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## **Harnessing the immunostimulatory properties of oncolytic reovirus for anticancer immunotherapy**

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PART

# C

**Blockade of TGF- $\beta$  signaling to improve  
reovirus-based immuno-therapy**



# CHAPTER 6

## Immunotherapeutic Potential of TGF- $\beta$ Inhibition and Oncolytic Viruses

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## ABSTRACT

In cancer immunotherapy, a patient's own immune system is harnessed against cancer. Immune checkpoint inhibitors release the brakes on tumor-reactive T cells and therefore are particularly effective in treating certain immune-infiltrated solid tumors. In contrast, solid tumors with immune-silent profiles show limited efficacy of checkpoint blockers due to several barriers. Recent discoveries highlight transforming growth factor- $\beta$  (TGF- $\beta$ )-induced immune exclusion and a lack of immunogenicity as examples of these barriers. In this review, we summarize preclinical and clinical evidence that illustrates how the inhibition of TGF- $\beta$  signaling and the use of oncolytic viruses (OVs) can increase the efficacy of immunotherapy and discuss the promise and challenges of combining these approaches with immune checkpoint blockade.

## HIGHLIGHTS

- Immune checkpoint blockade is not effective in immune-excluded and immune-desert tumors due to an immunosuppressive tumor microenvironment and the absence of activated T cells.
- TGF- $\beta$  is a pleiotropic cytokine that contributes to immune exclusion and evasion in various cancer types.
- The therapeutic efficacy of oncolytic viruses is built on the recruitment of T cells and the induction of tumor-reactive immunity.
- Oncolytic virotherapy and inhibition of TGF- $\beta$  signaling, either alone or in combination, are two emerging approaches to increase the susceptibility of immune-silent tumors to immune checkpoint therapy.

## THE IMMUNE PROFILE OF SOLID TUMORS CAN DETERMINE THE EFFICACY OF IMMUNOTHERAPY

Our immune system is able to respond to invading pathogens and initiate a protective immune response. Although malignant cells are much more similar to the host than pathogens are, they still differ genetically, metabolically, and morphologically from normal cells and can therefore be recognized by the adaptive immune system, a trait called **immunogenicity** (see Glossary). In **Box 1** we provide more information about processes involved in antitumor immunity. **Immunotherapy** is being extensively studied as a new modality of cancer treatment for a wide variety of tumors. In contrast to conventional therapies that directly target the proliferation, survival, or metabolic activity of tumor cells, cancer immunotherapy is directed towards immune cells with the purpose of eliciting a durable and effective anticancer immune response.

### Box 1: Priming of tumor-specific T cells

Recognition of tumors by T cells requires the expression of tumor antigens (TAs), aberrant proteins, or peptides beyond the normal repertoire that alert the adaptive immune system that the tumor cell is no longer healthy. Different classes of tumor antigens have been identified, of which **neoantigens** are the most tumor-specific. Neoantigens arise from genetic mutations in a tumor cell that give rise to a novel protein or peptide sequence. The number of mutations varies significantly per tumor type and it is believed that tumors with a high mutational burden are more immunogenic, display a higher immune infiltrate, and are more responsive to immune checkpoint inhibitors than tumors with a low mutational burden (1).

Whereas CTLs are believed to be the main T-cell subset responsible for eliminating cancer cells, CD4<sup>+</sup> T helper cells are of vital importance in shaping the tumor-specific T-cell response (2). Priming of naive CD4<sup>+</sup> and CD8<sup>+</sup> T cells towards effective tumor-specific CD4<sup>+</sup> helper and CD8<sup>+</sup> cytotoxic T cells, respectively, is a multifaceted process that requires uptake, processing, and presentation of TAs by dendritic cells (DCs) in the context of inflammation (3). The sensing of inflammatory signals, derived from **pathogen-associated molecular patterns** (PAMPs) or **damage-associated molecular patterns** (DAMPs) by a DC induces a differentiation process called maturation. DC maturation is characterized by upregulated amounts of costimulatory molecules, antigen processing, and presentation pathways, and the production of type 1 helper T cell (Th1) skewing cytokines.

The tumor microenvironment (TME) is usually not inflammatory in nature, and additionally, tumors use several strategies to actively suppress the immune system, leading to T-cell ignorance. Activating tumor-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells by applying TAs in the optimal context together with a DC maturing agent is in some cases achieved by cancer vaccine platforms, such as synthetic peptide-based vaccines or dendritic cell-based vaccines (4-6). However, tumors often have the ability to escape immune recognition and destruction, which highlights the need for effective immunotherapeutic strategies to overcome these barriers.

The tumor immune profile is an important determinant to guide immunotherapeutic strategies (7,8). Clinical responses to immune checkpoint inhibition mostly occur in patients with an **immune-infiltrated** tumor phenotype, which displays a pre-existing but often dysfunctional immune response (9). In contrast to immune-infiltrated tumors, **immune-excluded or immune-desert** (also described as immune-silent) tumors are less susceptible to checkpoint inhibition because **tumor-infiltrating T cells** (TILs) are absent (10). Strategies to convert immune-silent tumors into immune-active tumors are desperately needed to broaden the fraction of patients that might benefit from immune checkpoint therapy. In this review, we discuss two emerging approaches that

might be harnessed on their own or in combination to enhance the efficacy of immune checkpoint inhibition in immune-silent tumors (**Figure 1, Graphical Abstract**).

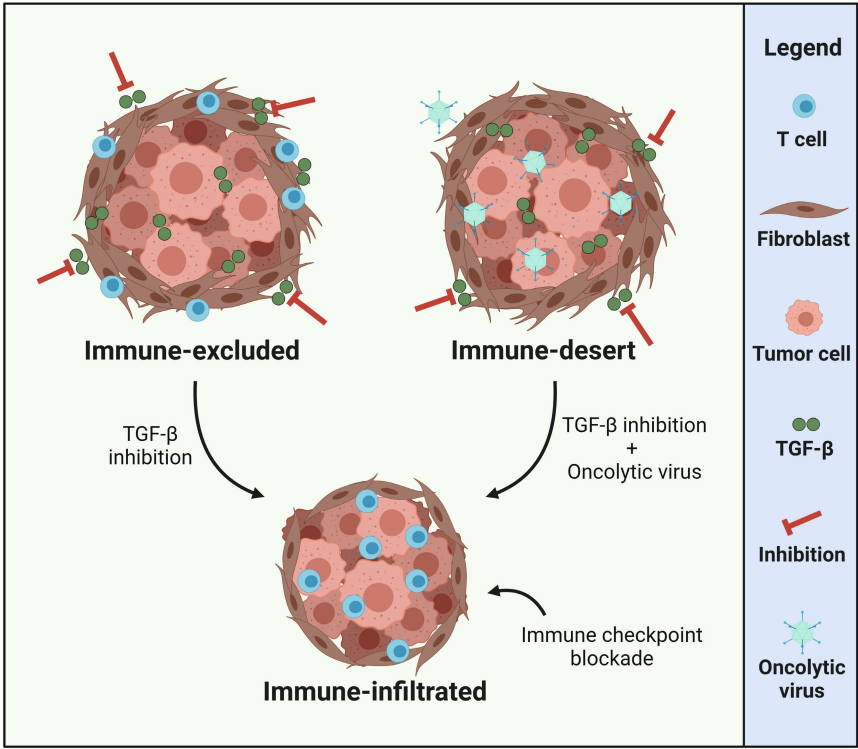


**Figure 1, Graphical Abstract. Combining TGF- $\beta$  inhibition with oncolytic viruses to increase efficacy of immune checkpoint blockade in solid tumors.** Immune checkpoint blockade is mostly effective in immune-infiltrated tumors where T cells (blue) are present in the tumor nests (red) but may be dysfunctional. In immune excluded tumors, T cells are present but remain trapped in the stromal regions (brown) surrounding the tumor nests. TGF- $\beta$  (dark green) inhibition is expected to change the phenotype of immune-excluded tumors towards an immune-infiltrated phenotype. In immune-desert tumors, a T cell response is absent. Combination strategies of oncolytic viruses with TGF- $\beta$ -inhibition may also convert immune-desert tumors to immune-infiltrated tumors, facilitating effective immune checkpoint blockade for all immune phenotypes in solid tumors.

First, we discuss the inhibition of **transforming growth factor  $\beta$  (TGF- $\beta$ )** signaling to enhance the efficacy of checkpoint blockade therapy given that recent evidence suggests that TGF- $\beta$  may be a key factor in regulating immune exclusion and immunosuppression in solid tumors. Additionally, we highlight the potency of **oncolytic viruses (OVs)** to convert solid tumors from an immune-silent phenotype towards an immune-infiltrated phenotype. Lastly, we theorize how a combination of TGF- $\beta$  inhibition, OV and immune checkpoint blockade may be superior in efficacy compared to strategies that contain only two of these three aspects.

## IMMUNE CHECKPOINT INHIBITION CAN REINVIGORATE DYSFUNCTIONAL ANTITUMOR RESPONSES IN IMMUNE-INFILTRATED TUMORS

The discovery of immune checkpoints boosted the development of immunotherapeutic strategies against certain cancers. **Programmed cell death protein 1** (PD-1) and **cytotoxic T lymphocyte-associated antigen 4** (CTLA-4) are well-recognized immune checkpoint receptors that can limit antitumor immunity using distinct mechanisms. CTLA-4 prevents T-cell activation by competing with the costimulatory molecule CD28 for binding to their common ligands CD80 and CD86 (11). In contrast, PD-1 induces **T-cell anergy** or **T-cell exhaustion** after binding to one of its ligands, PD-L1 or PD-L2, expressed on the surface of tumor cells and/or immune cells (10,12). The use of blocking antibodies specific for these immune checkpoint axes can prevent or overcome T lymphocyte dysfunction and reinvigorate potent CD8<sup>+</sup> T-cell-mediated antitumor immune responses, as has been demonstrated in clinical practice for hematological malignancies such as acute myeloid leukemia, as well as in solid tumors such as melanoma, lung, bladder and head and neck cancers (10,13). In addition to the CTLA4-CD80/86 and the PD-1-PD-L1/L2 axes, other coinhibitory receptor targets, such as lymphocyte activation gene 3 (LAG-3) (14), T-cell immunoglobulin and mucin-domain containing protein 3 (TIM-3) (15) and C-type lectin receptor NKG2A (16,17), are currently being investigated in either preclinical studies or clinical trials for a wide variety of both hematological and solid cancers. Although checkpoint inhibition is able to induce dramatic responses in some types of cancer, the response rate in general ranges from 10-40% and heavily depends on the cancer type and the development of resistance during disease progression (18). Factors associated with a beneficial response to checkpoint blockade therapies include a high total number of mutations in tumor cell DNA (1), the presence of an interferon gene signature, the expression of proinflammatory and T-cell-recruiting chemokines such as CXCL9 and CXCL10, the presence of CD8<sup>+</sup> T lymphocytes in close proximity to tumor cells, and high PD-L1 expression, in particular on infiltrating immune cells (10,19-22). An immune cell-infiltrated tumor without a clinical response may suggest a pre-existing but dysfunctional tumor-specific CD8<sup>+</sup> T-cell response (23).

One category of immune-silent tumors with relatively low susceptibility to checkpoint inhibition includes tumors with an immune-excluded phenotype, such as colorectal cancer, ovarian cancer, pancreatic ductal adenocarcinoma, and vulvar squamous cell carcinoma (24,25, reviewed in 26). Immune-excluded tumors are characterized by the presence of CD8<sup>+</sup> T cells in the tumor-surrounding tumor stromal regions, but these T cells fail to infiltrate into the tumor beds (**Figure 1**) (27). The presence of stroma including **cancer-associated fibroblasts (CAFs)**, extracellular matrix components such as collagen, and cells of the myeloid lineage, such as the so-called **myeloid-derived suppressor cells (MDSCs)** (28) and **tumor-associated macrophages (TAMs)** (29), not only represents a physical barrier but also induces an

immunosuppressive **tumor microenvironment (TME)**, which limits T-cell infiltration into tumor nests (26,30). Hence, it is necessary to overcome the physical barrier and modify the immunosuppressive TME in immune-excluded tumors to facilitate T-cell migration through the stromal region into the tumor cell nests, where these immune cells can fully exert their tumoricidal function. An additional type of immune-silent tumors that exhibits low susceptibility to checkpoint blockade is the immune-desert phenotype. Immune-desert tumors lack the presence of T cells completely and require preceding T-cell activation (31). Below, we discuss promising methods for achieving T-cell infiltration into immune-desert tumors.

## **OVERCOMING IMMUNOSUPPRESSION VIA TGF- $\beta$ SIGNALING INHIBITION FOR IMMUNE-EXCLUDED TUMORS**

### ***TGF- $\beta$ as a mediator of immunosuppression***

The secreted cytokine TGF- $\beta$  is one of the key factors believed to be responsible for immune exclusion and suppression in certain types of cancer, such as pancreatic cancer, non-small cell lung cancer, and colon cancer (32-34). In premalignant lesions, TGF- $\beta$  signaling suppresses tumor growth by inducing apoptosis and inhibiting cell proliferation (35). However, during tumor progression, tumor cells become insensitive to TGF- $\beta$ -induced cytostatic effects, and TGF- $\beta$  functionally switches into acting as a tumor-promoting cytokine by promoting cancer cell migration and invasion, extracellular matrix (ECM) remodeling, **epithelial-to-mesenchymal transition (EMT)** and the formation of an immunosuppressive TME (36). TGF- $\beta$  induces its prometastatic programs directly via cell surface TGF- $\beta$  type I and type II serine/threonine kinase receptors (TGF- $\beta$ RI and TGF- $\beta$ RII) and intracellular SMAD-transcriptional effector proteins. Especially in human colon and pancreatic cancers, the TGF- $\beta$ -induced cytostatic response is often inactivated by mutation of TGF- $\beta$  receptors or SMADs (37). However, TGF- $\beta$  is still produced in high amounts by cancer and stromal cells, which is associated with relapse and reduced survival (32,33,38). In **Box 2**, we provide further details regarding the TGF- $\beta$  signaling pathway in cancer progression and metastasis, and how this pathway can be inhibited.

In addition to the regulation of tumor-promoting processes described above, TGF- $\beta$  also inhibits the generation and function of CD4<sup>+</sup> and CD8<sup>+</sup> effector T cells and dendritic cells (DCs), while promoting the expansion of **regulatory T cells (Tregs)** and MDSCs (recently reviewed in 39). Early, pivotal studies showed that CD4-dnTGF $\beta$ RII transgenic mice engineered to express a dominant-negative version of TGF- $\beta$ RII in their CD4<sup>+</sup> and CD8<sup>+</sup> T cells rendered these mice resistant to tumor challenge with B16.F10 murine melanoma cells or EL-4 murine lymphoma cells (40). TGF- $\beta$  inhibits the differentiation of CD4<sup>+</sup> T cells into effector cells by silencing the expression of master transcription factor T-bet (41), while stimulating the transition of naive CD4<sup>+</sup> cells into Tregs by inducing FoxP3 expression (42). In CD8<sup>+</sup> T cells, TGF- $\beta$  represses eomesodermin (EOMES), an

important transcription factor that regulates the effector program of cytotoxic CD8<sup>+</sup> T cells (43). In a murine B16.F10 melanoma model, treatment with various small molecule kinase inhibitors specific for TGF- $\beta$ RI not only directly inhibited phosphorylation of receptor-regulated SMAD proteins, but also induced ubiquitin-mediated degradation of SMAD4 mainly in CD8<sup>+</sup> **cytotoxic T lymphocytes (CTLs)**, and thereby increased their effector function and suppressed tumor growth (43). The important role of TGF- $\beta$  in T-cell suppression was further illustrated by the observation that TGF- $\beta$  induced the surface expression of PD-1 on both activated human peripheral blood mononuclear cells (PBMCs) and murine B16.F10 tumor-infiltrating CD8<sup>+</sup> and CD4<sup>+</sup> T cells through SMAD3-dependent transcriptional activation, thereby reducing T-cell effector function and limiting the antitumor response (44). Additionally, T cells genetically modified to be resistant to TGF- $\beta$  showed significantly enhanced tumor control in an adoptive T-cell transfer setting in a syngeneic murine B16.F10 melanoma model in comparison with T cells that could still respond to TGF- $\beta$  (45).

### Box 2: TGF- $\beta$ signaling in cancer and metastasis

Transforming growth factor  $\beta$  (TGF- $\beta$ ) is a pleiotropic cytokine produced in a latent form by cancer cells, various immune cells, platelets, and stromal cells (39). The signaling pathways activated by TGF- $\beta$  and its family members are highly conserved among species and are involved in development, homeostasis, and regeneration (46). Dysfunction of the TGF- $\beta$  signaling pathway might lead to various pathologies such as fibrosis, congenital defects, dysfunction of the immune system, and cancer. In short, all three TGF- $\beta$  isoforms, i.e. TGF- $\beta$ 1, - $\beta$ 2, and - $\beta$ 3 are cleaved into their active form by integrins or matrix metalloproteinases, resulting in a TGF- $\beta$  dimer. Signaling takes place via TGF- $\beta$  type I and II serine/threonine kinase receptors (TGF- $\beta$ RI and TGF- $\beta$ RII) that are expressed on the plasma membrane (**Figure I**). Upon TGF- $\beta$ -induced heteromeric complex formation of TGF- $\beta$ RI and TGF- $\beta$ RII, TGF- $\beta$ RII phosphorylates TGF- $\beta$ RI, which subsequently leads to the phosphorylation of receptor-regulated SMADs (R-SMADs). R-SMADs form complexes with the common mediator SMAD4, which drives transcriptional regulation of various TGF- $\beta$  target genes. Additionally, TGF- $\beta$  family members can also signal in SMAD-independent manners by using non-canonical pathways such as the phosphatidylinositol-3 kinase (PI3K)-AKT and the p38 MAP kinase pathway (47).

TGF- $\beta$  acts as a tumor suppressor in the early stages of tumor development, but this function is lost during the later phases of cancer progression. Instead, TGF- $\beta$  signaling promotes epithelial-to-mesenchymal transition (EMT) by reducing the expression of epithelial markers, such as E-cadherin, while increasing the expression of mesenchymal markers, such as vimentin (48). The TGF- $\beta$ -induced exploitation of EMT during cancer progression is assumed to contribute to the growth of the primary tumor as well as metastasis. Because of the various functions of TGF- $\beta$  during tumor progression and metastasis, multiple strategies

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>> have been developed to block TGF- $\beta$  signaling (**Figure I**). Current TGF- $\beta$  pathway inhibitors work on different levels by either **(A)** preventing TGF- $\beta$  production or expression of its receptor by antisense oligonucleotides (synthetic nucleic acids with a complementary sequence that prevents mRNA translation), **(B)** preventing TGF- $\beta$  activation via integrin-blocking antibodies (49), **(C)** inhibiting the interaction between TGF- $\beta$  and its receptor with neutralizing antibodies to TGF- $\beta$ , blocking antibodies to TGF- $\beta$ RII or ligand traps (engineered soluble forms of the receptor that compete with the cell-bound receptor) or **(D)** preventing intracellular TGF- $\beta$  receptor signal transduction via **small molecule kinase inhibitors** such as galunisertib (reviewed in 50).

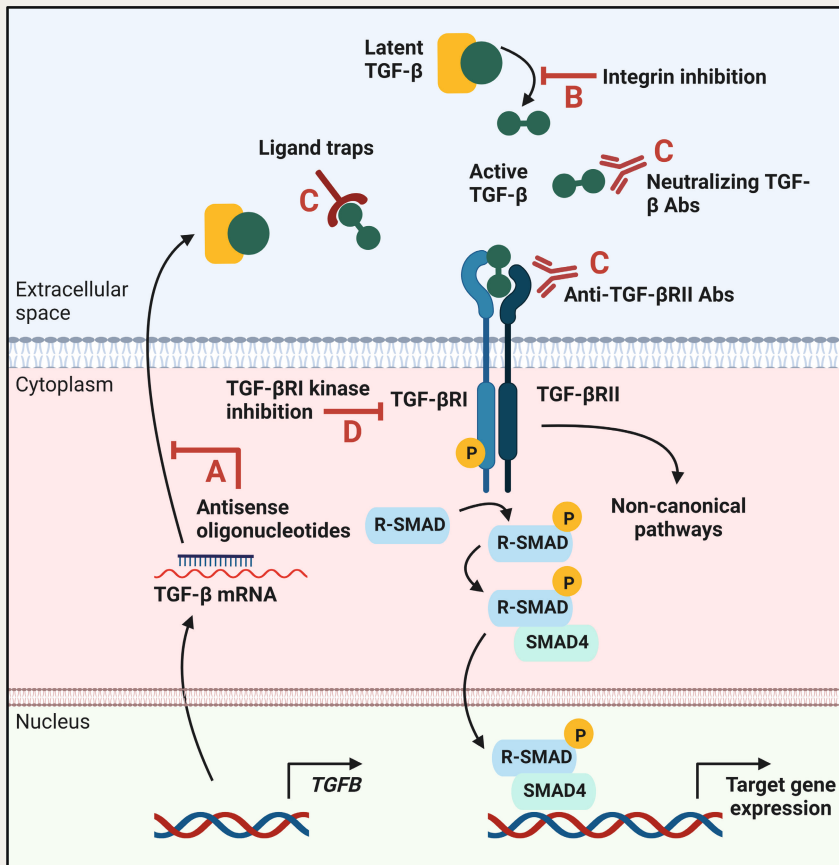


**Figure I. Overview of TGF- $\beta$  signaling across species.** Arrows indicate processes. Latent TGF- $\beta$  (dark green) is cleaved into its active form by integrins or matrix metalloproteinases, resulting in a TGF- $\beta$  dimer. Active TGF- $\beta$  induces heterodimerisation of TGF- $\beta$ RI and TGF- $\beta$ RII, ultimately leading to the phosphorylation of TGF- $\beta$ RI. Subsequent phosphorylation of R-SMADs initiates the SMAD-mediated transcription of TGF- $\beta$  target genes. TGF- $\beta$  also signals via non-SMAD pathways, via the so-called non-canonical pathways. Interference in TGF- $\beta$  signaling is possible using various strategies (indicated in dark red) by inhibiting **A**: TGF- $\beta$  (receptor) production, **B**: TGF- $\beta$  activation, **C**: ligand-receptor interaction or **D**: TGF- $\beta$ RI receptor activation.

The results of these *in vivo* studies hint towards a potential beneficial effect of TGF- $\beta$  inhibition on the induction of a potent antitumor response. Indeed, antibody-mediated inhibition of TGF- $\beta$  was able to induce complete tumor regression when given as monotherapy in up to 20% of animals in the subcutaneous CCK168 model of chemically-induced cutaneous squamous cell carcinoma engrafted in FVB/NJ mice (51). Furthermore, rechallenge experiments suggested that TGF- $\beta$  blockade induced immunological memory and long-term protection since both the parental cell line or similar chemically-induced cutaneous squamous cell carcinoma cell lines failed to grow in the animals that underwent complete regression (51). Similar effects were observed in a mouse model of murine 4T1-luciferase breast cancer, where complete regression was observed in 50% of animals after treatment with galunisertib (LY2157299 monohydrate), a small molecule that inhibits the kinase activity of TGF- $\beta$ RI (52). Mice with durable regressions also rejected tumor rechallenge with both the 4T1-luciferase cell line and the parental, less immunogenic 4T1 cell line, thereby demonstrating established immunological memory (52). In addition, inhibition of TGF- $\beta$  signaling using the same compound unleashed a potent and enduring CTL response in murine metastatic colorectal cancer models, reducing both primary tumor growth and blocking the appearance of liver metastases (53). Rechallenge experiments with the same tumor model demonstrated rejection of most tumors in the absence of any treatment, an effect that was mitigated upon antibody-mediated depletion of CTLs, again suggesting that TGF- $\beta$  could limit adaptive immune responses by inhibiting CTL responses (53). Overall, TGF- $\beta$  can heavily impair CTL responses and induce a generally immunosuppressed TME, thereby promoting tumor progression and metastasis.

### ***TGF- $\beta$ inhibition can increase the efficacy of immune checkpoint therapy***

As described above, TGF- $\beta$  inhibition induces regression of primary tumors, prevents metastasis formation, and induces protection against tumor rechallenge in various mouse tumor models when applied as a monotherapy. However, can TGF- $\beta$  inhibition provide an added therapeutic effect to immune checkpoint therapy? A rationale for this strategy was demonstrated by a genomic and transcriptomic analysis that revealed enrichment in markers of EMT, cell adhesion, and ECM remodeling in PD-1 therapy-resistant melanoma patients in comparison to therapy-responding patients (54). All of these cellular processes are known to be regulated via TGF- $\beta$  signaling (55). Moreover, transcriptomic analysis of human tumors from The Cancer Genome Atlas (TCGA) suggested that upregulation of ECM gene expression, such as genes encoding matrix metalloproteinases (MMPs) and collagen, was linked to the activation of TGF- $\beta$  target genes in CAFs and that this pan-cancer signature predicted unresponsiveness to PD-1 blockade (56). Additionally, single-cell sequencing studies identified a population of TGF- $\beta$ -driven CAFs that was associated with poor response to anti-PD-L1 therapy in human immune-excluded tumors, such as pancreatic cancer and bladder cancer (57). Finally, gene set enrichment analysis identified the genes *TGFB1* (encoding TGF- $\beta$ 1) and *TGFBR2* (encoding TGF- $\beta$ RII) to be associated with nonresponse to anti-PD-L1 therapy and reduced overall survival in patients with urothelial cancer (58). Altogether, these

studies support the use of TGF- $\beta$  signaling pathway inhibitors to sensitize immune-excluded tumors for immunotherapy. Indeed, combined treatment with anti-PD-L1 and anti-TGF- $\beta$  antibodies in the immune-excluded EMT6 mouse mammary carcinoma model led to a significant decrease in the tumor burden, reprogramming of stromal fibroblasts and increased infiltration of CD8<sup>+</sup> T cells in comparison to either treatment alone (58). These effects were lost after antibody-mediated depletion of CD8<sup>+</sup> T cells, indicating that the effect of this combination therapy was based on a potent CD8<sup>+</sup> T-cell-driven antitumor immune response (58). In the 4T1 mouse model of metastatic breast cancer, TGF- $\beta$  neutralization using the pan-isoform 1D11 monoclonal antibody during radiotherapy successfully decreased both primary tumor growth and the occurrence of metastasis and increased CD4<sup>+</sup> and CD8<sup>+</sup> T-cell infiltration (59). The addition of checkpoint blockade to this regimen led to complete tumor regression in 75% of mice, delayed tumor recurrence, and prolonged survival. Similar beneficial effects of combined checkpoint inhibition and TGF- $\beta$  inhibition on tumor regression were observed in mouse models of 4T1 breast cancer (52), progressive metastatic liver disease (53), MC38 colorectal cancer (60), and on the metastatic spread to the lung of the colorectal tumor model CT26 (61). Additionally, *in vivo* treatment using the bifunctional fusion protein M7824, composed of an antibody targeting PD-L1 and a TGF- $\beta$  ligand trap, has shown promising antitumor activity in a vast number of preclinical models, including **orthotopic** and subcutaneous mouse models of breast cancer, colon cancer, and renal adenocarcinoma, as well as in a xenograft model of human pharyngeal carcinoma (62). Last, a similar bifunctional fusion protein targeting CTLA-4 instead of PD-L1 was shown to inhibit tumor growth more efficiently than anti-CTLA-4 alone in human melanoma and triple-negative breast cancer models established in immunodeficient, humanized mice (63). Based on the promising effects observed in preclinical studies, various clinical trials are ongoing in which the combination of TGF- $\beta$  inhibition and checkpoint blockade is investigated. For example, an ongoing Phase 1b/2 dose-escalation and cohort-expansion study with 75 participants (NCT02423343)<sup>1</sup> aims to evaluate the safety, tolerability, and efficacy of the combination of galunisertib and anti-PD-1 in advanced refractory solid tumors (Phase 1b) and in recurrent or refractory non-small cell lung cancer or hepatocellular carcinoma patients (Phase 2). This trial and others may provide more information about the ability of dual inhibition of immune checkpoint axes and the TGF- $\beta$  pathway to establish tumor growth control and prevent metastasis.

## **RECRUITING TUMOR-SPECIFIC T CELLS IS THE FIRST PRIORITY IN IMMUNE-DESERT TUMORS**

While immune-excluded tumors may benefit from combined checkpoint blockade and TGF- $\beta$  inhibition, tumors with the immune-desert phenotype are less likely to benefit from this combination therapy (10,64). Immune-desert tumors are characterized by an absence of T lymphocytes in both the tumor and the surrounding stromal regions

(10). The absence of pre-existing antitumor immunity is the first barrier that needs to be overcome before checkpoint inhibitors and TGF- $\beta$  blockade can be used.

**Using oncolytic viruses to induce antitumor immunity**

A promising immunotherapeutic strategy that may promote antitumor immunity is treatment with oncolytic viruses (OVs) (65). The use of OVs as anticancer agents is emerging and driven by the FDA approval of **talimogene laherparepvec (T-VEC)**, a modified herpes simplex virus type 1 (HSV-1) that increased survival and demonstrated favorable tolerability in advanced-stage melanoma patients (66). OVs selectively replicate in transformed cells, either naturally or after genetic modification (**Figure 2A**). Accumulating evidence suggests that beyond their oncolytic activity, OVs have broad immunostimulatory properties. Mechanisms of action include the induction of local inflammation and priming and recruitment of tumor-reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells (**Figure 2B**) (67-70). In addition to their oncolytic and immunostimulatory properties, OVs can also be used as a delivery platform for tumor-specific expression of immunostimulatory transgenes such as cytokines, chemokines, costimulatory ligands, immune checkpoint inhibitors and TAs (**Figure 2C**) (71). More background on OVs is provided in **Box 3**.

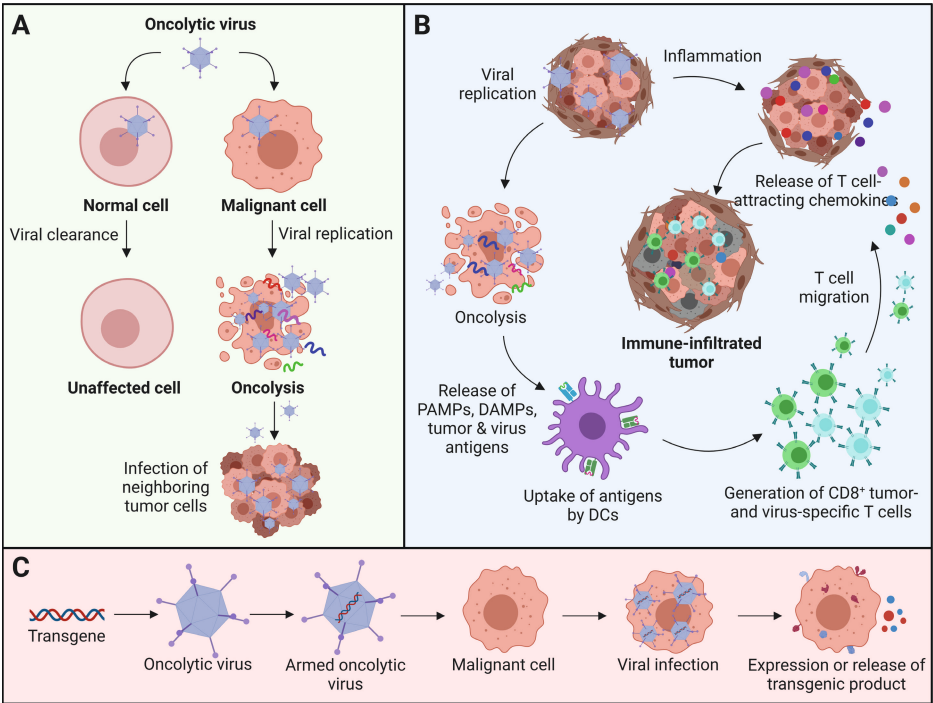


**Figure 2. Properties of oncolytic viruses. (A) Oncolytic properties.** Oncolytic viruses (OVs) selectively replicate in malignant cells, either naturally or after genetic modification. Normal cells remain unaffected due to viral clearance. Viral replication together with the induction of cell death pathways leads to lysis of tumor cells. Oncolysis causes the release of virus progeny, which infects new tumor cells.

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>> (B) *Immuno-stimulatory properties.* Virus replication causes oncolysis, which induces the release of tumor-specific and virus-specific antigens and **pathogen- and damage-associated molecular pattern molecules (PAMPs and DAMPs**, respectively). On the one hand, the subsequent uptake and presentation of antigens by dendritic cells leads to the induction of tumor- and virus-specific T cells. On the other hand, viral infection and replication induces an inflammatory response which causes the release of T cell-attracting chemokines. The tumor- and virus-specific T cells are attracted by these chemokines and migrate towards the tumor to exert their function. (C) *OVs as transgene delivery platform.* Some OVs (such as adenovirus and vaccinia virus) can be modified to encode transgenes (armed oncolytic viruses) such as cytokines or antibodies, ensuring specific delivery to the tumor microenvironment and further stimulation of an antitumor immune response.

### Box 3: Oncolytic viruses as antitumor agents

Oncolytic viruses (OVs) are able to selectively infect, replicate in and lyse tumor cells that initially gained interest because of their tumor cell-lysing (oncolytic) capabilities. In contrast to normal cells, where virus infection initiates a type I interferon (IFN)-driven antiviral response program, deficiency of this pathway in many cancer cells favors cancer-specific OV replication (85,86). Tumor-specific driver mutations, such as an activated RAS pathway, and upregulation of cell-entry receptor expression can further promote selective replication in tumor cells (87,88). Furthermore, some OVs, including adenovirus and HSV, have been engineered to increase their tumor specificity (89,90), whereas a strain of oncolytic reovirus has been bioselected by growing on cells that lack expression of the entry receptor to broaden its tropism for different tumor cells (91).

Beyond their oncolytic activity, OVs are able to induce a tumor-reactive T-cell response by acting as *in situ* vaccines (70,79,92). The process of T-cell priming is particularly effective during virus infection because virus-derived nucleic acids optimally induce the maturation of dendritic cells (67,70). Simultaneously, dying tumor cells are a source of TAs, leading to the priming of tumor-reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells (93,94). This process is further enhanced by the OV-induced IFN response, which recruits immune cells to the tumor and promotes antigen presentation (78). Noteworthy, the OV-induced *in situ* vaccine strategy does not rely on prior identification of TAs or neoantigens for a given tumor or patient, which conceptually would provide a major advantage over other types of cancer vaccines (71).

OVs can also be used as platforms for the specific delivery of transgenes into the tumor bed (71). Cytokines and chemokines represent attractive transgenes because they have pleiotropic effects and are encoded by small genes (95). The cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF), which promotes DC recruitment and maturation, has been the most widely studied transgene and has been encoded in many different OV platforms (96-98), including the leading clinically approved OV T-VEC (99). Other cargoes used to promote tumor-reactive

T-cell responses include ligands for costimulatory receptors such as CD40 ligand (100,101) or inducible co-stimulator (ICOS) ligand (102), checkpoint blockers such as anti-PD-1 antibodies (103), or **bispecific T-cell-engagers** (BiTEs) (104). Alternatively, OV<sub>s</sub> can also be armed with enzymes such as DNase or hyaluronidase to promote intratumoral penetration (105), or TAs such as MAGE-A3, which turn OV<sub>s</sub> into oncolytic vaccine vectors (106).

In addition to the direct elimination of primary treated tumors, OV<sub>s</sub> can induce long-term protection against secondary tumors (72-74). In an elegant rechallenge model, primary 4T1, EMT6, and E0771 murine breast cancer tumors were treated intratumorally with unarmed oncolytic Maraba virus MG1, after which the primary tumor was surgically resected (72). Thereafter, secondary tumors were implanted in the mammary fat pad and left untreated. Mice that were previously treated with Maraba virus showed significantly better tumor control of the untreated secondary tumor, and at least 20% of the animals showed complete tumor control. When T-cell deficient nude mice were used in a similar experiment, this effect was completely lost, suggesting that a functional adaptive immune system was necessary to induce T-cell memory and subsequent protection from secondary tumors. The capacity to confer immunological memory was similarly demonstrated for vesicular stomatitis virus (VSV), adenovirus, and HSV-1 in the 4T1 breast cancer model (73). Presurgical treatment with reovirus was only effective against the primary tumor in this study (73), but did induce protective memory in the EMT6 murine breast cancer model in another study (75). The OV-induced tumor-reactive immunity is believed to be not only a crucial aspect of the therapeutic efficacy of OV<sub>s</sub> (76,77) but may also be utilized to sensitize tumors for other types of immunotherapy by enhancing immunogenicity or by attracting activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells to nonresponsive tumors (65,68).

### ***Oncolytic virotherapy can synergize with immune checkpoint blockade***

Several OV platforms have been demonstrated to increase the number of TILs and sensitize tumors for checkpoint therapy, both in preclinical studies and in clinical trials (68,72,75,78-81). For example, a randomized Phase 1b clinical trial (NCT02263508)<sup>11</sup> that investigated the combination of FDA-approved oncolytic HSV-1 T-VEC and pembrolizumab (anti-PD-1) in 21 patients with unresectable melanoma patients has shown promising results with a 61.9% objective response and a 33.3% complete response (82). Of note, responses have also occurred in patients whose tumors displayed a low CD8<sup>+</sup> T-cell density and no PD-L1 expression at baseline, which originally emerged as the first potential predictive biomarker for insensitivity to immune checkpoint blockade (83). This trial is currently continued as a Phase 3 trial to investigate the effect of combined treatment with T-VEC and pembrolizumab on progression-free survival and overall survival in comparison with pembrolizumab alone (NCT02263508)<sup>11</sup>. Furthermore, the combination of T-VEC and ipilimumab (anti-CTLA4 monoclonal antibody) has shown promising results in a randomized Phase 2 clinical

trial (NCT01740297)<sup>111</sup> with 198 patients with unresectable stage IIIB or IV melanoma that were randomly assigned half-half to combination therapy or ipilimumab alone (84). The combination therapy resulted in an objective response of 35.7% compared to 17.5% in the ipilimumab-only treated group (84). Driven by these encouraging initial studies and additional preclinical data, there are more than twenty ongoing clinical programs involving different OV platforms in combination with immune checkpoint inhibitors (65). Collectively, these studies not only highlight the potent role of OVs as anticancer agents but also illustrate their capacity to sensitize tumors for subsequent immunotherapy, although further robust testing is evidently warranted.

## **COMBINING OVS WITH TGF- $\beta$ INHIBITION TO SENSITIZE SOLID TUMORS FOR IMMUNOTHERAPY**

The lack of immunogenicity and the presence of stromal and immunosuppressive barriers are 2 major hurdles to effective immunotherapy for immune-desert tumors. Combined modulation of the stromal barrier by TGF- $\beta$  inhibition and increasing immunogenicity using OVs might therefore be a potent strategy to sensitize immune-desert tumors for T-cell-based immunotherapy. Indeed, systemic treatment with a small molecule TGF- $\beta$ RI inhibitor in combination with a single intratumoral injection of oncolytic HSV-1 variant MG18L resulted in complete tumor regression in 60% of treated subjects in an orthotopic model of patient-derived recurrent glioblastomas established in severe combined immunodeficient (SCID) mice lacking mature B and T cells (107). In a human MDA-MB-231 breast cancer xenograft model established in nude mice, 3 intratumoral injections of an oncolytic adenovirus armed with a soluble form of TGF- $\beta$  receptor type II (sTGF- $\beta$ RII) that functions as a ligand trap for TGF- $\beta$  caused complete tumor regression in 7 out of 8 mice, which was better than the efficacy of the unarmed virus (3 out of 8 mice) and sTGF- $\beta$ RII only (1 out of 8 mice) (108). Additionally, intravenous delivery of the same armed virus in this MDA-MB-231 breast cancer xenograft model significantly inhibited the progression of bone metastasis and prolonged survival when compared with the unarmed virus (109). A limitation of the studies performed in immunodeficient mice is that the role of T cells during the OV and TGF- $\beta$  inhibition combination therapies remains underexplored. Combination treatment with intratumorally injected HSV1716, an attenuated unarmed oncolytic HSV-1, and a small molecule inhibitor of TGF- $\beta$ RI was evaluated in immunocompetent models of murine rhabdomyosarcoma, resulting in tumor growth stabilization, significantly prolonged survival and even some complete responses compared to the single agents alone (89). In this study, the removal of T-cell responses via antibody-mediated depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells or the use of athymic nude mice as recipients completely abolished the antitumor effect, indicating the importance of the T-cell response underlying efficacy of this combination treatment (89). Together, these preclinical studies suggest that the combination of TGF $\beta$  inhibition and OV therapy may be considered to putatively treat tumors with low immunogenicity and stromal or immunosuppressive barriers.

## CONCLUDING REMARKS

In this review, we discussed two promising therapeutic strategies to overcome barriers to effective immunotherapy in relation to the tumor immune phenotype. For the classification of tumor immune profiles, we relied on the three main tumor immune phenotypes postulated by Chen and Mellman (9). We recognize that other classification strategies are possible, and more detailed profiles based on immunophenotyping of tumors are being investigated (26,110). Immune-infiltrated tumors have an ongoing T-cell response, but the dysfunctional state of these T cells needs to be overcome by immune checkpoint therapy. Clinical successes in various tumors with this immune phenotype have already been reported, and many efforts to identify novel targets, find biomarkers of efficacy, and understand secondary resistance mechanisms are ongoing, and more breakthroughs are anticipated. Tumors with an immune-excluded phenotype require modification of the immunosuppressive TME to allow T-cell infiltration into the tumor before checkpoint therapy can be applied. As discussed above, TGF- $\beta$  inhibition has emerged as a multifunctional strategy to increase the efficacy of immunotherapy due to its capacity to modify the desmoplastic TME, increase the cytotoxic activity of CD8<sup>+</sup> (and possibly CD4<sup>+</sup>) T cells, and reduce the frequency of Tregs. However, due to the pleiotropic effects on different cell types and the heterogeneity of the TGF- $\beta$  superfamily, TGF- $\beta$  is a challenging target in terms of pharmacology.

For immune-desert tumors, immunotherapy is a different, much harder, challenge. Treating these tumors with immune checkpoint blockade and TGF- $\beta$  inhibition may be useful only when a prior treatment strategy has increased the immunogenicity of the tumor and induced tumor-reactive T-cell responses. OVs may represent potent tools to evoke potent CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses, as has been demonstrated by multiple preclinical studies mentioned above. The addition of TGF- $\beta$  blockade may increase the efficacy of this combination therapy even further, but this remains to be vigorously investigated. TGF- $\beta$  inhibition can not only lift the immunosuppressive and physical barriers to allow T-cell infiltration into the tumor bed, but also lift a physical barrier for penetration of OVs into tumors. Previous studies have shown that stromal components in the TME, such as TGF- $\beta$ -producing CAFs and collagen, may impair viral spread in tumors, limiting the efficacy of OVs (111). Indeed, an oncolytic vaccinia virus armed with a **bispecific T-cell-engager (BiTE)** directed against fibroblast activation protein (FAP) and murine CD3 decreased the number of FAP-expressing CAFs, increased the viral titer and T-cell accumulation in the tumor and enhanced antitumor efficacy in comparison with the unarmed virus in the murine B16.F1 melanoma model (112). Furthermore, in a similar approach with oncolytic Adenovirus that secretes FAP-targeting BiTEs, T-cell accumulation and antitumor efficacy were enhanced in xenograft models of subcutaneous human lung carcinoma and pancreatic adenocarcinoma established in NSG mice supplemented with pre-stimulated human T cells (113). Nevertheless, a great deal of caution needs to be taken with these interpretations since TGF- $\beta$  and CAFs can also promote the efficacy of OV replication. A study performed in xenografts

derived from patients with pancreatic cancer showed that tumor-derived TGF- $\beta$  made the CAFs more sensitive to infection with various OV, such as Vaccinia virus, VSV, and Maraba virus by downregulating their antiviral program (114). In turn, CAFs produced high amounts of fibroblast growth factor 2, which impeded the ability of the pancreatic cancer cells to detect and respond to virus infection.

Because of this complex interplay, the interference between TGF- $\beta$  signaling and OV treatment needs to be investigated further in the context of checkpoint blockade therapy. In particular, the rational choice of targets and the timing of the combination strategy might be of key importance to effectively sensitize tumors for immunotherapy (see **Outstanding Questions**). For instance, in an inducible murine model of BRAF<sup>V600E</sup>PTEN<sup>-/-</sup> melanoma with modest baseline responses to PD-1/PD-L1 blockade, TGF- $\beta$  inhibition failed to augment the response to anti-PD-1 immunotherapy whereas anti-CTLA-4 immunotherapy did benefit from the combination, resulting in tumor growth control and increased survival (115). Mechanistic studies in mice with subcutaneously implanted BRAF<sup>V600E</sup>PTEN<sup>-/-</sup> melanomas in C57BL/6 mice revealed that inhibition of TGF- $\beta$  signaling promoted the proliferative expansion of stromal fibroblasts and increased the production of MMP9, which subsequently facilitated cleavage of PD-L1 on the surface of melanoma cells, ultimately leading to resistance to anti-PD-1 therapy (115). The authors also demonstrated that TGF- $\beta$  inhibition following anti-PD-1 treatment had superior therapeutic efficacy compared to a continuous combination of TGF- $\beta$  inhibition and PD-1 blockade (115).

Additionally, whether combinations of three separate strategies are achievable in terms of cost and the accumulating burden of adverse events in patients remains undetermined. Although side effects may be limited for all monotherapies (116-118), the question arises as to whether adding up these therapies still has manageable adverse effects. Encoding checkpoint blockers and TGF- $\beta$  blocking agents in a single OV for intratumoral delivery may limit the therapeutic burden and systemic adverse effects (71), however, it remains to be assessed whether the antitumor efficacy of this strategy reaches its full potential when all agents are delivered to the tumor simultaneously. Additionally, not all OVs have sufficient space in their genome to allow the encoding of complicated and large molecules (119). Extensive preclinical studies need to be performed to elucidate the putative therapeutic effect of combined TGF- $\beta$  inhibition and OV therapy to sensitize immune-desert tumors for immune checkpoint blockade or other immunotherapeutic strategies and to determine for which specific cancers these combinations can be helpful.

Although multiple challenges and questions remain to be addressed, combining immune checkpoint inhibition with strategies to overcome immune evasion and exclusion is expected to result in the induction of strong antitumor immune responses in a variety of cancers. It will be exciting to follow future progress in this area.

## DECLARATIONS

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### Resources.

<sup>i</sup>This trial is listed in <https://clinicaltrials.gov/ct2/show/NCT02423343>

<sup>ii</sup>This trial is listed in <https://clinicaltrials.gov/ct2/show/NCT02263508>

<sup>iii</sup>This trial is listed in <https://clinicaltrials.gov/ct2/show/NCT01740297>

## OUTSTANDING QUESTIONS

- Can we develop approaches to selectively inhibit TGF- $\beta$  signaling in immune cells or in a specific CAF subset to restore immune surveillance and overcome immune evasion in solid tumors?
- Is replication of oncolytic virus required for its expected synergistic effect with TGF- $\beta$  signaling inhibition and immune checkpoint therapy?
- Which biomarkers can predict susceptibility to the combination therapy of oncolytic viruses, TGF- $\beta$  inhibition, and immune checkpoint inhibition?
- Which criteria should be used to select the appropriate oncolytic virus and immune checkpoint inhibitor for application in combination therapy? Does this differ between tumor types or even between patients?
- What would be the optimal timing for a combination approach of oncolytic virus therapy, TGF- $\beta$  signaling inhibition, and checkpoint blockade?
- Would it be technically feasible and therapeutically effective to genetically engineer a single oncolytic virus expressing TGF- $\beta$  signaling antagonists and immune checkpoint inhibitors to limit the therapy burden for patients?

## GLOSSARY

**Bispecific T-cell-engagers (BiTEs):** fusion proteins consisting of two different single-chain variable fragments of monoclonal antibodies for simultaneous tumor cell binding and T-cell activation.

**Cancer-associated fibroblasts (CAFs):** cell type within the tumor stroma that can promote tumor progression by extracellular matrix remodeling and secretion of cytokines.

**Cytotoxic T lymphocytes (CTL):** CD8<sup>+</sup> effector T cells, important for the elimination of intracellular pathogens and malignant cells.

**Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4):** immune checkpoint receptor that downregulates T-cell responses.

**Damage-associated molecular patterns (DAMPs):** endogenous molecules that are released from damaged cells, initiating a noninfectious inflammatory response.

**Dendritic cells (DCs):** antigen-presenting cells that are specialized in priming of naive T cells.

**Epithelial-to-mesenchymal transition (EMT):** a process by which epithelial cells de-differentiate towards migratory and invasive mesenchymal stem cells.

**Extracellular matrix (ECM):** a network of extracellular macromolecules such as collagen.

**Immune-desert:** tumor phenotype without an evident immune response.

**Immune-excluded:** tumor phenotype where tumor-reactive T cells are unable to infiltrate into the tumor beds due to a physical or immunosuppressive barrier.

**Immune-infiltrated:** tumor phenotype where inflammation is present and T lymphocytes have infiltrated the tumor.

**Immunogenicity:** the ability to evoke an adaptive immune response.

**Immunotherapy:** treatment focused on mobilizing the host immune system to combat disease.

**Myeloid-derived suppressor cells (MDSCs):** cells of the myeloid lineage with strong immunosuppressive properties that are associated with tumor progression.

**Neoantigens:** antigens that result from tumor-specific mutations and are absent from the normal genome.

**Oncolytic viruses (OVs):** viruses that preferentially replicate in and kill cancer cells.

**Orthotopic tumor model:** an experimental model where a transplanted tumor is placed in the organ of the original tumor.

**Pathogen-associated molecular patterns (PAMPs):** molecules derived from bacteria or viruses that evoke an inflammatory reaction.

**Priming:** process in which naive T cells encounter an antigen in the context of an activated dendritic cell and start clonal expansion.

**Programmed cell death protein-1 (PD-1):** immune checkpoint receptor expressed on the cell surface of T cells, which negatively regulates T-cell responses.

**Regulatory T cells:** FoxP3-expressing CD4<sup>+</sup> T lymphocytes that functionally suppress effector T cells.

**Small molecule kinase inhibitors:** low molecular weight compounds that block the action of one or more enzymes called protein kinases.

**Talimogene laherparepvec (T-VEC):** genetically modified oncolytic herpes simplex virus type 1, designed to produce GM-CSF. FDA-approved for the treatment of melanoma.

**T-cell anergy:** functionally inactivated state of T cells after antigen encounter.

**T-cell exhaustion:** progressive loss of effector function in T cells due to prolonged antigen stimulation

**Transforming growth factor  $\beta$ :** multifunctional secreted protein with three isoforms, involved in regulating and mediating many cellular processes.

**Tumor antigens:** proteins or substances produced in tumor cells that can be recognized by the adaptive immune system.

**Tumor-associated macrophages (TAMs):** macrophages found in tumors that exhibit immunosuppressive properties.

**Tumor-infiltrating lymphocytes (TILs):** white blood cells that have migrated into the tumor.

**Tumor microenvironment (TME):** the molecules, cells, and vessels that surround and interact with the tumor cells.

## REFERENCES

1. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, *et al.* Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. *Mol Cancer Ther* **2017**;16:2598-608
2. Ahrends T, Spanjaard A, Pilzecker B, Bąbala N, Bovens A, Xiao Y, *et al.* CD4+ T Cell Help Confers a Cytotoxic T Cell Effector Program Including Coinhibitory Receptor Downregulation and Increased Tissue Invasiveness. *Immunity* **2017**;47:848-61.e5
3. Prlic M, Williams MA, Bevan MJ. Requirements for CD8 T-cell priming, memory generation and maintenance. *Current Opinion in Immunology* **2007**;19:315-9
4. van der Burg SH, Arens R, Ossendorp F, van Hall T, Melief CJM. Vaccines for established cancer: overcoming the challenges posed by immune evasion. *Nature Reviews Cancer* **2016**;16:219
5. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, *et al.* Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N Engl J Med* **2009**;361:1838-47
6. Aarntzen EHJG, De Vries IJM, Lesterhuis WJ, Schuurhuis D, Jacobs JFM, Bol K, *et al.* Targeting CD4+ T-Helper Cells Improves the Induction of Antitumor Responses in Dendritic Cell-Based Vaccination. *Cancer Res* **2013**;73:19-29
7. Ribas A, Tumei PC. The Future of Cancer Therapy: Selecting Patients Likely to Respond to PD1/L1 Blockade. *Clin Cancer Res* **2014**;20:4982-4
8. Tumei PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJM, Robert L, *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **2014**;515:568-71
9. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature* **2017**;541:321-30
10. Herbst RS, Soria J-C, Kowanetz M, Fine GD, Hamid O, Gordon MS, *et al.* Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* **2014**;515:563-7
11. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* **1996**;271:1734-6
12. Gordon J, Freeman AJL, Yoshiko Iwai, Karen Bourque, Tatyana Chernova, Hiroyuki Nishimura, Lori J. Fitz, Nelly Malenkovich, Taku Okazaki, Michael C. Byrne, Heidi F. Horton, Lynette Fouser, Laura Carter, Vincent Ling, Michael R. Bowman, Beatriz M. Carreno MC, Clive R. Wood, and Tasuku Honjo. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *Journal of Experimental Medicine* **2000**;192:1027-34
13. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* **2015**;27:450-61
14. Maruhashi T, Okazaki I-m, Sugiura D, Takahashi S, Maeda TK, Shimizu K, *et al.* LAG-3 inhibits the activation of CD4+ T cells that recognize stable pMHCII through its conformation-dependent recognition of pMHCII. *Nature Immunology* **2018**;19:1415-26
15. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* **2010**;207:2187-94
16. van Montfoort N, Borst L, Korner MJ, Sluijter M, Marijt KA, Santegoets SJ, *et al.* NKG2A Blockade Potentiates CD8 T Cell Immunity Induced by Cancer Vaccines. *Cell* **2018**;175:1744-55 e15
17. Andre P, Denis C, Soulas C, Bourbon-Caillet C, Lopez J, Arnoux T, *et al.* Anti-NKG2A mAb Is a Checkpoint Inhibitor that Promotes Anti-tumor Immunity by Unleashing Both T and NK Cells. *Cell* **2018**;175:1731-43 e13
18. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. *Sci Transl Med* **2016**;8:328rv4

19. Gajewski TF. The Next Hurdle in Cancer Immunotherapy: Overcoming the Non-T-Cell-Inflamed Tumor Microenvironment. *Semin Oncol* **2015**;42:663-71
20. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, *et al.* Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clin Cancer Res* **2014**;20:5064-74
21. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, *et al.* Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* **2014**;371:2189-99
22. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, *et al.* Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* **2015**;350:207-11
23. Miller BC, Sen DR, Al Abosy R, Bi K, Virkud YV, LaFleur MW, *et al.* Subsets of exhausted CD8(+) T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol* **2019**;20:326-36
24. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, *et al.* Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome. *Science* **2006**;313:1960-4
25. Kortekaas KE, Santegoets SJ, Abdulrahman Z, van Ham VJ, van der Tol M, Ehsan I, *et al.* High numbers of activated helper T cells are associated with better clinical outcome in early stage vulvar cancer, irrespective of HPV or p53 status. *Journal for ImmunoTherapy of Cancer* **2019**;7:236
26. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nature Reviews Drug Discovery* **2019**;18:197-218
27. Salmon H, Franciszkiewicz K, Damotte D, Dieu-Nosjean MC, Validire P, Trautmann A, *et al.* Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J Clin Invest* **2012**;122:899-910
28. Lesokhin AM, Hohl TM, Kitano S, Cortez C, Hirschhorn-Cymerman D, Avogadri F, *et al.* Monocytic CCR2(+) myeloid-derived suppressor cells promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment. *Cancer research* **2012**;72:876-86
29. Beatty GL, Winograd R, Evans RA, Long KB, Luque SL, Lee JW, *et al.* Exclusion of T Cells From Pancreatic Carcinomas in Mice Is Regulated by Ly6C(low) F4/80(+) Extratumoral Macrophages. *Gastroenterology* **2015**;149:201-10
30. Feig C, Jones JO, Kraman M, Wells RJB, Deonarine A, Chan DS, *et al.* Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci U S A* **2013**;110:20212-7
31. Kather JN, Suarez-Carmona M, Charoentong P, Weis C-A, Hirsch D, Bankhead P, *et al.* Topography of cancer-associated immune cells in human solid tumors. *Elife* **2018**;7:e36967
32. Shen W, Tao GQ, Zhang Y, Cai B, Sun J, Tian ZQ. TGF-beta in pancreatic cancer initiation and progression: two sides of the same coin. *Cell & Bioscience* **2017**;7:39
33. Calon A, Espinet E, Palomo-Ponce S, Tauriello DV, Iglesias M, Cespedes MV, *et al.* Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. *Cancer Cell* **2012**;22:571-84
34. Chae YK, Chang S, Ko T, Anker J, Agte S, Iams W, *et al.* Epithelial-mesenchymal transition (EMT) signature is inversely associated with T-cell infiltration in non-small cell lung cancer (NSCLC). *Scientific reports* **2018**;8:2918
35. David CJ, Massagué J. Contextual determinants of TGFβ action in development, immunity and cancer. *Nature Reviews Molecular Cell Biology* **2018**;19:419-35
36. Connolly EC, Freimuth J, Akhurst RJ. Complexities of TGF-beta targeted cancer therapy. *Int J Biol Sci* **2012**;8:964-78
37. Oyanagi H, Shimada Y, Nagahashi M, Ichikawa H, Tajima Y, Abe K, *et al.* SMAD4 alteration associates with invasive-front pathological markers and poor prognosis in colorectal cancer. *Histopathology* **2019**;74:873-82

38. Zhao J, Liang Y, Yin Q, Liu S, Wang Q, Tang Y, *et al.* Clinical and prognostic significance of serum transforming growth factor-beta1 levels in patients with pancreatic ductal adenocarcinoma. *Braz J Med Biol Res* **2016**;49:e5485
39. Batlle E, Massague J. Transforming Growth Factor-beta Signaling in Immunity and Cancer. *Immunity* **2019**;50:924-40
40. Gorelik L, Flavell RA. Immune-mediated eradication of tumors through the blockade of transforming growth factor- $\beta$  signaling in T cells. *Nat Med* **2001**;7:1118-22
41. Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. *J Exp Med* **2002**;195:1499-505
42. Chen W, Jin W, Hardegen N, Lei K-J, Li L, Marinos N, *et al.* Conversion of peripheral CD4+CD25-naïve T cells to CD4+CD25+ regulatory T cells by TGF- $\beta$  induction of transcription factor Foxp3. *J Exp Med* **2003**;198:1875-86
43. Yoon J-H, Jung SM, Park SH, Kato M, Yamashita T, Lee I-K, *et al.* Activin receptor-like kinase5 inhibition suppresses mouse melanoma by ubiquitin degradation of Smad4, thereby derepressing eomesodermin in cytotoxic T lymphocytes. *EMBO Mol Med* **2013**;5:1720-39
44. Park BV, Freeman ZT, Ghasemzadeh A, Chattergoon MA, Rutebemberwa A, Steigner J, *et al.* TGF $\beta$ 1-Mediated SMAD3 Enhances PD-1 Expression on Antigen-Specific T Cells in Cancer. *Cancer discovery* **2016**;6:1366-81
45. Zhang L, Yu Z, Muranski P, Palmer DC, Restifo NP, Rosenberg SA, *et al.* Inhibition of TGF- $\beta$  signaling in genetically engineered tumor antigen-reactive T cells significantly enhances tumor treatment efficacy. *Gene therapy* **2013**;20:575-80
46. Zheng S, Long J, Liu Z, Tao W, Wang D. Identification and Evolution of TGF- $\beta$  Signaling Pathway Members in Twenty-Four Animal Species and Expression in Tilapia. *Int J Mol Sci* **2018**;19:1154
47. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF- $\beta$  family signalling. *Nature* **2003**;425:577-84
48. Xu J, Lamouille S, Derynck R. TGF- $\beta$ -induced epithelial to mesenchymal transition. *Cell Res* **2009**;19:156-72
49. Takasaka N, Seed RI, Cormier A, Bondesson AJ, Lou J, Elattma A, *et al.* Integrin  $\alpha\beta$ 8-expressing tumor cells evade host immunity by regulating TGF- $\beta$  activation in immune cells. *JCI Insight* **2018**;3
50. Neuzillet C, Tijeras-Raballand A, Cohen R, Cros J, Faivre S, Raymond E, *et al.* Targeting the TGFbeta pathway for cancer therapy. *Pharmacol Ther* **2015**;147:22-31
51. Dodagatta-Marri E, Meyer DS, Reeves MQ, Paniagua R, To MD, Binnewies M, *et al.*  $\alpha$ -PD-1 therapy elevates Treg/Th balance and increases tumor cell pSmad3 that are both targeted by  $\alpha$ -TGF $\beta$  antibody to promote durable rejection and immunity in squamous cell carcinomas. *Journal for immunotherapy of cancer* **2019**;7:10.1186/s40425-018-0493-9
52. Holmgaard RB, Schaer DA, Li Y, Castaneda SP, Murphy MY, Xu X, *et al.* Targeting the TGFbeta pathway with galunisertib, a TGFbetaRI small molecule inhibitor, promotes anti-tumor immunity leading to durable, complete responses, as monotherapy and in combination with checkpoint blockade. *J Immunother Cancer* **2018**;6:47
53. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llargo A, Badia-Ramentol J, Iglesias M, *et al.* TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature* **2018**;554:538-43
54. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, *et al.* Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* **2016**;165:35-44
55. Akhurst RJ, Hata A. Targeting the TGF $\beta$  signalling pathway in disease. *Nat Rev Drug Discov* **2012**;11:790-811
56. Chakravarthy A, Khan L, Bensler NP, Bose P, De Carvalho DD. TGF- $\beta$ -associated extracellular matrix genes link cancer-associated fibroblasts to immune evasion and immunotherapy failure. *Nature Communications* **2018**;9:4692

57. Dominguez CX, Muller S, Keerthivasan S, Koeppen H, Hung J, Gierke S, *et al.* Single-cell RNA sequencing reveals stromal evolution into LRRC15+ myofibroblasts as a determinant of patient response to cancer immunotherapy. *Cancer Discov* **2019**:CD-19-0644
58. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, *et al.* TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* **2018**;554:544-8
59. Vanpouille-Box C, Diamond JM, Pilonis KA, Zavadil J, Babb JS, Formenti SC, *et al.* TGFbeta Is a Master Regulator of Radiation Therapy-Induced Antitumor Immunity. *Cancer Res* **2015**;75:2232-42
60. Sow HS, Ren J, Camps M, Ossendorp F, ten Dijke P. Combined Inhibition of TGF- $\beta$  Signaling and the PD-L1 Immune Checkpoint Is Differentially Effective in Tumor Models. *Cells* **2019**;8
61. Terabe M, Robertson FC, Clark K, De Ravin E, Bloom A, Venzon DJ, *et al.* Blockade of only TGF-beta 1 and 2 is sufficient to enhance the efficacy of vaccine and PD-1 checkpoint blockade immunotherapy. *Oncoimmunology* **2017**;6:e1308616
62. Lan Y, Zhang D, Xu C, Hance KW, Marelli B, Qi J, *et al.* Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-beta. *Sci Transl Med* **2018**;10
63. Ravi R, Noonan KA, Pham V, Bedi R, Zhavoronkov A, Ozerov IV, *et al.* Bifunctional immune checkpoint-targeted antibody-ligand traps that simultaneously disable TGFbeta enhance the efficacy of cancer immunotherapy. *Nat Commun* **2018**;9:741
64. Duan J, Wang Y, Jiao S. Checkpoint blockade-based immunotherapy in the context of tumor microenvironment: Opportunities and challenges. *Cancer Med* **2018**;7:4517-29
65. Harrington K, Freeman DJ, Kelly B, Harper J, Soria J-C. Optimizing oncolytic virotherapy in cancer treatment. *Nature Reviews Drug Discovery* **2019**
66. Andtbacka RHI, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, *et al.* Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. *J Clin Oncol* **2015**;33:2780-8
67. Benencia F, Courrèges MC, Conejo-García JR, Mohamed-Hadley A, Zhang L, Buckanovich RJ, *et al.* HSV oncolytic therapy upregulates interferon-inducible chemokines and recruits immune effector cells in ovarian cancer. *Molecular Therapy* **2005**;12:789-802
68. Zamarin D, Holmgaard RB, Subudhi SK, Park JS, Mansour M, Palese P, *et al.* Localized Oncolytic Virotherapy Overcomes Systemic Tumor Resistance to Immune Checkpoint Blockade Immunotherapy. *Sci Transl Med* **2014**;6:226ra32-ra32
69. Atherton MJ, Stephenson KB, Tzelepis F, Bakhshinyan D, Nikota JK, Son HH, *et al.* Transforming the prostatic tumor microenvironment with oncolytic virotherapy. *Oncoimmunology* **2018**;7:e1445459-e
70. Brown MC, Holl EK, Boczkowski D, Dobrikova E, Mosaheb M, Chandramohan V, *et al.* Cancer immunotherapy with recombinant poliovirus induces IFN-dominant activation of dendritic cells and tumor antigen-specific CTLs. *Sci Transl Med* **2017**;9:eaan4220
71. Twumasi-Boateng K, Pettigrew JL, Kwok YYE, Bell JC, Nelson BH. Oncolytic viruses as engineering platforms for combination immunotherapy. *Nature Reviews Cancer* **2018**;18:419-32
72. Bourgeois-Daigneault M-C, Roy DG, Aitken AS, El Sayes N, Martin NT, Varette O, *et al.* Neoadjuvant oncolytic virotherapy before surgery sensitizes triple-negative breast cancer to immune checkpoint therapy. *Sci Transl Med* **2018**;10:eaao1641
73. Martin NT, Roy DG, Workenhe ST, van den Wollenberg DJM, Hoebe RC, Mossman KL, *et al.* Pre-surgical neoadjuvant oncolytic virotherapy confers protection against rechallenge in a murine model of breast cancer. *Sci Rep* **2019**;9:1865
74. Jiang H, Shin DH, Nguyen TT, Fuego J, Fan X, Henry V, *et al.* Localized Treatment with Oncolytic Adenovirus Delta-24-RGDOX Induces Systemic Immunity against Disseminated Subcutaneous and Intracranial Melanomas. *Clin Cancer Res* **2019**;25:6801-14

75. Mostafa AA, Meyers DE, Thirukkumaran CM, Liu PJ, Gratton K, Spurrell J, *et al.* Oncolytic Reovirus and Immune Checkpoint Inhibition as a Novel Immunotherapeutic Strategy for Breast Cancer. *Cancers (Basel)* **2018**;10
76. Diaz RM, Galivo F, Kottke T, Wongthida P, Qiao J, Thompson J, *et al.* Oncolytic Immunovirotherapy for Melanoma Using Vesicular Stomatitis Virus. *Cancer Res* **2007**;67:2840-8
77. Moesta AK, Cooke K, Piasecki J, Mitchell P, Rottman JB, Fitzgerald K, *et al.* Local Delivery of OncoVEXmGM-CSF Generates Systemic Antitumor Immune Responses Enhanced by Cytotoxic T-Lymphocyte-Associated Protein Blockade. *Clin Cancer Res* **2017**;23:6190-202
78. Samson A, Scott KJ, Taggart D, West EJ, Wilson E, Nuovo GJ, *et al.* Intravenous delivery of oncolytic reovirus to brain tumor patients immunologically primes for subsequent checkpoint blockade. *Sci Transl Med* **2018**;10:eaam7577
79. Woller N, Gürlevik E, Fleischmann-Mundt B, Schumacher A, Knocke S, Kloos AM, *et al.* Viral Infection of Tumors Overcomes Resistance to PD-1-immunotherapy by Broadening Neoantigenome-directed T-cell Responses. *Molecular therapy : the journal of the American Society of Gene Therapy* **2015**;23:1630-40
80. Cervera-Carrascon V, Siurala M, Santos JM, Havunen R, Tahtinen S, Karell P, *et al.* TNF $\alpha$  and IL-2 armed adenoviruses enable complete responses by anti-PD-1 checkpoint blockade. *Oncoimmunology* **2018**;7:e1412902
81. Feola S, Capasso C, Fucciello M, Martins B, Tahtinen S, Medeot M, *et al.* Oncolytic vaccines increase the response to PD-L1 blockade in immunogenic and poorly immunogenic tumors. *Oncoimmunology* **2018**;7:e1457596
82. Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RHI, Michielin O, *et al.* Oncolytic Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. *Cell* **2017**;170:1109-19.e10
83. Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. *Journal for ImmunoTherapy of Cancer* **2019**;7:278
84. Chesney J, Puzanov I, Collichio F, Singh P, Milhem MM, Glaspy J, *et al.* Randomized, Open-Label Phase II Study Evaluating the Efficacy and Safety of Talimogene Laherparepvec in Combination With Ipilimumab Versus Ipilimumab Alone in Patients With Advanced, Unresectable Melanoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2018**;36:1658-67
85. Allagui F, Achard C, Panterne C, Combredet C, Labarriere N, Dreno B, *et al.* Modulation of the Type I Interferon Response Defines the Sensitivity of Human Melanoma Cells to Oncolytic Measles Virus. *Curr Gene Ther* **2017**;16:419-28
86. Kohlhapp FJ, Kaufman HL. Molecular Pathways: Mechanism of Action for Talimogene Laherparepvec, a New Oncolytic Virus Immunotherapy. *Clin Cancer Res* **2016**;22:1048-54
87. Shmulevitz M, Pan L-Z, Garant K, Pan D, Lee PWK. Oncogenic Ras Promotes Reovirus Spread by Suppressing IFN- $\beta$  Production through Negative Regulation of RIG-I Signaling. *Cancer Res* **2010**;70:4912-21
88. Lin L-T, Richardson CD. The Host Cell Receptors for Measles Virus and Their Interaction with the Viral Hemagglutinin (H) Protein. *Viruses* **2016**;8:250
89. Hutzen B, Chen CY, Wang PY, Sprague L, Swain HM, Love J, *et al.* TGF-beta Inhibition Improves Oncolytic Herpes Viroimmunotherapy in Murine Models of Rhabdomyosarcoma. *Mol Ther Oncolytics* **2017**;7:17-26
90. Fuego J, Gomez-Manzano C, Alemany R, Lee PSY, McDonnell TJ, Mitlianga P, *et al.* A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. *Oncogene* **2000**;19:2-12
91. van den Wollenberg DJ, Dautzenberg IJ, van den Hengel SK, Cramer SJ, de Groot RJ, Hoebe RC. Isolation of reovirus T3D mutants capable of infecting human tumor cells independent of junction adhesion molecule-A. *PLoS One* **2012**;7:e48064

92. Prestwich RJ, Ilett EJ, Errington F, Diaz RM, Steele LP, Kottke T, *et al.* Immune-mediated antitumor activity of reovirus is required for therapy and is independent of direct viral oncolysis and replication. *Clin Cancer Res* **2009**;15:4374-81
93. Gauvrit A, Brandler S, Sapede-Peroz C, Boisgerault N, Tangy F, Gregoire M. Measles virus induces oncolysis of mesothelioma cells and allows dendritic cells to cross-prime tumor-specific CD8 response. *Cancer Res* **2008**;68:4882-92
94. Delaunay T, Violland M, Boisgerault N, Dutoit S, Vignard V, Münz C, *et al.* Oncolytic viruses sensitize human tumor cells for NY-ESO-1 tumor antigen recognition by CD4+ effector T cells. *Oncoimmunology* **2017**;7:e1407897-e
95. de Graaf JF, de Vor L, Fouchier RAM, van den Hoogen BG. Armed oncolytic viruses: A kick-start for anti-tumor immunity. *Cytokine & Growth Factor Reviews* **2018**;41:28-39
96. Kemp V, van den Wollenberg DJM, Camps MGM, van Hall T, Kinderman P, Pronk-van Montfoort N, *et al.* Arming oncolytic reovirus with GM-CSF gene to enhance immunity. *Cancer Gene Therapy* **2018**
97. Cerullo V, Pesonen S, Diaconu I, Escutenaire S, Arstila PT, Ugolini M, *et al.* Oncolytic adenovirus coding for granulocyte macrophage colony-stimulating factor induces antitumoral immunity in cancer patients. *Cancer Res* **2010**;70:4297-309
98. Grossardt C, Engeland CE, Bossow S, Halama N, Zaoui K, Leber MF, *et al.* Granulocyte-macrophage colony-stimulating factor-armed oncolytic measles virus is an effective therapeutic cancer vaccine. *Hum Gene Ther* **2013**;24:644-54
99. Andtbacka RHI, Collichio F, Harrington KJ, Middleton MR, Downey G, Öhrling K, *et al.* Final analyses of OPTiM: a randomized phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III–IV melanoma. *Journal for ImmunoTherapy of Cancer* **2019**;7:145
100. Diaconu I, Cerullo V, Hirvinen ML, Escutenaire S, Ugolini M, Pesonen SK, *et al.* Immune response is an important aspect of the antitumor effect produced by a CD40L-encoding oncolytic adenovirus. *Cancer Res* **2012**;72:2327-38
101. Pesonen S, Diaconu I, Kangasniemi L, Ranki T, Kanerva A, Pesonen SK, *et al.* Oncolytic Immunotherapy of Advanced Solid Tumors with a CD40L-Expressing Replicating Adenovirus: Assessment of Safety and Immunologic Responses in Patients. *Cancer Res* **2012**;72:1621-31
102. Zamarin D, Holmgaard RB, Ricca J, Plitt T, Palese P, Sharma P, *et al.* Intratumoral modulation of the inducible co-stimulator ICOS by recombinant oncolytic virus promotes systemic anti-tumour immunity. *Nature Communications* **2017**;8:14340
103. Kleinpeter P, Fend L, Thioudellet C, Geist M, Sfrontato N, Koerper V, *et al.* Vectorization in an oncolytic vaccinia virus of an antibody, a Fab and a scFv against programmed cell death -1 (PD-1) allows their intratumoral delivery and an improved tumor-growth inhibition. *Oncoimmunology* **2016**;5:e1220467-e
104. Wing A, Fajardo CA, Posey AD, Shaw C, Da T, Young RM, *et al.* Improving CART-Cell Therapy of Solid Tumors with Oncolytic Virus-Driven Production of a Bispecific T-cell Engager. *Cancer Immunol Res* **2018**;6:605-16
105. Tedcastle A, Illingworth S, Brown A, Seymour LW, Fisher KD. Actin-resistant DNase I Expression From Oncolytic Adenovirus Enadenotucirev Enhances Its Intratumoral Spread and Reduces Tumor Growth. *Molecular therapy : the journal of the American Society of Gene Therapy* **2016**;24:796-804
106. Pol JG, Acuna SA, Yadollahi B, Tang N, Stephenson KB, Atherton MJ, *et al.* Preclinical evaluation of a MAGE-A3 vaccination utilizing the oncolytic Maraba virus currently in first-in-human trials. *Oncoimmunology* **2018**;8:e1512329-e
107. Esaki S, Nigim F, Moon E, Luk S, Kiyokawa J, Curry W, Jr., *et al.* Blockade of transforming growth factor-beta signaling enhances oncolytic herpes simplex virus efficacy in patient-derived recurrent glioblastoma models. *Int J Cancer* **2017**;141:2348-58

108. Seth P, Wang ZG, Pister A, Zafar MB, Kim S, Guise T, *et al.* Development of oncolytic adenovirus armed with a fusion of soluble transforming growth factor-beta receptor II and human immunoglobulin Fc for breast cancer therapy. *Hum Gene Ther* **2006**;17:1152-60
109. Hu Z, Gerseny H, Zhang Z, Chen YJ, Berg A, Zhang Z, *et al.* Oncolytic adenovirus expressing soluble TGFbeta receptor II-Fc-mediated inhibition of established bone metastases: a safe and effective systemic therapeutic approach for breast cancer. *Mol Ther* **2011**;19:1609-18
110. Thommen DS, Schumacher TN. T Cell Dysfunction in Cancer. *Cancer Cell* **2018**;33:547-62
111. McKee TD, Grandi P, Mok W, Alexandrakis G, Insin N, Zimmer JP, *et al.* Degradation of fibrillar collagen in a human melanoma xenograft improves the efficacy of an oncolytic herpes simplex virus vector. *Cancer Res* **2006**;66:2509-13
112. Yu F, Hong B, Song X-T. A T-cell engager-armed oncolytic vaccinia virus to target the tumor stroma. *Cancer Transl Med* **2017**;3:122-32
113. de Sostoa J, Fajardo CA, Moreno R, Ramos MD, Farrera-Sal M, Alemany R. Targeting the tumor stroma with an oncolytic adenovirus secreting a fibroblast activation protein-targeted bispecific T-cell engager. *Journal for immunotherapy of cancer* **2019**;7:19-
114. Ilkow CS, Marguerie M, Batenchuk C, Mayer J, Ben Neriah D, Cousineau S, *et al.* Reciprocal cellular cross-talk within the tumor microenvironment promotes oncolytic virus activity. *Nat Med* **2015**;21:530-6
115. Zhao F, Evans K, Xiao C, DeVito N, Theivanthiran B, Holtzhausen A, *et al.* Stromal Fibroblasts Mediate Anti-PD-1 Resistance via MMP-9 and Dictate TGFβ Inhibitor Sequencing in Melanoma. *Cancer Immunol Res* **2018**;6:1459-71
116. Bajwa R, Cheema A, Khan T, Amirpour A, Paul A, Chaughtai S, *et al.* Adverse Effects of Immune Checkpoint Inhibitors (Programmed Death-1 Inhibitors and Cytotoxic T-Lymphocyte-Associated Protein-4 Inhibitors): Results of a Retrospective Study. *J Clin Med Res* **2019**;11:225-36
117. Kovacs RJ, Maldonado G, Azaro A, Fernández MS, Romero FL, Sepulveda-Sánchez JM, *et al.* Cardiac Safety of TGF-β Receptor I Kinase Inhibitor LY2157299 Monohydrate in Cancer Patients in a First-in-Human Dose Study. *Cardiovasc Toxicol* **2015**;15:309-23
118. Perez MC, Miura JT, Naqvi SMH, Kim Y, Holstein A, Lee D, *et al.* Talimogene Laherparepvec (TVEC) for the Treatment of Advanced Melanoma: A Single-Institution Experience. *Ann Surg Oncol* **2018**;25:3960-5
119. Bommareddy PK, Shettigar M, Kaufman HL. Integrating oncolytic viruses in combination cancer immunotherapy. *Nature Reviews Immunology* **2018**;18:498-513



