Overcoming melanoma drug resistance: Mechanisms and clinical progress of oncolytic viruses combined with immune checkpoint inhibitors (Review)

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Abstract. Although immune checkpoint inhibitors (ICIs) have revolutionized the management of advanced melanoma, a major clinical challenge persists: Nearly 50% of patients develop primary or acquired resistance and therefore derive no meaningful clinical benefit. As ICIs are increasingly used in earlier disease settings, including adjuvant and neoadjuvant therapy, the population of patients with ICI-resistant disease continues to grow. For patients whose disease progresses after first-line combined programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) blockade, effective salvage options remain limited, highlighting an urgent unmet medical need. Oncolytic viruses (OVs), which exert antitumor effects through the dual mechanisms of selective tumor cell lysis and immunomodulatory remodeling of the tumor microenvironment, represent a compelling therapeutic strategy for overcoming ICI resistance. Recent clinical and preclinical advances have moved OV-ICI combination regimens from conceptual strategies to clinically evaluable interventions for refractory melanoma. Nevertheless, several translational barriers impede their broader implementation, including interpatient heterogeneity in treatment response, uncertainty regarding the optimal sequencing and dosing of combination therapies, and the lack of validated predictive biomarkers. The present review systematically synthesizes emerging clinical evidence and mechanistic insights into OV-ICI synergy to inform rational trial design, refine combination strategies, and accelerate the development of precision immuno-oncology approaches.

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1. Introduction

Melanoma, the most aggressive form of skin cancer, continues to increase in incidence worldwide (1). Although immune checkpoint inhibitors (ICIs), including antibodies targeting programmed cell death 1 (PD-1)/programmed death ligand 1 (PD-L1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), have revolutionized the treatment of advanced melanoma, substantial clinical challenges remain (2). Clinical data indicate that anti-PD-1 monotherapy achieves an objective response rate (ORR) of only ~38% in advanced melanoma, with >50% of patients developing primary or acquired resistance (3). This resistance is closely associated with characteristics of the tumor microenvironment (TME); a considerable proportion of melanomas exhibit a 'cold tumor' phenotype characterized by sparse T-cell infiltration and an immunosuppressive milieu (4).

Oncolytic viruses (OVs) genetically engineered to replicate selectively in tumor cells represent a promising strategy for overcoming ICI resistance (5). In addition to directly lysing tumor cells, OVs promote the release of tumor-associated antigens (TAAs) and damage-associated molecular patterns (DAMPs), thereby activating innate and adaptive immune responses. This process can convert immunologically 'cold' tumors into 'hot' tumors and enhance their sensitivity to ICIs and other immunotherapies (6,7). In recent years, multiple OV platforms have been developed, including oncolytic herpes simplex virus (oHSV), oncolytic adenoviruses (oAds), vesicular stomatitis virus (VSV), and novel chimeric viruses such as VSV-Chikungunya virus (CHIKV) (8,9).

The rationale for combining OVs with ICIs is based on their complementary mechanisms of action. OV-induced immune activation promotes T-cell infiltration and alleviates immunosuppression within the TME, whereas ICIs block inhibitory checkpoints and thereby amplify OV-induced antitumor immune responses (10). Preliminary evidence from preclinical and clinical studies supports this synergistic interaction (11,12). Emerging evidence further suggests that this combination can substantially improve therapeutic efficacy while maintaining a manageable safety profile (13). The present review examines the mechanisms by which OVs remodel the melanoma immune microenvironment, systematically summarizes clinical advances in combination strategies, and discusses future directions for clinical translation.

To minimize selection bias and ensure comprehensive coverage, a predefined search of PubMed (https://pubmed.ncbi.nlm.nih.gov), Web of Science Core Collection (https://www.webofscience.com), and ClinicalTrials.gov (https://clinicaltrials.gov) from database inception through March 10, 2026 was conducted. Additionally, an updated supplementary search was performed in July 2026 during the revision process to incorporate the most recent clinical evidence. The search strategy combined MeSH terms with free-text keywords as follows: ('oncolytic viruses' OR 'OV' OR 'T-VEC' OR 'RP1' OR 'talimogene laherparepvec') AND ('melanoma') AND ('immune checkpoint inhibitors' OR 'anti-PD-1' OR 'anti-CTLA-4') AND ('drug resistance' OR 'immune evasion' OR 'tumor microenvironment'). The inclusion criteria were as follows: i) Preclinical mechanistic studies or clinical trials evaluating OVs combined with ICIs in melanoma, with priority given to randomized controlled trials (RCTs), phase I-III trials with complete data, and high-quality retrospective analyses; and ii) original studies investigating resistance mechanisms, TME remodeling, or predictive biomarkers. Articles published in languages other than English, conference abstracts, and expert opinions without original data were excluded. For clinical evidence extraction, trials involving ≥10 evaluable patients, with robust methodological quality and clearly defined efficacy endpoints, were prioritized. Negative findings were systematically incorporated to ensure a balanced interpretation. Following this selection process, 44 preclinical mechanistic studies and 43 clinical or translational studies were included. The remaining articles comprised reviews and commentaries; collectively, these publications form the core evidentiary basis of the present review.

2. ICI resistance in melanoma

Primary resistance. Primary resistance refers to the failure of tumors to respond upon initial exposure to ICIs and arises from intrinsic genomic alterations and signaling characteristics of tumor cells. Mitogen-activated protein kinase (MAPK) pathway-mediated immune exclusion is a central driver of primary resistance (14). The B-Raf proto-oncogene, serine/threonine kinase (BRAF) V600E mutation constitutively activates MAPK signaling, thereby promoting tumor proliferation and suppressing secretion of the chemokine C-C motif chemokine ligand 4 (CCL4) through upregulation of β-catenin signaling. This markedly impairs the recruitment of cluster of differentiation (CD)8^+ T cells and dendritic cells into the TME, resulting in the so-called 'immune-excluded' phenotype (15). MAPK pathway activation can also directly upregulate PD-L1 expression on the tumor cell surface, further reinforcing immunosuppression (16). Consistent with these findings, a dedifferentiated phenotype characterized by a low microphthalmia-associated transcription factor (MITF)/AXL (AXL receptor tyrosine kinase) ratio has been closely associated with cross-resistance to targeted therapy and immunotherapy in melanoma (17,18). Furthermore, phosphatase and tensin homolog (PTEN) loss has been shown to promote the development of an immunosuppressive microenvironment through activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway (19). Activation of Wnt/β-catenin signaling constitutes another important mechanism of primary resistance. The Wnt/β-catenin-driven melanocytic state is closely associated with immune exclusion. Aberrant β-catenin accumulation has been found to limit the infiltration of $CD103^+$ dendritic cells (DCs) by suppressing CCL4 expression, thereby impairing T-cell priming and activation (20,21). Neuroblastoma RAS viral oncogene homolog (NRAS)-mutant melanoma exhibits a distinct mechanism of primary resistance. Previous studies have shown that, although NRAS-mutant melanoma displays high immune gene activity within tumor cells, the inducibility of these genes is attenuated under immune stimulation. This intrinsic failure of immune activation may limit the response to immune checkpoint blockade (22,23).

Adaptive resistance. Adaptive resistance is a reversible, treatment-induced response that tumors develop following initial exposure to ICIs. Unlike resistance driven by fixed genetic lesions, it depends on sustained therapeutic pressure. Compensatory interferon (IFN)-γ signaling is a central driver of this process (24). Following ICI-induced T-cell activation, secreted IFN-γ exerts opposing effects. It enhances antitumor immunity but also induces transcriptional upregulation of PD-L1 on the tumor cell surface through Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling, thereby establishing a negative feedback loop that facilitates immune evasion (25,26). This dynamic interplay provides a mechanistic explanation for the clinical pattern of an initial response followed by later disease progression in patients receiving anti-PD-1 therapy. Studies of sequential regimens combining anti-PD-1/PD-L1 therapy with MAPK inhibitors in preclinical melanoma models have shown that upfront ICI administration promotes proinflammatory macrophage repolarization and clonal expansion of $CD8^+$ cytotoxic T cells, thereby producing more durable responses to combination therapy (27,28). Transforming growth factor (TGF)-β signaling represents another major axis of adaptive resistance. Paradoxically, inhibition of this pathway has been shown to promote stromal fibroblast expansion, which induces matrix metalloproteinase-9 (MMP-9)-dependent shedding of cell-surface PD-L1 and ultimately leads to therapeutic failure despite continued anti-PD-1 treatment (29).

Acquired resistance. Acquired resistance is a durable loss of sensitivity to ICIs following prolonged therapeutic pressure and is typically driven by genetic mutations or stable epigenetic

reprogramming. It represents the predominant mechanism underlying late-stage disease relapse (30).

Genetic inactivation of the IFN-γ signaling axis is among the earliest and best-characterized mechanisms of acquired resistance (31). Recurrent *JAK1*/*JAK2* alterations have been documented in patients with melanoma who experience disease progression following anti-PD-1 therapy (32). These alterations render tumor cells entirely refractory to IFN-γ, resulting in loss of responsiveness to both its antiproliferative effects and downstream immunostimulatory signaling.

Disruption of antigen presentation represents a second major mechanism of acquired resistance. In the study by Zaretsky *et al* (32), a truncating mutation in the β2-microglobulin (B2M) gene was identified in a third patient, resulting in complete loss of cell-surface major histocompatibility complex class I (MHC-I) expression. B2M deficiency has been found to not only abolish MHC-I-mediated recognition by $CD8^+$ T cells but may also impair antitumor immunity by disrupting CD1D-dependent natural killer (NK) T-cell surveillance (33,34). Notably, even in the absence of MHC-I loss, downregulation of alternative immunomodulatory molecules such as CD109 can confer resistance to tumor-infiltrating lymphocyte (TIL)-mediated cytotoxicity in melanoma cells (35).

Genomic evolution promoting apoptotic evasion has recently emerged as an important nonimmunologic determinant of acquired resistance. Through genomic profiling of progressive melanomas, Wu *et al* (36) identified recurrent copy-number amplifications of antiapoptotic genes and concurrent deletions of proapoptotic genes relative to matched pretreatment tumors (36). Melanoma cell lines and murine models with acquired resistance induced by chronic exposure to T cells or ICIs recapitulated the co-occurring patterns of apoptotic gene copy-number alterations observed in clinically progressive disease. Restoring the expression of deleted proapoptotic factors increased mitochondrial priming and resensitized tumor cells to cytotoxic T-cell- or ICI-mediated killing (36,37). Collectively, these findings identify the intrinsic apoptotic threshold as a key determinant of the durability of responses to ICIs.

Moreover, a range of novel resistance-associated genetic alterations has recently been identified. For instance, SEC24C and SEC24D mutations, detected in a subset of patients with acquired resistance, are associated with attenuated stimulator of interferon genes (STING) signaling, reduced type I interferon (IFN-I) production, and impaired antigen presentation (38). Gain-of-function isocitrate dehydrogenase (IDH) mutations, which are associated with aberrant methylation patterns, were found to be linked with poor responses to anti-PD-1 blockade in patients with melanoma (39). Zinc finger protein 180 (ZNF180) has been characterized as a tumor-intrinsic transcriptional driver of melanoma plasticity. Its overexpression has been shown to promote a dedifferentiated phenotype, resulting in loss of immunogenicity, activation of the T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT)/CD155 checkpoint axis, and exclusion of $CD4^+$ helper T cells (40).

Intrinsic mechanisms of tumors. Tumor-intrinsic mechanisms encompass immune-evasion strategies encoded within tumor cells that operate independently of external therapeutic selection. Epigenetic reprogramming is a major driver of immune evasion in melanoma (41). Tumor cell plasticity, characterized by dynamic and reversible transitions among distinct cellular states, underlies both immune escape and therapeutic resistance through mechanisms extending beyond static genetic mutations. Core epigenetic regulatory mechanisms include DNA methylation and active demethylation (42), noncoding RNA-mediated gene regulation, post-translational histone modifications such as methylation/demethylation and acetylation/deacetylation, and epigenetic regulation of nonhistone proteins. Pharmacologic inhibition of DNA methyltransferases in melanoma cell lines induces genome-wide DNA demethylation, thereby promoting dedifferentiation and enhancing molecular signatures of viral mimicry, both of which predict favorable responses to IFN-γ-based immunotherapies (43). Tumor-intrinsic defects in the antigen-processing and presentation machinery represent another important mechanism. In addition to well-documented loss-of-function mutations in B2M and MHC-I genes, transcriptional downregulation of key components, including transporter associated with antigen processing (TAP)1/TAP2, is prevalent in melanoma. Moreover, epigenetic silencing of the cyclic GMP-AMP synthase (cGAS)-STING pathway, particularly through promoter hypermethylation leading to functional ablation, is frequently observed in acral and mucosal melanomas. This silencing has been found to compromise tumor-intrinsic sensing of cytosolic self-DNA and the subsequent activation of innate immune signaling (44,45).

Microenvironmental mechanisms. Non-tumor cellular and stromal components of the TME contribute substantially to resistance to ICIs. A hallmark of TME-mediated therapeutic resistance is the selective enrichment of immunosuppressive cell populations, including myeloid-derived suppressor cells (MDSCs), M2-polarized tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and cancer-associated fibroblasts (CAFs). Together, these populations form a spatially organized, multilayered immune-exclusion network (46). MDSCs exert immunosuppressive effects primarily by secreting soluble mediators such as interleukin (IL)-17, IL-6, IL-1β, and TGF-β (47). TAMs promote ICI resistance through multiple molecular pathways, notably the V-set and immunoglobulin domain-containing protein 4 (VSIG4)/$CD8^+$ T-cell axis and IL-11/STAT3 signaling (48,49). CAFs establish an immunosuppressive niche through periostin (POSTN)-dependent and β-catenin-mediated signaling while simultaneously remodeling the extracellular matrix (ECM) into a dense physical barrier that impedes immune-cell trafficking (50). Recent single-cell and spatial transcriptomic analyses have further shown that CAFs establish a functionally hierarchical immune-exclusion architecture in melanoma, characterized by spatial clustering and coordinated, multitiered immunoregulatory signaling (50).

Metabolic reprogramming represents a second major mechanism of TME-mediated ICI resistance (51). Melanoma cells frequently shift toward aerobic glycolysis, leading to substantial lactate production. Elevated lactate levels directly impair the function of cytotoxic $CD8^+$ T cells and NK cells while promoting the expansion and activation of MDSCs

and Tregs. The accumulation of lactate and lipids, coupled with tryptophan catabolism, further disrupts DC maturation, antigen processing and presentation, and subsequent T-cell priming and activation (52). Melanoma cells export lactate through the CD147-monocarboxylate transporter 1 (MCT1) axis, after which it is taken up by TAMs, thereby reinforcing local immunosuppression. Concurrently, aberrant tumor vasculature, characterized by structural immaturity, leakiness, and dysfunctional perfusion, causes hypoperfusion and elevated interstitial fluid pressure, limiting the delivery of both endogenous immune effectors and exogenous therapeutics (53).

Therapy-induced immune evasion. Certain tumors acquire resistance to immune surveillance following PD-1/PD-L1 blockade through compensatory immunoregulatory mechanisms. Among these mechanisms, therapy-induced upregulation of alternative immune checkpoints represents the predominant adaptive escape pathway. Preclinical and translational evidence indicates that PD-1 inhibition induces the coordinated upregulation of alternative inhibitory receptors, including T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte activation gene 3 (LAG-3), and TIGIT, in both melanoma cells and tumor-infiltrating $CD8^+$ T cells, thereby restoring immunosuppression. In murine melanoma models, tumors enriched in terminally exhausted $CD8^+$ T cells were shown to markedly respond to triple immune checkpoint blockade targeting PD-1, LAG-3, and TIM-3 (54). Notably, adding TIM-3 blockade to combined PD-1 and LAG-3 inhibition was found to increase the magnitude and functional specificity of the antitumor T-cell response. Furthermore, elevated B7-H3 expression was shown to be strongly associated with clinical resistance to checkpoint inhibition. Emerging evidence suggests that therapy-induced androgen receptor signaling may drive or amplify B7-H3-mediated immunosuppressive programs (55).

In parallel, tumor-cell phenotypic plasticity, specifically, the transition from a differentiated, proliferative state to a dedifferentiated, invasive phenotype, represents a distinct, nonimmune mechanism of treatment-induced immune evasion. This dynamic transition enables tumors to remodel their antigenic and functional landscapes under therapeutic selection, thereby impairing T-cell recognition and effector function (56,57).

3. OVs reverse ICI resistance

Induction of immunogenic cell death (ICD) to overcome primary and adaptive resistance. OVs have been shown to selectively infect melanoma cells and induce ICD (58). In melanoma, the therapeutic relevance of ICD lies in its ability to overcome both primary and adaptive resistance mechanisms, particularly those driven by hyperactivated MAPK signaling and impaired antigen-processing and presentation machinery. During ICD, dying tumor cells release TAAs and expose or release DAMPs, including calreticulin (CRT), high-mobility group box 1 (HMGB1), a nonhistone chromatin-binding protein, adenosine 5'-triphosphate (ATP), and heat shock proteins (HSPs) (59). Collectively, these molecules act as endogenous danger signals that engage pattern-recognition receptors (PRRs) on innate and adaptive immune cells, thereby initiating DC activation, antigen uptake and processing, and TAA cross-presentation (60). TAAs provide antigenic substrates for neoepitope generation and the priming of robust, tumor-specific $CD8^+$ T-cell responses. In addition, DAMPs act as endogenous adjuvants that promote DC maturation and enhance the efficiency and durability of antigen presentation, ultimately facilitating cytotoxic T-lymphocyte (CTL) differentiation and expansion (61). Recognition of these signals by PRRs activates downstream inflammatory cascades closely associated with the establishment of long-lasting antitumor immune memory (61).

The translocation of CRT to the plasma membrane, surface exposure of HSPs, extracellular release of ATP, and secretion of HMGB1 collectively constitute the canonical hallmarks of bona fide ICD. However, to fulfill the functional criteria for immunogenicity, these events must occur in a temporally coordinated and synergistic manner rather than in isolation. Specifically, CRT exposure acts as an 'eat-me' signal that promotes phagocytosis by DCs (62); ATP functions as a chemotactic 'find-me' signal that recruits DCs and other leukocytes to the tumor site (63); and HMGB1, released during late-stage apoptosis or necrosis, binds to Toll-like receptor 4 (TLR4) and receptor for advanced glycation end products (RAGE), promoting DC maturation and proinflammatory cytokine production (64). This sequential, multimodal signaling cascade coordinates DC recruitment to the TME, phagocytosis of tumor debris, antigen processing, DC maturation, and subsequent priming of tumor-reactive CTLs. The resulting IFN-γ-dominant response engages both conventional $CD8^+$ T cells and γδ T cells, promoting their infiltration into the TME, suppression of tumor progression, and direct lysis of tumor cells.

Constitutive activation of the BRAF/mitogen-activated protein kinase kinase (MEK) pathway, a hallmark of numerous melanomas, suppresses CCL4 expression and contributes to the exclusion of $CD8^+$ T cells from the TME. OV-induced ICD counteracts this immunosuppressive phenotype through the release of ATP and HMGB1, which act as potent inflammatory signals capable of activating the NLR family pyrin domain-containing 3 (NLRP3) inflammasome in myeloid cells. This activation induces caspase-1-dependent secretion of IL-1β and IL-18, thereby promoting the recruitment and activation of IL-17-producing γδ T cells and cytotoxic $CD8^+$ T cells (64,65). Mechanistically, this process restores immune-cell trafficking and reverses MAPK-driven T-cell exclusion. Preclinical studies using the B16 murine melanoma model demonstrated that the magnitude of ICD induction is positively associated with responsiveness to anti-PD-1 immunotherapy, supporting the translational relevance of ICD as an important contributor to ICI efficacy (Fig. 1) (66,67).

Activation of innate immune signaling to reverse epigenetic and IFN pathway deficiencies. OVs activate innate immune responses through PRRs, conferring distinct therapeutic advantages in melanomas with epigenetic silencing of the cGAS-STING pathway or loss-of-function mutations in the IFN-γ signaling cascade. The cGAS-STING pathway is frequently inactivated in melanoma, predominantly through promoter hypermethylation, particularly in acral

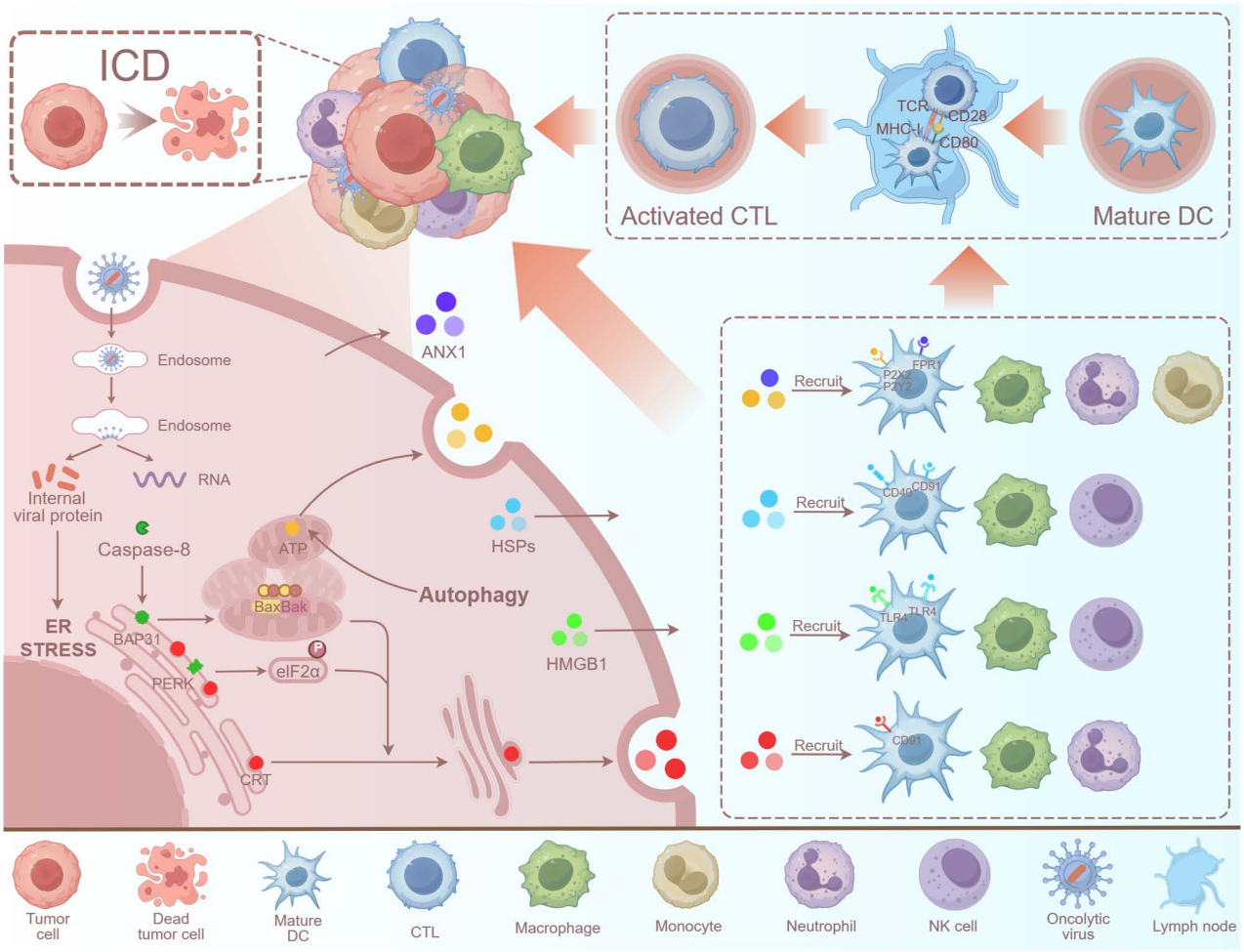

Figure 1. Mechanisms of immunogenic cell death in tumor cells and recruitment of immune cells. ICD, immunogenic cell death; CTL, cytotoxic T lymphocyte; TCR, T-cell receptor; MHC-I, major histocompatibility complex class I; DC, dendritic cell; ANX1, Annexin A1; HSPs, heat shock proteins; HMGB1, high-mobility group box 1; ER, endoplasmic reticulum; BAP31, B-cell receptor-associated protein 31; PERK, protein kinase R-like endoplasmic reticulum kinase; ATP, adenosine-5'-triphosphate; Bax, Bcl-2-associated X protein; Bak, Bcl-2 antagonist/killer 1; eIF2α, eukaryotic initiation factor 2α; CRT, calreticulin; NK, natural killer; FPR1, formyl peptide receptor 1; P2X2, purinergic receptor P2X 2; P2Y2, purinergic receptor P2Y 2; TLR4, Toll-like receptor 4. This figure was created using Figdraw (https://www.figdraw.com).

and mucosal subtypes. OVs circumvent STING deficiency by directly engaging the TANK-binding kinase 1 (TBK1)-interferon regulatory factor 3 (IRF3) axis through viral double-stranded DNA intermediates. Alternatively, they compensate for impaired STING function by inducing the release of high levels of DAMPs, thereby restoring IFN-I production (44,58,68). Nevertheless, ~11% of melanomas harbor biallelic loss-of-function mutations in *JAK1* or *JAK2*, resulting in profound attenuation of IFN-I signal transduction (69). Multiple preclinical and translational studies have shown that anti-PD-1-resistant tumor cells with *JAK1*/*JAK2* deficiency exhibit heightened susceptibility to OV-mediated oncolysis (31,69,70). For example, melanoma cells harboring *JAK2* mutations display marked hypersensitivity to the engineered oAd-suppressor of cytokine signaling 3 (SOCS3). This hypersensitivity is attributable to SOCS3 overexpression, which suppresses residual STAT1/STAT2 phosphorylation, thereby weakening intrinsic antiviral defenses while concurrently downregulating PD-L1 expression (70). Comparative functional profiling further shows that melanoma cell lines derived from post-progression biopsies following anti-PD-1 therapy are significantly more sensitive to OVs than matched baseline-derived cell lines, exhibiting 7-fold greater susceptibility to herpes simplex virus type 1 (HSV-1)-deleted infected cell protein 0 (dICP0) and 22-fold greater sensitivity to VSV-Δ51 (69).

Remodeling the immunosuppressive TME to combat microenvironment-mediated resistance

Eliminate immunosuppressive cell populations. OVs remodel the immunosuppressive TME by selectively depleting or functionally reprogramming key immunosuppressive cell populations, thereby attenuating microenvironment-mediated resistance to immunotherapy. In patients with melanoma, reduced responsiveness to ICIs is strongly associated with increased infiltration by MDSCs and M2-polarized tumor-associated macrophages (M2-TAMs) (71). DAMPs released during OV-induced ICD promote the repolarization of M2-like TAMs toward a proinflammatory, antitumor M1-like phenotype (72). While certain first-generation oAds may paradoxically promote M2 polarization under specific conditions, rationally engineered variants expressing signal

regulatory protein α (SIRPα)-fragment crystallizable region (Fc) fusion proteins have been shown in preclinical models to enhance macrophage-mediated phagocytosis of melanoma cells by competitively blocking the CD47-SIRPα 'don't eat me' axis (73). The dense ECM characteristic of melanoma also creates a substantial physical barrier to ICI penetration and T-cell trafficking. To overcome this barrier, enadenotucirev, a genetically modified oAd engineered to express bispecific T-cell engagers (BiTEs), has demonstrated selective cytotoxicity against CAFs in mixed-tumor melanoma models, thereby disrupting stromal architecture and restoring immune cell accessibility (74). An *in vivo* study conducted in a pancreatic cancer model further demonstrated that oHSV induces transcriptional and functional reprogramming of CAF subsets. Specifically, oHSV reduced the abundance of immunosuppressive myofibroblastic CAFs (myCAFs) while expanding immunostimulatory antigen-presenting CAFs (apCAFs), thereby converting immunologically inert 'cold tumors' into T-cell-inflamed 'hot tumors' (75).

Reprogrammed immunoregulatory factors. OVs modulate the IFN-γ signaling axis and chemokine networks in melanoma in a coordinated but bidirectional manner. IFN-γ serves as a central immunological mediator linking OV-induced antitumor immunity to the therapeutic efficacy of ICIs. Infection of melanoma cells with recombinant VSV expressing lymphocytic choriomeningitis virus glycoprotein (rVSV-LCMVG) was shown to activate the cytosolic retinoic acid-inducible gene I (RIG-I)/melanoma differentiation-associated protein 5 (MDA5) RNA-sensing pathway, inducing robust production of IFN-γ and the T-cell-recruiting chemokine C-X-C motif chemokine ligand 10 (CXCL10). This response is essential for $CD8^+$ T-cell infiltration into the TME (76).

However, IFN-γ signaling has context-dependent effects, particularly in the development of adaptive immune resistance in melanoma. IFN-γ enhances tumor immunogenicity by upregulating MHC class I expression and the antigen-processing machinery. Conversely, persistent IFN-γ stimulation through the canonical JAK-STAT pathway was found to induce transcriptional upregulation of PD-L1 on tumor cells (32). Clinical evidence indicates that OV-mediated T-cell infiltration is frequently accompanied by PD-L1 upregulation in patient tumor biopsies, establishing a state of adaptive resistance. This dynamic provides a biological rationale for combining OVs with PD-1 blockade: OVs initiate innate and adaptive immune activation, whereas subsequent PD-1 blockade interrupts the resulting inhibitory feedback, thereby sustaining cytotoxic T-cell function through synergistic mechanisms (77,78).

Acquired resistance to ICIs in melanoma also commonly results from genetic or epigenetic downregulation of key antigen-processing and presentation components, including TAP1, TAP2, and B2M, often accompanied by impaired downstream IFN-γ signal transduction. Although loss-of-function mutations in *JAK2* compromise IFN-γ-induced MHC class I expression and antigen presentation, they concurrently increase the susceptibility of tumor cells to direct OV-mediated oncolysis (69), revealing a vulnerability that can be exploited through virotherapy.

OVs can also be engineered to target alternative immune checkpoints and thereby counter therapy-induced immune evasion. To address the compensatory upregulation of non-PD-1 checkpoints following prolonged PD-1 blockade, next-generation OVs have been designed to deliver immunomodulatory transgenes targeting TIM-3, LAG-3, and TIGIT. For instance, the oncolytic vaccinia virus Yolk sac tumor (YST)-ovarian high-grade (OVH) has been shown to restore the tumor-immune balance in melanoma models characterized by coexpression of CTLA-4 and TIM-3 on terminally exhausted $CD8^+$ T cells and increased infiltration of $CTLA\text{-}4^+$ Tregs within the TME (79). Combining YST-OVH with anti-CTLA-4 or anti-TIM-3 antibodies has been shown to further enhance tumor immunogenicity and promote *de novo* antitumor T-cell responses. Single-cell T-cell receptor (TCR) sequencing and preclinical validation have also demonstrated that concurrent TIGIT blockade with PD-1 and LAG-3 blockade overcomes established resistance to standard ICI regimens in melanoma. Encoding an anti-TIGIT single-chain variable fragment (scFv) within the OV genome enables sustained, spatially restricted intratumoral delivery and produces significantly greater antitumor efficacy than systemic scFv administration in syngeneic melanoma models (80). Clinically, the phase II trial NCT02519322 demonstrated that the anti-LAG-3 antibody relatlimab combined with nivolumab induces durable pathological responses in patients with advanced melanoma (81). Building on these findings, engineering OVs to express LAG-3-blocking biologics for localized reversal of T-cell exhaustion represents a promising but still nascent strategy that warrants rigorous clinical investigation (Fig. 2).

Overcoming subtype-specific resistance in melanoma. The major melanoma subtypes, cutaneous melanoma (CM), acral melanoma (AM), mucosal melanoma (MM), and uveal melanoma (UM), differ substantially in their genomic architecture, tumor immune microenvironment, and clinical responsiveness to ICIs (82-84). These biological differences inform the rationale, design, and therapeutic potential of OV-based interventions. Whereas CM is characterized by a high tumor mutational burden (TMB) and relatively favorable sensitivity to ICIs, AM, MM, and UM consistently exhibit primary resistance to standard ICI regimens. This resistance reflects subtype-specific immunobiological constraints, including low TMB, impaired antigen presentation, and profoundly immunosuppressive or immune-excluded microenvironments, making patients with these subtypes high-priority populations for novel virotherapeutic strategies (85-87).

AM, the most prevalent melanoma subtype among Asian populations, has a distinct molecular profile characterized by a low prevalence of BRAF mutations and a high frequency of KIT mutations (88,89). Although AM has lower ORRs to ICIs than CM, OVs have demonstrated clinical activity in this subtype. Talimogene laherparepvec (T-VEC), the first OV approved by both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), has shown efficacy in AM. In 2020, Franke *et al* (90) reported the first documented case of histopathologically confirmed complete remission in an older patient with unresectable primary AM after ten intratumoral T-VEC injections; surgical resection was contraindicated in this patient (90). Nevertheless, robust prospective evidence remains limited. A phase Ib trial (NCT04197882) evaluating neoadjuvant T-VEC plus

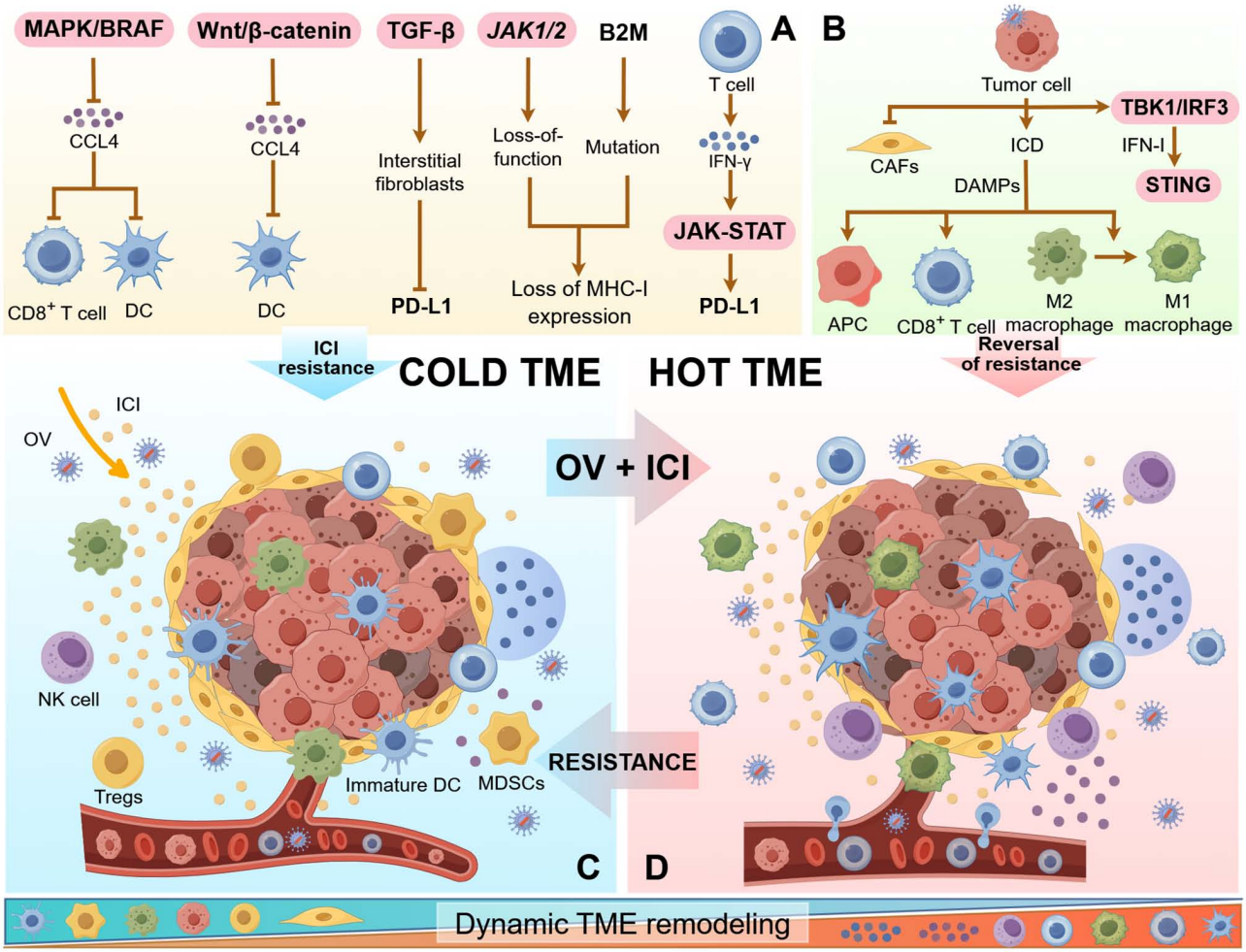

Figure 2. OVs reprogram the TME and synergize with ICIs to overcome resistance in melanoma. (A) Major mechanisms of resistance to ICIs. (B) Core mechanisms underlying OV-mediated reversal of ICI resistance. (C) Cold tumor microenvironment (Cold TME) without OV therapy. (D) Hot tumor microenvironment (Hot TME) following combination therapy with OV and ICI. TME, tumor microenvironment; ICIs, immune checkpoint inhibitors; OV, oncolytic virus; MAPK, mitogen-activated protein kinase; BRAF, B-Raf proto-oncogene, serine/threonine kinase; TGF-β, transforming growth factor-β; *JAK1/2*, Janus kinase 1/2; B2M, β2-microglobulin; CCL4, C-C motif chemokine ligand 4; IFN-γ, interferon-γ; DC, dendritic cell; PD-L1, programmed death ligand 1; MHC-I, major histocompatibility complex class I; STAT, signal transducer and activator of transcription CAFs, cancer-associated fibroblasts; ICD, immunogenic cell death; DAMPs, damage-associated molecular patterns; TBK1, TANK-binding kinase 1; IRF3, interferon regulatory factor 3; IFN-I, type I interferon; STING, stimulator of interferon genes; APC, antigen presenting cell; NK, natural killer; Tregs, regulatory T cells; MDSCs, myeloid-derived suppressor cells. This figure was created using Figdraw (https://www.figdraw.com).

pembrolizumab in 30 patients with resectable stage IIIB-IV M1a AM reported a pathological response rate (pRR) of 77.8%, substantially higher than the radiographic ORR of 36.6%, with a pathological complete response (pCR) rate of 14.8%. At the reported median follow-up, the 1- and 2-year recurrence-free survival (RFS) rates were 85.2 and 81.5%, respectively (91). Subsequent studies have identified an important role for STING signaling in AM-associated resistance to OV-based combination therapy, providing a mechanistic basis for understanding this resistance and developing new combination strategies (92-94).

MM is a rare and aggressive subtype for which the ORR to anti-PD-1/PD-L1 monotherapy is <15%, contributing to its poor prognosis (95). Igrelimogene litadenorepvec (TILT-123), an armed oAd engineered to replicate selectively within tumors while secreting TNF-α and IL-2, was evaluated in combination with TIL therapy in a phase I trial (NCT04217473). Among 17 patients with metastatic melanoma refractory to previous ICIs, the regimen achieved an ORR of 11.7% and a disease control rate (DCR) of 35%, with a median overall survival (OS) of 447 days and six long-term survivors (>600 days) (96). Notably, one patient with metastatic MM achieved a durable complete remission lasting >4 years. This protocol eliminated the need for myeloablative conditioning and high-dose systemic IL-2, both of which are standard components of conventional TIL therapy, thereby substantially reducing treatment-related toxicity. Biomarker analyses confirmed intratumoral persistence and systemic biodistribution of TILT-123 DNA, accompanied by increased $CD8^+$ T-cell infiltration in both injected and noninjected lesions. These findings indicate the induction of abscopal antitumor immunity, a feature of particular relevance given the frequent multicentric metastatic pattern of MM (97).

UM, the most common primary intraocular malignancy in adults, carries an ~50% risk of distant metastasis and a median OS of ~12 months after the diagnosis of metastatic disease. Response rates to single-agent ICIs are <10%, making UM the melanoma subtype with the greatest unmet need and

the poorest outcomes with immunotherapy (98,99). RP2, a second-generation HSV-1-based oncolytic immunotherapy, is genetically engineered to express human granulocyte-macrophage colony-stimulating factor (GM-CSF), a fusogenic glycoprotein, and a CTLA-4-targeting single-chain variable fragment (anti-CTLA-4 scFv). In the phase I RP2-101 trial (NCT04336241), RP2 monotherapy or RP2 plus nivolumab achieved an ORR of 33.3% among 17 evaluable patients with metastatic UM, many of whom had previously received ICI therapy (100,101). Building on these findings, the global, randomized, open-label phase II/III RP2-202 trial (NCT06581406) is enrolling ~280 treatment-naïve patients with metastatic UM and randomly assigning them in a 1:1 ratio to RP2 plus nivolumab or ipilimumab plus nivolumab. The primary endpoints are OS and progression-free survival (PFS), and the results are expected to inform future standards of care for metastatic UM (102). Beyond RP2, several OV platforms have undergone early-phase evaluation in metastatic UM, including VSV-IFNβ-tyrosinase-related protein 1 (TYRP1), systemically administered ICOVIR-5 [an arginine-glycine-aspartic acid (RGD)-modified oAd], and coxsackievirus A21 (CVA21) combined with ipilimumab (103-105). While objective radiographic responses were not consistently observed in these initial studies, they provided important safety, pharmacokinetic, and immunomodulatory data to support further OV development in this particularly challenging disease setting.

The biological differences among melanoma subtypes require a precision-guided, mechanism-informed approach to OV development. For AM, priority should be given to optimizing neoadjuvant multimodal regimens, particularly OV-ICI combinations, to deepen pathological responses and sustain long-term recurrence-free survival. For MM, rational integration of OVs with adoptive cellular therapies or molecularly targeted agents represents a promising strategy that warrants accelerated clinical evaluation. For UM, the principal unmet need is the development of next-generation armed OVs capable of both disrupting its immune-privileged, T-cell-excluded microenvironment and delivering coordinated immunostimulatory payloads, including cytokines, immune checkpoint modulators, and ECM-remodeling effectors.

4. Clinical translation and efficacy analysis of combination therapy

The preceding mechanistic analysis indicates that OVs may overcome resistance to ICIs through multiple mechanisms, including the induction of ICD, activation of innate and adaptive immune signaling pathways, and remodeling of the TME. However, these mechanistic insights are derived predominantly from preclinical models, including immortalized cancer cell lines, subcutaneous syngeneic or xenograft mouse models, and selected patient-derived xenograft systems. These models have inherent limitations in recapitulating the human tumor ecosystem and frequently fail to reproduce the spatial heterogeneity, stromal complexity, ECM architecture, and dynamic immunobiological processes, including immune cell infiltration, retention, and exclusion, observed in clinical tumors. Consequently, while these findings support the biological plausibility of OV-ICI combination strategies, they do not provide direct evidence of clinical efficacy. Robust validation in rigorously designed clinical trials remains essential.

Early-phase clinical studies focused primarily on establishing the safety and preliminary efficacy of OV monotherapy. The phase III trial of T-VEC, the first OV approved by the U.S. FDA for unresectable melanoma, reported an ORR of 31.5%, with CRs observed in 16.9% of patients (106). Response durations ranged from several months to multiple years, suggesting the induction of durable, antigen-specific immune memory following OV administration. Similarly, a clinical study evaluating the intratumoral administration of a recombinant human adenovirus serotype 5 vector demonstrated tumor regression in 70% of enrolled patients, with a favorable tolerability profile (107). Collectively, these findings established the clinical feasibility of OVs while highlighting a major limitation of monotherapy: Although local control of injected lesions was substantial, systemic disease, particularly distant metastases, remained inadequately controlled. This limitation prompted the development of rational combination strategies.

In recent years, the clinical success of ICIs has shifted therapeutic development toward potentially synergistic OV-ICI combinations. Preclinical studies have shown that co-administration of VSV-CHIKV and anti-PD-1 monoclonal antibodies significantly prolongs survival in murine tumor models (108,109). Mechanistically, OV-induced pyroptosis initiates local inflammatory responses, converts immunologically 'cold' tumors into 'hot' microenvironments, increases $CD8^+$ T-cell infiltration, and thereby enhances responsiveness to checkpoint blockade (108). Nevertheless, successful clinical translation requires several practical challenges to be addressed, including optimization of treatment sequence, route of administration, dosing interval, and management of OV-associated adverse events. The current clinical development landscape encompasses a heterogeneous range of OV platforms, from the FDA-approved HSV-1-derived T-VEC to emerging candidates such as CVA21 and the adenovirus-based ONCOS-102. These agents are being evaluated in phase I-III trials as monotherapies or in combination with anti-PD-1/PD-L1 or anti-CTLA-4 antibodies. Beyond conventional endpoints such as the ORR in treatment-naïve melanoma, ongoing trials are assessing whether OV-ICI combinations can overcome primary resistance in PD-1 inhibitor-refractory populations and delay the development of acquired resistance. Given the substantial heterogeneity among trials in viral backbone, dosing regimen, patient selection, and concomitant therapy, the following section provides a systematic analysis of the available clinical evidence stratified by virus class, with emphasis on clinically meaningful outcomes, current translational limitations, and potential strategies for refining combination immunotherapy (Table I) (10,77,78,80,91,101,103,105,110-118).

Collectively, the phase I/II trials described above provide cautiously encouraging evidence. OV-ICI combinations have produced durable objective responses in patients with melanoma across distinct resistance phenotypes, including primary, acquired, and adjuvant-refractory disease, and have demonstrated abscopal responses in non-injected metastatic lesions. Next-generation OV platforms such as T-VEC and RP1 have shown clinically meaningful antitumor activity in patients previously treated with anti-PD-1 therapy, potentially

Table I. Results of major clinical trials of oncolytic viruses in combination with immune checkpoint inhibitors.

Virus platform	Combination Regimen	Phase	Sample size	Patient population	Prior PD-1 exposure	ORR	CR	Median DOR	Key limitations	(Refs.)
T-VEC (HSV-1) (NCT01740297)	+Ipilimumab	Ib	19	Treatment-naïve, unresectable stage IIIB-IVM1c	0%	50.0%	22.0%	NR	Small sample size	(110)
T-VEC (HSV-1) (NCT01740297)	+Ipilimumab	II	98	Predominantly treatment-naïve (PD-1-refractory rare)	≤2.5%	39.0%	20.4%	69.2 mo	Did not enroll PD-1-refractory patients	(111)
T-VEC (HSV-1) (NCT02263508)	+Pembrolizumab	Ib	21	Stage IIIB-IV melanoma	0%	61.9%	42.9%	NR	Small sample; limited M1c enrollment	(77,112)
T-VEC (HSV-1) (NCT02263508)	+Pembrolizumab	III	346	Anti-PD-1-naïve advanced melanoma	0%	48.6%	17.9%	43.7 mo	Enrollment bias toward stage III/IVM1a (potentially less aggressive disease)	(10)
T-VEC (HSV-1) (NCT04068181)	+Pembrolizumab	II	72	PD-1-refractory advanced (4 cohorts: primary/acquired resistance, adjuvant relapse)	100%	0%-46.7% (marked cohort variation)	0%-13.3%	NR	Cohort 1 (primary resistance) ORR 0%; single-arm design	(113)
T-VEC (HSV-1) (NCT04330430)	+Nivolumab	II	24	Treatment-naïve, resectable stage IIIB-IVM1a	0%	65.0% (MPR)	NR	NR	Injectable lesion required; single-arm	(114)
ONCOS-102 (adenovirus)	+Pembrolizumab	Pilot	20	Anti-PD-1-resistant advanced	100%	35.0%	5.0%	NR	Very small sample; pilot study	(78)
RP1 (HSV-1) (NCT03767348)	+Nivolumab	I/II	140	Advanced melanoma progressing after anti-PD-1 failure	100% (65.7% primary resistance)	32.9% (34.1% in primary resistance)	15.0%	33.7 mo	Single-arm design	(115,116)
V937 (Coxsackievirus A21) (NCT02565992)	+Pembrolizumab	Ib	36	Metastatic/unresectable stage IIIB-IV (8 had prior ICI)	22.2%	47.0% (38% in prior-ICI subset)	22.0%	NR	Small sample; single-arm	(117)
Gebasaxturev (Coxsackievirus A21) (NCT04303169)	+Pembrolizumab	I/II	25	Treatment-naïve, resectable stage IIIB-IIID	0%	32.0%	0%	NR	Small sample size	(80)
V937 (Coxsackievirus A21) (NCT02307149)	+Ipilimumab	Ib	50	Metastatic/unresectable stage IIIB/C or IV	66.0%	30.0% (21% in prior-ICI subset)	10.0% (6% in prior-ICI subset)	8.8 mo	Single-arm; small sample	(118)

Table I. Continued.

Virus platform	Combination Regimen	Phase	Sample size	Patient population	Prior PD-1 exposure	ORR	CR	Median DOR	Key limitations	(Refs.)
V937 (Coxsackievirus A21) (NCT03408587)	+Ipilimumab	Ib	11	Metastatic UM	NR	0%	0%	NR	Very small sample	(105)
RP2 (HSV-1) (NCT03767348)	+Nivolumab	I	85 (17 UM;11 CM)	Advanced solid tumors	42%	17.3% overall (33.3% in UM subset)	NR	22.1 mo	Broad tumor types; modest UM subset	(101)
VSV-IFNβ-TYRP1 (VSV)	+Ipilimumab/ Nivolumab	I	8	Metastatic UM	83.3%	12.5%	0	NR	Extremely small; no disaggregated data	(103)
orienX010 (HSV) (NCT04197882)	+Toripalimab	Ib	30	Resectable AM	0%	36.6%	3.3%	NR	Small sample; single-center	(91)

HSV-1, herpes simplex virus type 1; PD-1, programmed cell death protein 1; ORR, objective response rate; CR, complete response; DOR, duration of response; NR, not reached; MPR, major pathological response; ICI, immune checkpoint inhibitor; UM, uveal melanoma; CM, cutaneous melanoma; VSV, vesicular stomatitis virus; IFNβ, interferon β; TYRP1, tyrosinase-related protein 1; AM, acral melanoma; mo, months.

providing additional options for those with refractory disease after standard-of-care treatment (113,115,116). Nevertheless, rigorous evaluation of the current evidence reveals several methodological limitations, including non-randomized study designs, heterogeneous patient selection criteria, variable dosing schedules, and endpoint definitions without standardized immunologic or survival-based validation. These limitations may inflate preliminary efficacy estimates and restrict the external validity and generalizability of the findings to phase III settings. Therefore, despite the potential of these regimens, the phase I/II trials require careful appraisal with respect to sample-size adequacy, patient heterogeneity, selection bias related to injected lesions, and limitations of the selected endpoints. Such evaluation is necessary to provide a more objective evidence base for optimizing subsequent phase III trial designs and informing clinical decision-making.

From a methodological perspective, most landmark trials enrolled relatively small cohorts. The phase Ib/II study of T-VEC plus pembrolizumab (NCT04068181) enrolled 72 patients across four prespecified cohorts, each comprising only 17-19 participants; the ONCOS-102 plus pembrolizumab study enrolled 21 patients; and the V937 plus ipilimumab trial (NCT03408587) included only 11 evaluable patients (78,105,113). Although such early-phase studies are appropriately designed to generate hypotheses rather than provide definitive estimates of efficacy, their limited statistical power results in wide 95% confidence intervals and imprecise effect estimates. For example, cohort 3 of the T-VEC-pembrolizumab trial reported an ORR of 40.0% (95% CI, 16.3-67.7%), whereas cohort 4 reported an ORR of 46.7% (95% CI, 21.3-73.4%). These intervals span nearly 50 percentage points, precluding reliable estimation of the true treatment benefit (113). Even the comparatively larger trial of RP1 plus nivolumab (NCT03767348), which enrolled 140 patients, reported an ORR of 33.3% (95% CI, 25.2-41.3%) (115,116), indicating persistent uncertainty around the point estimate. Small-sample studies are also inherently susceptible to effect-size inflation, as reflected by the frequent failure of promising phase I/II findings to be reproduced in adequately powered phase III confirmatory trials.

Substantial inter-cohort and inter-trial heterogeneity also exists in treatment line, prior therapeutic exposure, and the biological basis of resistance. In NCT04068181, cohort 1, comprising patients with primary resistance to anti-PD-1 therapy, had an ORR of 0%, whereas cohort 2, comprising patients with acquired resistance, had an ORR of 6.7%. These cohorts represent mechanistically distinct resistance phenotypes. By contrast, cohorts 3 and 4, which included patients with recurrence after adjuvant anti-PD-1 therapy, achieved markedly higher ORRs of 40.0-46.7% (113). Similarly, in the RP1-nivolumab trial (NCT03767348), 65.7% of participants had primary resistance, and 46.4% had previously received dual ICI therapy with anti-PD-1 and anti-CTLA-4 antibodies (115,116). This pronounced clinical and biological heterogeneity complicates cross-study comparisons and suggests that the efficacy of OV-ICI combinations is context-dependent, varying according to the underlying resistance mechanism, composition of the tumor immune microenvironment, and cumulative treatment burden. Moreover, phase I/II trials commonly apply stringent eligibility criteria that favor patients with preserved

performance status [Eastern Cooperative Oncoclogy Group (ECOG) 0-1], limited comorbidity, and fewer previous lines of systemic therapy. Although these characteristics may improve treatment tolerability and observed response rates, they substantially limit extrapolation to real-world and phase III populations, which often include patients with greater frailty and more treatment-refractory disease.

Second, methodological limitations, particularly selection bias in lesion selection, pose substantial challenges to the interpretation and generalizability of OV clinical trials. Nearly all trials of intratumoral OVs require patients to have at least one accessible superficial or otherwise injectable tumor lesion. This requirement introduces systematic selection bias because patients with exclusively deep visceral metastases and no concomitant superficial disease are routinely excluded. Furthermore, lesion selection is generally nonrandom and guided by clinical considerations. Investigators preferentially select lesions that are anatomically accessible, moderate in size, and associated with lower procedural risk rather than those with the greatest immunogenic potential or clinical urgency. Consequently, the high response rates observed in injected lesions, which may reflect localized exposure to high viral concentrations and direct oncolysis, do not necessarily indicate systemic antitumor activity. For instance, in the phase III OPTiM trial, the CR rate was 47% in injected lesions but only 22% in noninjected, nonvisceral lesions (119). Similarly, in the T-VEC plus pembrolizumab trial (NCT04068181), cohort 3 had an immune-related ORR (iORR) of 53.3%, compared with a Response Evaluation Criteria in Solid Tumors (RECIST)-defined ORR of 40.0% (113). Conversely, reliance on patient-level ORR, as defined by RECIST criteria requiring assessment across all target lesions, may attenuate the apparent therapeutic signal arising from the potent local effects of OV therapy. The RP1 plus nivolumab trial (NCT03767348) partially addressed this limitation by incorporating injections into deep or visceral lesions. Patients who received such injections (n=22) achieved an ORR of 40.9%, compared with 29.8% among those who received injections into superficial lesions only (115,116). However, deep injections involve non-negligible procedural risks, including pneumothorax in 5.8% of lung injection procedures (3/52), which may limit their feasibility and scalability. Although intravenous systemic delivery could theoretically overcome these anatomical constraints, it remains limited by pharmacokinetic barriers, including rapid neutralization by pre-existing antibodies, nonspecific hepatic sequestration, and inadequate tumor vascular permeability.

Notably, approximately half of the cited trials enrolled exclusively patients previously treated with anti-PD-1 therapy, a population that differs substantially from the heterogeneous cohort of treatment-naïve and previously treated patients that supported the initial regulatory approval of T-VEC monotherapy. Although this focus addresses an important unmet need for effective salvage treatment after PD-1 inhibitor failure, it also introduces substantial confounding. Patients who experienced early progression or severe immune-related adverse events during prior PD-1 therapy are likely to be underrepresented, resulting in a selected survivor population. Moreover, primary and acquired resistance arise through distinct biological mechanisms, and pooling these groups in efficacy analyses may obscure differential treatment effects. In the T-VEC plus pembrolizumab trial (NCT04068181), as aforementioned, cohort 1, comprising patients with primary resistance, had an ORR of 0%, whereas cohort 2, comprising patients with acquired resistance, achieved an ORR of 6.7% (113), underscoring the importance of stratifying patients by resistance phenotype.

The use of ORR as a primary endpoint in early-phase OV trials has inherent conceptual and practical limitations. ORR captures static volumetric changes at a single time point and does not reflect the dynamic immunobiological processes central to the mechanisms of action of OVs, including the kinetics of immune activation, clonal expansion of tumor-specific T cells, and establishment of durable immunological memory. Moreover, discrepancies between lesion-level and patient-level responses persist across studies. High local response rates in injected lesions do not necessarily translate into systemic efficacy. Because patient-level ORR requires designated target lesions to meet RECIST criteria for a partial response or CR, it may underestimate the potent local effects of OVs. As noted above, cohort 3 of the T-VEC plus pembrolizumab trial showed a marked difference between the iORR (53.3%) and RECIST-defined ORR (40.0%) (113). In the ONCOS-102 plus pembrolizumab trial, 53% of patients exhibited shrinkage of noninjected lesions, although this reduction did not meet the formal criteria for a partial response (78). Similarly, the OPTiM trial demonstrated a clear gradient in efficacy: CR rates were 47% in injected lesions and 22% in noninjected, nonvisceral lesions, with substantially lower rates in visceral lesions (119). This hierarchical pattern supports the presence of a measurable but limited abscopal effect that is insufficient to eliminate the need for direct treatment of all clinically relevant lesions.

The phase III randomized, double-blind, placebo-controlled trial comparing T-VEC plus pembrolizumab with placebo plus pembrolizumab (NCT02263508) failed to meet its primary endpoints of PFS and OS, with no statistically significant improvement over pembrolizumab monotherapy (10). This outcome contrasts with the encouraging efficacy signals observed in the earlier phase II study (NCT04068181), highlighting the difficulty of translating preliminary activity into definitive clinical benefit under rigorous confirmatory trial conditions. Several interrelated factors may have contributed to this discrepancy. First, in the first-line metastatic setting, in which patients are generally responsive to ICIs, the incremental immunostimulatory effect of OV therapy may be limited while adding procedural complexity and potential safety risks. Conversely, in later-line settings, particularly among patients with immunologically 'cold' tumors refractory to previous ICIs, the combined oncolytic and immune-priming effects of OVs may be more apparent and clinically relevant. Second, phase I/II trials frequently enroll highly selected populations. Participants tend to be younger, have better ECOG performance status, typically 0-1, fewer comorbidities, and have a lower baseline tumor burden. These favorable prognostic characteristics, together with the use of ORR as a primary endpoint, which is sensitive to short-term antitumor activity, increase the likelihood of detecting an apparent treatment effect. In contrast, phase III trials assess clinically meaningful time-to-event endpoints, including PFS and OS, in larger and more heterogeneous populations and use active comparator arms consistent with contemporary standards of

care, which continue to evolve as new evidence emerges. Third, methodological differences further complicate interpretation. Numerous early-phase studies use single-arm designs or historical controls, whereas phase III trials require prospective randomized comparisons with current standard treatments.

Collectively, these considerations indicate that OV-ICI combination therapy remains investigational rather than an established standard of care, with potential benefits most likely confined to biologically and clinically defined subgroups. Candidate populations that may derive the greatest benefit include patients with at least one injectable superficial lesion, recurrence after adjuvant PD-1 inhibitor therapy, an ECOG performance status of 0-1, and an intermediate tumor burden.

5. Conclusions and prospects

The combination of OVs and ICIs is supported by a strong mechanistic rationale, and preclinical studies and early-phase clinical trials have demonstrated potentially synergistic antitumor activity. However, the phase III randomized, double-blind, placebo-controlled trial of intratumoral T-VEC plus pembrolizumab (NCT02263508) failed to meet its primary endpoints of PFS and OS in an unselected first-line melanoma population (10). These findings indicate that the combination did not provide superior efficacy to ICI monotherapy in this broad patient population. Therefore, despite encouraging exploratory signals, OV-ICI combination therapy remains investigational and has not received regulatory approval or guideline endorsement as a standard-of-care treatment for melanoma. Emerging evidence nevertheless suggests clinically meaningful activity in biologically defined subgroups, particularly patients with accessible superficial lesions who experience disease recurrence after adjuvant anti-PD-1 therapy, although substantial interpatient heterogeneity in treatment response remains.

In light of the current evidence, future clinical development should follow a stratified, hypothesis-driven framework. Near-term priorities should emphasize precision rather than broad expansion, with rigorous biomarker-informed patient selection and prospectively designed RCTs to establish incremental clinical benefit. Patients with acquired resistance to adjuvant anti-PD-1 therapy and injectable cutaneous or subcutaneous lesions represent a high-priority population for validation. Treatment sequencing should also be optimized rationally. For example, intratumoral OV administration could be used to prime the TME by inducing ICD and converting immunologically 'cold' tumors into 'hot' tumors before ICI initiation. Physical enhancement strategies, including low-dose radiotherapy or focused ultrasound, may further improve viral transduction and augment local immune activation. Nevertheless, several translational challenges remain unresolved. First, the lack of validated predictive biomarkers limits reliable patient stratification. Prospective biomarker-enriched trials are therefore needed to determine whether baseline expression of co-inhibitory receptors, such as TIGIT, LAG-3, and B7-H3, within the TME can guide treatment selection. Second, delivery constraints remain substantial. Intratumoral injection excludes patients with exclusively deep visceral metastases and introduces selection bias based on lesion accessibility, whereas intravenous delivery is limited by rapid neutralization by pre-existing antibodies, off-target hepatic sequestration, and inefficient tumor accumulation. Third, conventional RECIST-based criteria have limited sensitivity for evaluating therapies that combine localized oncolysis with systemic immune effects (120,121). The persistent discrepancy between lesion-level radiographic responses and patient-level ORRs highlights the need for consensus-based, mechanism-aligned endpoints tailored to local-systemic therapeutic strategies.

Promising but higher-risk directions include next-generation engineered OVs, such as the NeoViron platform encoding patient-specific neoantigens (122); stimuli-responsive delivery systems, including chimeric antigen receptor-modified cell membrane-coated vectors and ultrasound-triggered pyroptosis-inducing platforms (123); and multimodal strategies combining OVs with radiotherapy, TIL therapy, or dual blockade of emerging checkpoints, such as LAG-3 and TIGIT (80,81). These approaches are intended to overcome anatomical delivery barriers, compensate for limited neoantigen availability, and reverse severe T-cell dysfunction. Although scientifically plausible, their clinical translation will require prolonged development and may be associated with substantial attrition. Progress should therefore proceed incrementally through phased proof-of-concept studies conducted under rigorous scientific and ethical oversight.

In summary, OV-ICI combination therapy is supported by a strong biological rationale for melanoma treatment, together with mechanistic evidence and early clinical signals, including documented abscopal effects after intratumoral OV administration in selected patients. However, the available evidence remains heterogeneous and context-dependent. Major unresolved challenges include the absence of reliable predictive biomarkers, the technical limitations of systemic OV delivery, particularly for deep-seated metastases, and the lack of definitive phase III evidence demonstrating superiority over established first-line regimens, such as dual PD-1/CTLA-4 blockade or BRAF/MEK inhibition (124). Continued progress will require close integration of virology, tumor immunology, nanomedicine, and clinical oncology. Rational combination strategies and methodologically rigorous, biomarker-integrated clinical validation will be necessary to determine the therapeutic value of this approach for patients with treatment-resistant melanoma.

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Authors' contributions

YH was responsible for literature retrieval, screening and data extraction, writing the first draft of the paper and preparation

of the figures. DP was responsible for research conception and design, determining the review framework, guiding the research process, making key revisions to the manuscript and ultimately approving its publication. WL participated in literature retrieval and data analysis, assisting in writing some parts of the manuscript and preparation of Table I. HC participated in literature screening and data extraction and revised the manuscript. YG participated in literature collection and organization, assisting in data verification. CG participated in the technical design and editing of the figures and the table, and assisted in reference formatting, deduplication, and citation verification using literature management software. YZ participated in literature retrieval and preliminary analysis and revised the manuscript. All authors reviewed, read and approved the final manuscript and agreed to be responsible for all aspects of the work. Data authentication is not applicable.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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