapeutic potential, necessitating the identification of novel IO candidates as combination therapies to maximize the clinical benefits of individual ICIs.

2.1. Development of Oncolytic Viruses in Combination with Immune Checkpoint Inhibitors

Oncolytic viruses (OVs) are native or recombinant viruses that self-propagate, selectively replicate in cancer cells, and infect adjacent cancer cells to elicit a cytolytic effect. Specifically, OVs differ from conventional cancer therapies in that they have naturally evolved to effectively hijack and reprogram the host's cellular machinery, effectively expressing both virus and therapeutic transgenes at a high level [21]. OVs have been demonstrated to stimulate the immune system by infecting a tumor cell to induce immunogenic cell death, which triggers an inflammatory reaction. This particular form of apoptosis results in the release of immune stimulatory agents, which activate innate and direct adaptive immune responses against cancer cells by the release of pathogen-associated molecular patterns (PAMPS), tumor-associated antigens (TAAs), and danger-associated molecular patterns (DAMPS) from lysed tumor cells [22][23]. APCs in the TME then recognize these key metabolites and generate an immune response. Furthermore, this local stimulation of the immune system creates a systemic and enduring anti-cancer response [24]. Importantly, these attributes of OVs have been shown to revert immunologically ‘cold' or ‘non-inflamed' tumors with low TIL count into ‘hot' tumors, indicating them as promising candidates to synergize with cancer immunotherapeutics [24][25][26]. There is a strong line of evidence that suggests viruses, like rotavirus, influenza or yellow fever virus, can also prime TME of cold tumors to be more responsive toward immunotherapy [27]. In particular, efforts to synergize OVs with other IO drugs have demonstrated OV infections to increase the expression levels of several proinflammatory cytokines and cause an influx of natural killer cells (NKs), activated T cells, and APCs into tumor tissues [28], priming unresponsive TMEs into more conducive targets for other cancer immunotherapeutics.

OVs provide additional versatility as effective anticancer agents, as they can be genetically engineered to possess specificity toward tumor cells and to preferentially lyse malignant cells, focusing their therapeutic transgene delivery and simultaneously reducing potential off-target side effects [29]. Importantly, arming OVs with antitumor immune transgenes, like cytokines, can lead to localized and high expression levels of these genes in tumor tissues, with minimal systemic circulation. These attributes allow OVs to deliver potent immune stimulators, like IL-12 [30] and 4-1BBL [31], among others, which had failed in clinical development as protein therapeutics due to their high systemic toxicity [32]. Despite these remarkable advances in OV IO, these viruses must overcome certain challenges to achieve clinical application and commercialization. Namely, most early clinical trials that administered OVs intratumorally as a monotherapy were not able to demonstrate a sufficient therapeutic outcome [33][34][35]. OVs have been shown to be susceptible to selective pressure of heterogeneous tumor populations when administered over an extended period of time, as these viruses lose essential signaling pathways by mutation or lose important receptors that mediate their life cycle [36].

Due to the above limitations, first-generation OVs like Rigvir and ONYX-015 have shown limited therapeutic efficacy as monotherapies, and their approval for in-human use has been restricted to the countries (Latvia and China, respectively) that commercialized these products [37][38]. Rigvir, a non-pathogenic ECHO-7 virus with no genetic modifications, was the first OV approved by regulatory authorities, gaining approval in Latvia in 2004 for the treatment of melanoma [37].

However, Rigvir did not achieve global commercialization; most of its clinical studies were performed before 1991, its safety and efficacy parameters needed to be updated to modern standards, and its response rate needed to be increased by optimizing its biomarkers toward more specific target populations [37]. Since Rigvir, several other OVs, which are innately oncolytic without genetic modification of the virus genome, have entered clinical development in the US and EU [39]. Still, most first-generation OVs, such as Oncorine (previously designated as ONYX-015) and HSV1716, which began clinical trials in the 1990s, as well as those that are currently under clinical evaluation, harbor several genetic modifications to improve the cancer specificity of virus strains. The first such genetically modified OV to be approved by a regulatory authority was Oncorine, which was approved by the Chinese FDA in 2005 for the treatment of solid tumors [40]. Despite gaining Chinese FDA approval, Oncorine's development in the US and EU failed to induce significant tumor growth inhibition in patients when used as a monotherapy and only achieved meaningful clinical therapeutic responses when used in combination with chemotherapy [41] or radiotherapy [42], demonstrating its inadequacies and suboptimal potency as a monotherapy.

Since commercialization of Rigvir and Oncorine in the early 2000s, only one other OV, Imlygic, has been approved for human use. Imlygic (granulocyte-macrophage colony-stimulating factor (GM-CSF)-armed, herpes simplex virus type 1 (HSV10)), which was approved by the US and EU FDAs in 2015 and by the AUS FDA in 2016 [43][44], remains the only internationally available OV with a proven clinical track record as a monotherapy against melanoma [45]. Unlike Rigvir and Oncorine, developed in the 1990s, which were primarily evaluated in a clinical setting for direct oncolytic effects, Imlygic was designed to maximize its therapeutic potential as a next-generation IO therapeutic, armed with antitumor immune-boosting transgenes like GM-CSF, and clinically examined with a strong emphasis on immune-related evaluation parameters, such as immune cell infiltration into tumor tissues and cytokine profiling. In general, this IO-oriented clinical development process of Imlygic outlines the drastic change in the perception of OVs as cancer immunotherapeutics in the modern cancer therapy landscape. There is a growing amount of evidence supporting this IO-driven development process: for instance, several studies have shown that OVs exerting minimal cytolytic effect against tumors can effectively suppress the growth of injected and non-injected distal tumors by inducing an antitumor immune response [46]. The landmark approval of Imlygic has accelerated clinical development of other OVs. Imlygic has been evaluated in the largest number of ongoing and completed clinical trials for any particular OV, as either a monotherapy or a combination therapy, and has generated large quantities of clinical data [47][48]. Such trials have demonstrated that OVs can inflame the immunosuppressed TMEs of clinical tumors and have provided strong empirical evidence for combining ICIs with OVs [23][49][50]. A positive phase II clinical trial results from "Ipilimumab With or Without Imlygic in Unresected Melanoma (NCT01740297)" was the first to demonstrate that synergistic therapeutic efficacy can be achieved with combination of OV and ICI in cancer patients, as a higher level of cytotoxic T cell infiltration in tumors was observed following combination treatment [47] compared to that following ipilimumab monotherapy [47]. Notably, the combination of Imlygic and ipilimumab did not induce more severe side effects than monotherapy, whereas ipilimumab in combination with other ICIs has been reported to elevate the incidence rates and severities of adverse events [51]. Not only does this evidence support OVs as a viable and effective complementary therapy strategy in combination with ICIs, but it also sets a precedent for future combination therapies for clinical development, which are discussed in detail below.

3. Oncolytic Viruses in Combination with Immune Checkpoint Inhibitors in Clinical Trials

3.1. Adenovirus

Adenoviruses (Ads) are non-enveloped and double-stranded DNA viruses [52] with an icosahedral capsid. These viruses range from 70–90 nm in size and possess a genome of approximately 35 kb [53]. While 57 known Ad serotypes exist, serotype 5 is the most commonly used backbone in OVs [54]. Ads are one of the most commonly used viral vectors in cancer gene therapy due to their advantageous characteristics, which include high gene transfer efficiency in both dividing and non-dividing cells, low risk of insertional mutagenesis, and exponential viral replication capacity (one infectious virus can produce more than 10,000 progeny viruses in an infected cancer cell) [21][45][55][56][57]. Furthermore, Ads have been shown to elevate the expression level of immunostimulatory signals to enhance TAA presentation by APC to induce a strong, tumor-specific immune response [12], illustrating them as one of the most immunogenic OVs [58]. For this reason, several oncolytic Ads are under evaluation in clinical trials as monotherapies and in combination with ICIs.

3.2. Herpes Simplex Virus-1

HSV is a virus characterized by an icosahedral capsid, approximately 200 nm in size, and a linear, 150-kb double-stranded DNA genome [59][60]. Oncolytic HSV (oHSV) has been extensively used in clinical immunotherapy and is considered a useful oncolytic vector due to its large transgene capacity, lack of insertional mutagenesis, and ability to

activate innate and adaptive immune responses against tumors [61]. Furthermore, HSV infection can be controlled by well-established antiviral drug regimens, ensuring greater safety in the case of a vector that threatens the life of the patient [62][63]. To date, various strains of HSV are used as monotherapies and have successfully expressed various transgenes, such as p53 [64], IL-2 [65][66], and IFN-γ [67], in preclinical studies. Currently, Imlygic remains the only US FDA-approved OV, but several other oHSVs have progressed to phase II/III clinical trials.

3.3. Vaccinia Virus

Vaccinia virus is a member of the *Poxviridae* family and possesses a large double-stranded DNA of 190 kb, indicating its suitability for large transgene insertion [68]. Furthermore, vaccinia virus replicates in the cytoplasm, eliminating the risk of insertional mutagenesis [53]. In addition, vaccinia viruses have a rapid replication and infection cycle that starts as early as 2 h after infection, with cell lysis taking place between 12 and 48 h [69]. For these reasons, vaccinia viruses are useful in the development of oncolytic virotherapy.

3.4. Coxsackievirus

Coxsackievirus is a small, 30-nm-sized, non-enveloped, single-stranded RNA virus with an icosahedral capsid belonging to the *Picornaviridae* family [53]. While the virus is grouped into two subtypes, A and B, the serotype A21 has been most commonly utilized in clinical trials for its inherent cytotoxicity against cancer cells [70]. Serotypes A13, A15, and A18 are being explored for oncolytic potential [71]. Coxsackievirus A21 is a naturally occurring OV and has not been as extensively characterized as other virus strains like HSV or Ad, which have been clinically developed as OVs since the 1990s [70].

3.5. Reovirus

Reoviruses are non-enveloped and double-stranded RNA viruses belonging to the *Reoviridae* family with icosahedral capsids [72]. Reoviruses are 75–80 nm in size [53] and replicate in the cytoplasm [72]. The most extensively developed oncolytic strain of reovirus is Type 3 Dearing virus (Reolysin), which has been investigated in more than 30 clinical trials [73]. Reolysin selectively replicates in and exerts oncolytic effect against cancer cells with activating Ras mutation, which is observed in approximately 30% of all human cancers [74][75].

3.6. Vesicular Stomatitis Virus

Vesicular stomatitis virus (VSV) belongs to the *Rhabdoviridae* family and is an enveloped, 11-kb, single-stranded RNA virus with a size of 70 nm [76]. Recombinant strains of VSV, such as VSV(Delta51)-NIS and VSV-IFNβ, have shown oncolytic properties in preclinical myeloma models [77][78] and in other cancer models [79][80][81][82]. VSV exhibits wide species tropism, allowing preclinical validation in immunocompetent rodents [83]. Furthermore, Stojdl et al. have demonstrated attenuated versions of VSV, such as AV1 or AV2, to have oncolytic properties, as these viruses are capable of selective replication in IFN-deficient cancer cells, while normal cells have an intact IFN response to resist viral infection [84].

3.7. Maraba Virus

Maraba virus (MV) is a member of the Rhabdovirus family with an RNA genome [85]. MVs are relatively simple viruses but possess a number of properties that suggest their use as OV agents. For example, MVs are rarely associated with human disease, and pre-existing immunity against MV is rare in most populations [86]. In addition, the entire life cycle of MV occurs in the cytoplasm, preventing the opportunity for insertional mutagenicity [87]. Furthermore, despite the small genome size of the virus at 11 kb, MVs can be genetically manipulated for transgene insertion [87].

3.8. Poliovirus

Poliovirus belongs to the *Picornaviridae* family and has a 7.5-kb genome [88]. While the poliovirus is naturally oncolytic [89], it has been shown to be potently tropic toward CD155, an immune checkpoint molecule expressed in a wide range of malignant cells of solid tumors [90]. The pathogenicity, such as neurovirulence, of wild-type poliovirus is well known and must be adequately addressed before use as a therapeutic agent [90]. An example of an attenuated poliovirus is the Sabin vaccine, which was genetically modified to reduce its poliomyelitis effects while retaining its oncolytic activity [90].

3.9. Newcastle Disease Virus

Newcastle disease virus (NDV) is a neurotropic virus with a 15-kb, single-stranded RNA genome and belongs to the *Paramyxoviridae* family [91]. NDV has been shown to be an effective oncolytic agent, reported to replicate up to 10,000 times faster in human cancer cells than in most normal human cells [92]. Further, Schirrmacher et al. have shown NDV-

treated tumors to be infiltrated with T cells secreting IFN-γ [93]. In addition, Koks et al. reported NDV infection to induce long-term survival and tumor-specific immunity in an orthotopic glioblastoma multiforme model in immunocompetent mice, while no therapeutic effects were seen in immunodeficient Rag2 (−/−) mice or mice depleted of $CD8^+$ T cells [94].

A preclinical study revealed that intratumoral administration of NDV and systemic CTLA-4 blockade induced inflammatory responses and antitumor effects in both local and distant tumors via the induction of systemic and tumor-specific immune responses. Zamarin et al. also reported that localized administration of NDV induced a systemic tumor inflammatory response, as evidenced by the increased infiltration of activated effector T cells in non-injected distant tumors [91]. In addition, the authors found combination therapy of localized NDV and systemic CTLA-4 or PD-1 blockade to produce antigen-dependent tumor rejection in a tumor rechallenge model [92]. Zamarin et al. developed an NDV expressing an ICI molecule, ICOSL (NDV-ICOSL), which in combination with anti-CTLA-4 therapy induced a more effective rejection of distant tumors than NDV monotherapy, leading to the long-term survival of the majority of mice and providing protection against tumor relapse [95].

4. Conclusions

The use of IO has changed cancer treatment in recent years. Despite ICI revolutionizing the cancer therapy field, it is largely ineffective in patients with cold tumors. To address this limitation, OVs in combination with ICIs can be a strategy to address the inefficacy of ICI against cold tumors, as these viruses can inflame the TME into a more immunologically favorable environment for cancer immunotherapeutics. The number of clinical trials investigating combinations of OVs and ICIs continues to rise, and most of the available results from these trials have demonstrated promising therapeutic potentials with good safety profiles. Other immunotherapeutics (like CAR-T and ICI) in combination with ICIs are well known to compound adverse events, whereas OVs do not, and this superior safety profile is particularly promising in IO. Due to the recency of many of these clinical trials, in-depth results are not available to the public, but many of the interim and preclinical results indicate strong synergism of the combination therapy approaches, as evidenced by increased migration of antitumor immune effector cells to tumor tissues and elevated expression of antitumor cytokines. The detailed results of these studies are highly anticipated, as they will provide much needed insights that will be critical to maximizing the therapeutic potential of OV and ICI combination therapies for treatment of intractable cancers. With increasing numbers of OV pipelines and ICIs entering clinical development, there is a strong potential for this strategy to revolutionize cancer treatment in the near future.

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