Beyond cancer cells: Targeting the tumor microenvironment with gene therapy and armed oncolytic virus

Peter Kok-Ting Wan, Anderson J. Ryan, and Leonard W. Seymour

Cancer gene therapies are usually designed either to express wild-type copies of tumor suppressor genes or to exploit tumor-associated phenotypic changes to endow selective cytotoxicity. However, these approaches become less relevant to cancers that contain many independent mutations, and the situation is made more complex by our increased understanding of clonal evolution of tumors, meaning that different metastases and even regions of the same tumor mass have distinct mutational and phenotypic profiles. In contrast, the relatively genetically stable tumor microenvironment (TME) therefore provides an appealing therapeutic target, particularly since it plays an essential role in promoting cancer growth, immune tolerance, and acquired resistance to many therapies. Recently, a variety of different TME-targeted gene therapy and armed oncolytic strategies have been explored, with particular success observed in strategies targeting the cancer stroma, reducing tumor vasculature, and repolarizing the immunosuppressive microenvironment. Herein, we review the progress of these TME-targeting approaches and try to highlight those showing the greatest promise.

INTRODUCTION

Importance of the tumor microenvironment (TME)

Components of the TME play essential roles in cancer development and growth. In most carcinomas, the malignant tumor cells are supported by a heterogeneous population of non-transformed cells in the TME, including fibroblasts, endothelial cells, immune cells, adipocytes, and pericytes. Developing tumors can recruit these stromal cells from adjacent non-tumor tissue and also from the bone marrow to support the emerging neoplastic phenotype. Bi-directional communication between the tumor and these microenvironmental cells appears to be critical for promoting tumor progression and metastasis, with tumors secreting cytokines and chemokines that alter surface markers on endothelial cells and induce tumor tolerance. Moreover, the components of the TME can change over time, providing supportive environments for different stages of tumor growth, and therefore the TME has been increasingly recognized as a valid target for therapeutic intervention.

Gene therapy targeting the TME

Gene therapy has evolved rapidly in the last two decades. Early cancer gene therapy trials focused on the delivery of tumor suppressor genes, most notably wild-type p53, and later approaches often exploited the tumor phenotype to regulate a selective cytotoxic effect. However, with the availability of single-cell sequencing and recent insights into tumor heterogeneity and clonal evolution showing the multitude of tumor-associated mutations and phenotypic variation between different regions even of the same tumor, such “one size fits all” therapeutic strategies now appear less feasible. Accordingly, gene therapy approaches have recently been developed that aim to manipulate the relatively genetically stable TME, including modulation of the local immune system. In particular, progress has been made to deplete cancer stroma and to degrade the tumor vasculature, while cytokine-based gene therapies and vaccines have aimed to boost the antitumor response or suppress tumor tolerance. In addition, armed oncolytic viruses (OVs) are increasingly considered as appealing therapeutic agents because they mediate tumor-selective oncolytic cell-killing mechanisms, lysing tumor cells to expose proinflammatory signals (pathogen-associated and damage-associated molecular patterns [PAMPs and DAMPs]) and tumor antigens, combined with local expression of encoded immunomodulatory transgenes. This dual mechanism affords many innovations in therapeutic approach. In this review, we consider the most promising TME-targeting strategies using gene therapies and armed OVs, illustrated in Figure 1, and suggest which stromal cells and therapeutic modalities might provide the greatest therapeutic value.

Choice of appropriate preclinical model systems is challenging for agents targeting the TME, particularly for immunomodulatory armed OVs, as these are often human selective and do not complete their full life cycle in most rodent cells. Some immune-competent animals, such as Syrian hamsters, are semi-permissive for some OVs, and this provides a handful of tumor models, although it is not clear whether the stromal composition of model tumors or the balance of direct oncolysis and transgene expression has clinical relevance. Similarly, xenografted tumors in transgenic mice provide improving model systems, although the stromal compartment is of murine origin and often poorly developed, and strict clinical
relevance is still unclear. Patient-derived xenograft (PDX) models may provide better model systems going forward, since direct xenografts can retain some human stroma.\(^{15}\) Many of the pharmacodynamic studies discussed below make use of human ex vivo tumor culture systems, which usually contain all of the representative stromal cell compartments but suffer from limited viability and useful lifespan.\(^{16}\) All of these model systems bring their own technical challenges, and we have tried to interpret findings with this in mind throughout this review.

**Pharmacokinetic aspects of tumor-targeted expression of biologics**

One major advantage of encoding anti-cancer biologics within gene therapy vectors or OVs is the potential for selective expression within tumors. While this does not increase the amount of material being delivered to the tumor, local production in situ leads to high local concentrations of the secreted biologic within the TME and relatively low concentrations in the circulation, thereby maximizing the therapeutic effect and limiting systemic toxicities. This strategy is most effective when the expressed agents are relatively small macromolecules, improving their percolation through the TME and also minimizing their persistence in the bloodstream (by accelerated renal clearance) if they leak out of the tumor into the circulation. For example, small biologics such as nanobodies (~15 kDa) and single-chain variable fragment (scFv) (~28 kDa) have a relatively short half-life of \(<0.5\) h.\(^{17,18}\) This is the converse of the desirable physical properties for systemic delivery, where a long half-life (for example using an intact antibody) is normally seen as preferred to maximize the opportunity to access disseminated targets. Accordingly, from a pharmacokinetic perspective, encoding antibody fragments or small biologics within gene therapy vectors or OVs may comprise a particularly powerful approach.

For gene therapies, transgene expression is normally regulated using tumor- or tissue-selective promoters.\(^{19}\) However, OVs provide the opportunity to use endogenous viral promoters to regulate expression, linking expression to the virus life cycle and thereby exploiting

---

**Figure 1. Strategies of armed OVs for targeting the TME**

OVs infect and lyse cancer cells, releasing the armed biologics to the TME. Matrix-degrading enzymes such as hyaluronidase, collagenase, and matrix metalloproteinase are used to degrade the dense and viscous cancer stroma. Bispecific antibodies such as bispecific T cell engager targeting FAP and FR\(\beta\) are designed to selective deplete cancer-associated fibroblasts and immunosuppressive M2 macrophages, and activate endogenous T cells independent of the MHC class I molecule. Monovalent antibodies such as anti-CTLA-4 and anti-PD-L1 antibodies can also be encoded by OVs to block the inhibitory receptors on regulatory T cells and tumor cells. In addition, cytokines such as GM-CSF and TNF-\(\alpha\) can be armed in OVs to promote dendritic cell maturation and macrophage repolarization. IL-2 and IL-12 have also been used to promote T cell proliferation and activation. Soluble VEGFR receptor composed of VEGFR fused to an Fc domain is encoded by OVs to block the VEGF/VEGFR pathway for suppressing angiogenesis. CTLA-4, cytotoxic T lymphocyte-associated protein 4; FAP, fibroblast activation protein \(\gamma\); FR\(\beta\), folate receptor \(\beta\); GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-2, interleukin-2; IL-12, interleukin-12; PD-L1, programmed death-ligand 1; TNF-\(\alpha\), tumor necrosis factor \(\alpha\); VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.
the cancer selectivity of the OV to ensure cancer-selective expression of the transgene. Alternatively, tumor- or tissue-specific promoters such as telomerase reverse transcriptase promoter and prostate-specific antigen promoter have been used to regulate both conditional replication and transgene expression in selected tissues. This also gives considerable flexibility over the duration of transgene expression. The use of AAV (adeno-associated virus) for gene therapy can give extended transgene expression; however, for most cancer therapies, where the expressed agents are likely to cause cytotoxicity, the intrinsically limited duration of expression afforded by most OVs may be preferable.

**Cancer-associated fibroblasts as a therapeutic target**

Cancer-associated fibroblasts (CAFs) orchestrate several aspects of cancer biology that contribute to poor prognosis, often producing cytokines, chemokines, and extracellular matrix (ECM). Notably, they secrete vascular endothelial growth factor (VEGF) to drive angiogenesis and transforming growth factor (TGF)-β to polarize immune cells to become immunosuppressive. In addition, CAFs can express high levels of programmed cell death ligand 2 (PD-L2) and FAS ligand (FASLG) to promote tumor immune evasion.  

**Targeted degradation of protein components of the ECM**

While supporting tumor progression in diverse ways, CAFs can also act to inhibit the infiltration and dissemination of gene therapy vectors and OVs. Notably, CAFs secrete large quantities of extracellular proteins such as collagen and fibronectin, coupled with collagen cross-linking enzymes such as lysyl hydroxylases and prolyl hydroxylases, which become embedded within a rigid framework of the densely packed ECM. Interstitial penetration and spreading of gene therapy vectors and OVs rely on fluid convection, and the densely packed ECM acts as a physical barrier that hinders their movement and thereby their dissemination throughout the tumor mass. There have been several attempts to address this challenge, for example Kim et al. and Choi et al., respectively, engineered relaxin- and decorin-armed oncolytic adenoviruses (Ads) in order to degrade collagen components of the ECM. Relaxin and decorin are involved in the induction of matrix metalloproteinases (MMPs) and inhibition of collagen expression and assembly. Both armed Ads showed enhanced viral spread, replication, and transgene expression, remodeling the ECM composition by depleting collagen type I, type III, and elastin. Similarly, intratumoral expression of MMP-8 by oncolytic Ads effectively depleted collagen and broke down the fibrillar collagen matrix to enhance therapeutic efficacy. Improved viral distribution and therapeutic outcome were also observed using an oncolytic herpes simplex virus I (HSV-1) vector after co-injection with bacterial collagenase to degrade fibrillar collagen in a melanoma xenograft mouse model.

**Targeted degradation of glycan and nucleic acid components of the TME**

While extracellular proteins provide the structural framework for ECM, more flexible hydrophilic components can associate with it and increase viscosity, further restricting virus spread by providing even greater resistance to fluid convection. These components include hyaluronic acid and extracellular DNA released during non-apoptotic tumor cell death. Building on observations that PEG-PH20 (hyaluronidase derivatized with poly(ethylene glycol) to improve its stability in the bloodstream) could be used to decrease the interstitial fluid pressure (IFP) and enhance the delivery of macromolecules and viruses into tumors, several groups have explored hyaluronidase-armed Ad or Ad co-injected with hyaluronidase to degrade the hyaluronic-rich matrix in an attempt to improve virus penetration. Guedan et al. showed that hyaluronidase (PH20)-armed oncolytic Ad improved viral spread and inhibited growth of human melanoma xenograft with high hyaluronan content in nude mice. Co-injection of hyaluronidase showed similar results. Importantly, despite the degradation of hyaluronic acid, the protein matrix remained intact and PH20 only allowed penetration of particles up to 200 nm in diameter, facilitating the efficient spreading of Ad but not metastasis of cancer cells. This hyaluronidase-expressing oncolytic Ad has now completed a phase I clinical trial and demonstrated modulation of the TME to a more pro-inflammatory state with a good safety profile.

**CAFs can directly inhibit gene and oncolytic therapies**

Stroma-tumor interactions have been reported to directly suppress the uptake of adeno viral vectors and the associated transgene expression. Intriguingly, the expression of essential Ad receptors (the coxsackievirus and Ad receptor, integrins αvβ3 and αvβ5, as well as clathrin) was found to be downregulated in pancreatic cancer cells exposed to media that had been conditioned with patient-derived fibroblasts in vitro. The study implicated hepatocyte growth factor (HGF) in the conditioned-causing phosphorylation of Met in the cancer cells, since inhibition of HGF/Met restored receptor expression. CAFs can also provide physical barriers to virus infection. For example, Vähä-Koskela et al. reported that initial oncolysis by an oncolytic Semliki Forest virus (SFV) strain A7 led to the formation of CAF-encapsulated tumor nodules containing both dying and regrowing cancer cells that were resistant to subsequent virus challenge. These studies illustrate the diverse ways that CAFs can directly impact successful gene and virotherapy by preventing infection of target cells.

**Cytokines released by CAFs promote angiogenesis and immunosuppression**

CAFs also secrete a wide range of signaling proteins, MMPs, and angiogenic factors, all of which can contribute to tumor growth and...
metastasis. They can also drive repolarization of tumor-associated macrophages (TAMs) towards more immunosuppressive phenotypes, induce differentiation of regulatory T cells (Tregs), and attenuate cytotoxic T cell responses. For all of these reasons, CAFs provide an important therapeutic target, and gene therapy strategies to achieve depletion of CAFs are considered in more detail below.

**Strategies for targeting CAFs using gene therapies and armed OVs**

Although the central paradigm for OV is to achieve selective replication and lysis of cancer cells, some studies have shown the possibility to include the stromal compartment in this activity. This is attractive given the frequent association of high stromal content and poor prognosis. For example, an oncolytic Ad with Rb-binding-deleted E1A under the transcriptional control of a truncated promoter from the stroma-associated gene SPARC (secreted protein, acidic, rich in cysteine) was reported to infect and lyse both tumor and stromal cells in human ovary cancer explants and human stroma-containing xenograft tumors in mice but showed no activity in non-malignant tissue explants. To enhance the replication capability of the Ad, motifs responsive to hypoxia-responsive element (HRE) and inflammation (nuclear factor kB) were incorporated into the SPARC promoter. This modified Ad demonstrated superior activity leading to complete regression of human melanoma in nude mice. In another approach, recombinant measles virus retargeting to urokinase receptor, which is abundantly expressed on tumor and stromal cells, was shown to delay recombinant measles virus retargeting to urokinase receptor, which is abundantly expressed on tumor and stromal cells, was shown to delay

**Targeting fibroblast activation protein-α (FAP)**

Expression of FAP is a key characteristic of CAFs. FAP is upregulated in many cancer types and is frequently associated with poor patient prognosis. Although mechanisms underlying FAP-promoted tumorigenesis are not fully understood, a clear association of FAP with tumor progression, metastasis, and angiogenesis is observed. However, despite the considerable promise, phase II studies targeting FAP using the monoclonal antibody abrotuzumab or blocking the enzymatic activity of FAP by talabostat showed no therapeutic efficacy in phase II trials in colorectal cancer, and later studies in animal raised the possibility of causing cachexia by depleting stromal fibroblasts from normal tissues.

Bispecific antibodies are synthetic macromolecules that can be easily encoded into gene therapy vectors and OVs, and they provide great flexibility to orchestrate localized cellular functions within tumors. They represent a very popular emerging therapeutic strategy. Bispecific T cell engagers (BiTEs) are composed of two scFvs or nanobodies linked by a short serine-glycine linker with one arm binding to T cells, usually via T cell receptor (TCR) signaling complex CD3, and the other arm binding to the surface antigen on tumor cells or any target cells. The BiTE brings T cells and target cells into close proximity to form an immune synapse to initiate perforin/granzyme-killing mechanisms. An advantage of BiTE therapeutics is that the T cell-mediated cytotoxicity is independent of co-stimulatory signals or major histocompatibility complex class I (MHC class I), which is often downregulated in tumor cells, allowing them to evade immune surveillance.

Armed oncolytic EnAd encoding anti-FAP/CD3 BiTE has been shown to target T cell cytotoxicity towards FAP-expressing cells in the TME. Using patient-derived immunosuppressive malignant ascites as an *ex vivo* model, the armed EnAd showed powerful cytotoxicity toward endogenous CAFs by activation of tumor-resident T cells. This is intriguing because it suggests that BiTEs may be able to overcome local immunosuppressive effects, a phenomenon that is documented increasingly widely. BiTE treatment also led to repolarization of M2 to M1 TAMs, perhaps due to the level of interferon γ (IFNγ) produced by activated T cells, or perhaps reflecting a broader reprogramming of the TME. Global upregulation of various immune stimulatory pathways was also observed. EnAd has been showing good blood stability, low immunogenicity, and an acceptable safety profile in preclinical trials, and the armed version expressing the FAP BiTE is now undergoing clinical assessment (ClinicalTrials.gov: NCT04053283).

“Double-deleted” Western Reserve vaccinia virus (vVDD, missing thymidine kinase and vaccinia growth factor) expressing anti-FAP/CD3 BiTE has also been shown to improve viral replication and promote intratumoral infiltration of activated T cells in a melanoma syngeneic model. Local and distant tumor regression were also observed in a subcutaneous melanoma model with local OV injection. In addition, anti-FAP/CD3 BiTE-expressing Ad ICOVIR15K showed superior antitumor responses and intratumoral T cell accumulation compared to the parental virus in xenograft mouse models of lung and pancreatic cancer with adoptive transfer of preactivated human T cells. Interestingly, FAP-negative expressing cells proximal to FAP-positive expressing cells were targeted by T cell-induced bystander cell killing, suggesting the potential of killing hard-to-target pro-tumorigenic cell populations (e.g. with lack of identified surface markers and/or with low abundance) in the TME. Apart from the BiTE approach, vaccinia virus (VV) encoding an anti-FAP scFv induced modest regressions in tumor growth compared with unarm VV in lung and prostate cancer xenograft mouse models, although a significant reduction of CAFs was observed in both infected and uninfected areas. The modest antitumor activity may be due to the use of non-cytotoxic FAP-blocking antibody instead of the cytotoxic anti-FAP BiTE. The FAP-blocking antibody did not kill the CAFs, allowing them to regrow and restore the TME. These preclinical studies are summarized in Table 1.

**Vaccines for depleting CAFs**

Using a different approach, DNA vaccines recently showed preclinical success in depleting CAFs. This vaccine approach is designed to encourage antigen-presenting cells to process and present FAP and to activate both cell-mediated immune responses by cytotoxic T cells and humoral immune responses to produce antibodies. These studies showed the induction of FAP-specific CD8 T cells and decreased
production of stromal cytokines in syngeneic mice bearing colon and breast tumors. A modified DNA vaccine expressing the extracellular domain of human FAP with the tissue plasminogen activator signal sequence, to enhance antigen expression, also demonstrated a superior antitumor effect, particularly reducing the recruitment of myeloid-derived suppressor cells (MDSCs) and angiogenic cytokines to the TME.

**Tumor-associated endothelial cells (TECs) as a target for gene therapy**

Tumor vasculature exhibits fundamentally different phenotypes from normal vasculature due to its disorganized structure and uneven vessel lumen diameter. Tumor blood vessels are often heterogeneous, dysfunctional, and leaky due to discontinuous basement membranes, incomplete endothelial linings, and inadequate pericyte coverage. The increased transcapillary flow and the abnormal or absence of lymphatic vessels in the tumor lead to an accumulation of interstitial fluid and subsequent elevated IFP. Furthermore, increased IFP, inconsistent lumen resistance, and abnormal vasculature result in blood stagnation and bi-directional increased IFP, inconsistent lumen resistance, and abnormal vasculature in tumor regions. The leakiness of the vessels allows extravasation of the virus into the subendothelial regions, the high IFP and dense stromal matrix inhibit the convection of vectors and OV-s from the periphery to the core tumor regions.

**Strategies for targeting TECs using gene therapy and armed OVs**

**Targeting VEGF/VEGF receptor (VEGFR)**

In cancer, targeting angiogenesis to block oxygen and nutrient supplies has attracted considerable attention, and several inhibitors, including bevacizumab and sunitinib, have been licensed for cancer treatment. VEGF acts as the key mediator of physiological angiogenesis to support the growth and remodeling processes of blood vessels by binding to and activating VEGFR-1 or VEGFR-2 expressed on vascular endothelial cells. Nonetheless, targeting the vasculature with highly selective VEGF signaling inhibitors (such as bevacizumab) used as monotherapy does not show significant anti-tumor activity in many clinical settings. Several unarmed OVs have been reported to exhibit direct anti-vascular properties, and a recent publication from Yousaf et al. suggests that in the case of Ad this may be due to virus-mediated suppression of HIF-1α, decreasing local toxicity and unwanted cytokine release.

Replication-competent VV encoding anti-VEGF scFv has been shown to inhibit angiogenesis and formation of malignant effusion in xenograft mouse models of lung and prostate cancer. It also re-sensitized endothelial cells to ionizing irradiation. The combination of virus with irradiation facilitated viral replication and synergistically impaired the formation of tumor vasculature in a glioma xenograft mouse model. Arming vvDD with soluble VEGFR-1 fused with an Fc domain reduced the viral dose required to achieve a similar level of anti-tumor effect compared with unarmed vvDD in a xenograft mouse model of renal cancer, while lowering the potential toxicity and unwanted cytokine release.

Non-replicating Ad-Flk1-Fc expressing VEGFR-2-Fc also inhibited microvessel formation. The antiangiogenic and antitumor activity of Ad-Flk1-Fc was further enhanced upon co-infection with replication-competent E1A-attenuated Ad strain d922/924, which acts as

### Table 1. Preclinical studies of antibody-armed oncolytic viruses targeting cancer stroma

| Target     | Virus (species)                                      | Antibody (promoter)          | Effect ex vivo/in vivo                                      | Delivery route (frequency)               | Ref. |
|------------|-----------------------------------------------------|------------------------------|------------------------------------------------------------|----------------------------------------|------|
| FAP        | EnAd-SA-FAP-BiTE (Ad11p/Ad3; enadenotucirev)        | anti-CD3/FAP BiTE (MLP)      | malignant ascites: \( \downarrow \) CAF (FC); \( \uparrow \) T cell activation (FC, CD25, CD69); \( \uparrow \) global immune response (DNA microarray); \( \uparrow \) effector cytokines (IL-17F, IL-22, IFN-γ); \( \uparrow \) TAM repolarization (FC, D206, CD63, CD64) | N/A (ex vivo)                          | 57   |
|            |                                                     |                              | prostate biopsy: \( \downarrow \) CAF (CLSM, active caspase-3); \( \uparrow \) TIL activation (IHC, CD25, 7 dpi) | i.t. (1)                               |      |
| FAP        | ICO15K-FB7TE (Ad)                                    | anti-CD3/FAP BiTE (MLP)      | lung, pancreatic cancer/s.c. in NSG mice with pre-activated T cells: \( \downarrow \) tumor mass; \( \downarrow \) CAF (qPCR, IHC, FAP, 11 dpi); \( \uparrow \) T cell infiltration (IVIS) | i.t. (1)                               | 58   |
|            |                                                     |                              |                                                           | i.t. (3; 0, 3, and 6 dpi)               |      |
| FAP        | mFAP:TEA-VV (VV)                                     | anti-CD3/FAP BiTE (F17R late promoter) | melanoma/s.c. in C57 mice: \( \downarrow \) tumor mass; \( \uparrow \) TIL activation (FC, IL-2, IFN-γ, 8 dpi) | i.t. (1)                               | 60   |
| FAP        | GLV-1h282 (VV)                                       | anti-VEGF scFv (PSEL)        | lung, prostate/ s.c. in nude mice: \( \downarrow \) tumor mass; \( \downarrow \) CAF (IHC, FAP, 36 dpi) | r.o. (1)                               | 61   |

Ad, adenovirus; BiTE, bispecific T cell engager; CSLM, confocal laser scanning microscopy; dpi, days post-infection; ELISA, enzyme-linked immunosorbent assay; FAP, fibroblast activation protein-α; FC, flow cytometry; IFN-γ, interferon γ; IHC, immunohistochemistry; IL-2, interleukin 2; IL-17F, interleukin 17F; i.t., intratumoral; IVIS, in vivo imaging system; MLP, major late promoter; N/A, not applicable; PSEL, synthetic early/late promoter; Ref., reference; r.o., retro-orbital; s.c., subcutaneous; TAM, tumor-associated macrophage; TIL, tumor-infiltrating lymphocyte; VV, vaccinia virus.
**Table 2. Preclinical studies of antibody-armed oncolytic viruses targeting tumor vasculature**

| Target         | Virus (species) | Antibody form (promoter) | Effect ex vivo/in vivo | Delivery route (frequency) | Ref. |
|----------------|----------------|--------------------------|------------------------|---------------------------|------|
| VEGF/VEGFR     | GLV-1h107; 1h108; 1h109 (VV) | anti-VEGF scFv (PSE, PSEL, PSL) | lung, prostate cancer/s.c. in nude mice: ↓ tumor mass; ↓ MVD (IHC, CD31, 21 dpi) | i.v. (1) | 74 |
| VEGF/VEGFR     | GLV-1h109 (VV) | anti-VEGF scFv (PSL) | soft tissue sarcoma, prostate cancer/s.c. in nude mice: ↓ tumor mass; ↓ MVD (IHC, CD31, 7 dpi); ↑ intratumoral GR-1<sup>+</sup>CD11b<sup>+</sup> granulocyte, GR-1<sup>+</sup>CD11b<sup>+</sup> monocyte, and F4/80<sup>+</sup>CD45<sup>+</sup> macrophage (FC, 7 dpi) | i.v. (1) | 85 |
| VEGF/VEGFR     | GLV-180 (VV) | anti-VEGF scFv (PSEL) | lung cancer/s.c. in nude mice: ↓ tumor mass, malignant effusion | i.v. (1) | 75 |
| VEGF/VEGFR     | GLV-1h164 (VV) | anti-VEGF scFv (PSE) | lung, prostate cancer/s.c. in nude mice: ↓ tumor mass; ↓ MVD (IHC, CD31, 36 dpi) breast cancer/o.t. in nude mice: ↓ tumor mass | r.o. (1) | 83, 86 |
| VEGF/VEGFR     | GLV-5a451 (VV) | anti-VEFG scFv (PSEL) | soft tissue sarcoma/s.c. in nude mice: ↓ tumor mass; ↓ MVD (IHC, CD31, 17 dpi) mammary cancer/s.c. in nude mice: ↓ tumor mass; ↓ MVD (IHC, CD31, 28 dpi) | i.v. (1) | 87, 88 |
| VEGF/VEGFR     | AAVrh10.BevMab (AAV) | anti-VEGF IgG (bevacizumab) (CAG promoter) | ovarian cancer/i.p. in BALB/c nude mice: ↓ tumor mass; ↓ MVD (IHC, CD31, 24 dpi) | i.p. (1) | 89 |
| VEGF/VEGFR     | Ad5/3-9HIF-Δ24-VEGFR-1-Ig (Ad) | soluble VEGFR1-Fc fusion protein (E3 promoter) | renal cancer/s.c. in nude mice: ↓ tumor mass; ↓ MVD (IHC, VWF, 16 dpi) | i.t. (1) | 90 |
| VEGF/VEGFR     | vvDD-VEGF1-1-Ig (VV) | soluble VEGFR1-Fc fusion protein (PSEL) | renal cancer/s.c. in nude mice: ↓ tumor mass; ↓ MVD (IHC, VWF, 36 dpi) renal cancer/s.c. in nude mice: ↓ tumor mass; ↓ MVD (IHC, VWF, 24 dpi); ↑ effector cytokine (IFNγ, CCL5, MCP-1) renal cancer/s.c. in BALB/c mice: ↓ tumor mass; ↑ effector cytokine (IFNγ, CCL5, MCP-1) | i.t. (1) | 73 |
| VEGF/VEGFR     | Ad-Flik1-Fc (Ad) | soluble Flik1-Fc (CMV) | lung cancer, fibrosarcoma/s.c. in C57BL/6 mice: ↓ tumor mass; ↓ MVD (IHC, CD31; corneal micropocket assay) | i.v. (1) | 77 |
| VEGF/VEGFR     | Ad-Flik1-Fc (Ad) + d822/947 (Ad) | soluble Flik1-Fc (CMV) | prostate, colorectal cancer/s.c. in CD1, SCID mice: ↓ tumor mass; ↓ MVD (IHC, CD31, 23 dpi) | Ad-Flik-Fc: i.t. (1) | 78 |
| VEGF/VEGFR     | vvDD (VV) + Ad-Flik1-Fc (Ad) | anti-Flik1-Fc (CMV) | breast cancer/s.c. in nude mice: ↓ tumor mass | i.v. (1) | 79 |
| CXCR4/CXCL12   | OVV-CXCR4-A-Fc (VV) | soluble CXCR4-A-Fc (PSEL) | ovarian cancer/o.t. in C57BL/6 mice: ↓ tumor mass, metastasis, cancer-initiating cells; ↑ survival; ↓ ascitic CXCL12, VEGF (ELISA, ~33 dpi); ↓ ascitic EPCs, MDSCs, pDCs, Tregs (FC, ~33 dpi); ↓ ascitic IFNγIL-10 CD4 CD8 T cell ratio (FC, ~33 dpi) | i.p. (3; 0, 7, 14 dpi) | 84 |
| CXCR4/CXCL12   | OVV-CXCR4-A-Fc (VV) | soluble CXCR4-A-Fc (PSEL) | breast cancer/o.t. in BALB/c mice: ↓ metastasis; ↓ MVD (IHC, CD31, KS67, 14 dpi); ↓ intratumoral CXCL12, VEGF (ELISA, 14 dpi); ↓ intratumoral EPCs, MDSCs (FC, 14 dpi); ↑ serum antitumor antibody | i.p. (7; 0, 1, 2, 3, 4, 5, 6 dpi) | 83 |

(Continued on next page)
help virus to support replication and repackaging of Ad-Flk1-Fc by providing E1 functions in trans. In addition, Hou et al. reported that the timing of Ad-Flk1-Fc administration was also critical for the potency of the anti-tumor response in a xenograft mouse model of breast cancer. Intravenous injection of Ad-Flk1-Fc 7 days after the vvDD injection showed greater tumor regression than did coinjection, as Flk1-Fc blocked the revascularization after vvDD clearance.

**Antivascular strategies targeting chemokine receptor 4 (CXCR4)**

CXCL12 mediates an indirect angiogenesis program by recruiting CXCR4-expressing bone marrow-derived monocytes to the perivascular regions of the TME. The recruited monocytes then release angiogenic factors such as VEGF and angiopoietin, which subsequently recruit endothelial and pericyte progenitors for neovascularization. CXCL12-stimulated CXCR4 has also been shown to positively regulate VEGF promoter activity and VEGF secretion via AKT and also by inhibiting glycolytic enzyme phosphoglycerate kinase 1 (PGK1) expression and activating angiogenesis-associated genes.

Systemic delivery of a VV expressing CXCR4 fused with murine Fc domain (CXCR4-mFc) was reported to disrupt tumor vasculature in both the tumor core and periphery and reduce the levels of CXCL12, VEGF, endothelial progenitor cells (EPCs), and MDSCs in tumor perfusion in an orthotopic breast cancer model. The suppression of lung metastasis in this tumor model further suggested that both VV-mediated direct cytotoxicity and the anti-tumor immunity (as detected by a sustained and elevated level of tumor-specific antibody in serum) contributed to the observed tumor regressions. It also showed higher viral titers in the lung metastases than the pre-operative setting, further highlighting the importance of the timing of OV delivery. In addition, CXCR4-mFc enhanced VV-disrupted immunosuppression by reducing the recruitment of Tregs and augmenting the ratio of IFNγ/interleukin (IL)-10-producing CD4+ and CD8+ lymphocytes in an ovarian cancer syngeneic model. These and other studies targeting the TECs are summarized in Table 2.

**Vaccines for targeting TECs**

A DNA vaccine expressing VEGFR2 has been reported to break peripheral T cell tolerance and exploit upregulation of VEGFR2 to elicit T cell-mediated killing of tumor vascular endothelial cells without impairing hematopoiesis and fertility in a syngeneic tumor mice model. In addition, non-human primates vaccinated with VEGF protein combined with a proteoliposome adjuvant can also break peripheral tolerance and elicit VEGF-neutralizing antibodies and a T cell response to VEGF-presenting cells. DNA vaccines targeting endoglin and delta-like ligand 4 (DLL4), expressed on endothelial tip cells during angiogenic sprouting, have also been reported to regress tumor formation and neoangiogenesis without affecting wound healing in murine models.

In contrast to the monovalent vaccine, placenta-derived endothelial cells primed with IFNγ, named ValloVax, is a whole cell-based polyvalent vaccine. The idea of using placenta as the source of antigens is based on the immunological similarities between pregnancy and cancer. Endothelial cells from placenta and tumors not only share many about guidance receptor 4 (ROBO4), suggesting that they may share a relatively comparable angiogenic mechanism and microenvironment. Immunization with ValloVax regressed tumor formation in syngeneic mice bearing melanoma, lung, and breast cancer. Clinical trials showed that ValloVax immunization elicited antibody response
against antigens associated with angiogenesis, including VEGFR1, VEGFR2, endoglin, and fibroblast growth factor receptor (FGFR).\textsuperscript{95,96}

**Gene therapies encoding antiangiogenic proteins**

Several gene therapy strategies that express antiangiogenic proteins have shown promising results in preclinical and clinical trials. The recombinant pseudotyped AAV serotype 2/8 vector\textsuperscript{29} and human placenta-derived mesenchymal stem cells (MSCs) with fiber-modified Ad vector\textsuperscript{30} carrying the plasminogen kringle 1 domain (RDD) showed an inhibitory effect on endothelial cell growth in a cancer-specific manner in a melanoma syngeneic mouse model.\textsuperscript{101} In a related strategy, NK4 is an angiogenesis inhibitor that acts as a competitive antagonist of HGF, which induces VEGF expression\textsuperscript{102}. Gene transfer of NK4 by Ad vector,\textsuperscript{103} electrotransfer,\textsuperscript{104} or MSCs\textsuperscript{105} demonstrated significant suppression of angiogenesis with potent antitumor activity that could be usefully combined with cisplatin\textsuperscript{106} or gemcitabine.\textsuperscript{107}

Apart from expressing antiangiogenic proteins or soluble receptors that bind to angiogenic molecules, another interesting approach has been the silencing of the angiogenic protein endoglin, a co-receptor that bind to angiogenic molecules, another interesting approach has been the silencing of the angiogenic protein endoglin, a co-receptor of TGF-β, using small interference RNA (siRNA)\textsuperscript{108} and short hairpin RNA (shRNA)\textsuperscript{109} under the control of the endothelin-1 promoter. This new strategy was shown to suppress angiogenesis and tumor growth in mammary adenocarcinoma\textsuperscript{108} and melanoma\textsuperscript{109} syngeneic mouse models.

**Tumor immune cells as a target for gene and virotherapy**

During the early phase of tumor formation, innate and adaptive immune cells are thought to migrate to the tumor to destroy cancer cells and secrete cytokines to sustain a pro-inflammatory environment. However, these cells are later “educated,” directly or indirectly, by tumor-secreted factors, and acquire pro-tumorigenic phenotypes. For example, under the influence of tumor secreted CSF-1, type I cytokine-producing M1 macrophages repolarize into key immunosuppressive M2 TAMs.\textsuperscript{4} They secrete C-C motif chemokine ligand 22 (CCL22) to recruit Tregs to the TME.\textsuperscript{110} M2 TAMs and Tregs mediate tumor tolerance by secreting immunosuppressive cytokines such as TGF-β, IL-10 and by expressing ligands for programmed death 1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4).\textsuperscript{111} Moreover, not all activated T cells are similarly potent in killing tumor cells. Persistent activation drives T cells to an “exhausted” state, in which they have high levels of inhibitory receptors and a transcription profile distinctly different from their effector or memory counterparts, resulting in a loss of proliferative and cytotoxic potential.\textsuperscript{112} In addition, MDSCs promote immune suppression by impairing lymphocyte homing, upregulating immune regulatory molecules B7 and PD-L1, and suppressing the expression of TCR through free radical production and metabolic depletion.\textsuperscript{113} The respective functions of immune cells in the TME were summarized by Gajewski et al.\textsuperscript{114} Tumor-associated immune cells therefore provide compelling target populations for OV, with key studies identified in Table 3.

**OVs creating a proinflammatory environment**

OVs augment the process of antigen presentation and T cell priming by providing three crucial signals, i.e., release of cancer antigens following cell lysis, co-stimulation by release of PAMPs and DAMPs, and production of proinflammatory cytokines.\textsuperscript{128} Following the recognition of OV as “foreign invaders” by the innate immune system, the release of type I IFNs often enhances the expression of MHC class I and II molecules and costimulatory signals on dendritic cells (DCs).\textsuperscript{129,130} Meanwhile, tumor antigens are released by cell lysis and may be cross-presented by DC-activated tumor-specific cytotoxic T cells. This process of uptake and cross-presentation of antigen by DCs is the central mechanism of epitope spreading, and it is essential for the recognition of tumor neoantigens.\textsuperscript{131,132} Epitope spreading facilitates the development of more diversified T cell clones against the tumors. Notably, an oncolytic Ad was reported to promote an oligoclonal T cell response against a panel of neoepitopes, while anti-PD-1 antibody was only able to elicit immune response against a single neoepitope in a murine lung adenocarcinoma model.\textsuperscript{133}

Impairment of antigen presentation is one of the primary methods for tumor immune evasion. OV have some potential to reverse this escape mechanism and thereby promote the APC-T cell interaction.\textsuperscript{128} For example, reovirus infection induced the expression of MHC class I, β2-microglobulin (B2M), transporter associated with antigen processing 1 (TAP-1), and TAP-2 on the tumor surface.\textsuperscript{134} Reovirus also restored the expression of MHC class II, CD40, CD80, and CD86 expression on DCs, which are initially downregulated by the tumors, and restored their overall antigen presentation ability to T cells. In addition, T cell priming was augmented by reovirus-induced proinflammatory cytokines such as TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-6, which promoted DC maturation, antigen presentation, and trafficking of T cells and macrophages to the tumor region.\textsuperscript{135} This induction of type I IFN by OV can provide the third signal necessary for naïve T cells to acquire effector phenotypes.\textsuperscript{136,137} OV also promoted the tumor infiltration of DCs and promote their homing to the lymphoid organs for T cell priming.\textsuperscript{138}

Nevertheless, one major challenge facing the field is how to direct the adaptive immune response towards recognition of tumor neoantigens rather than viral epitopes,\textsuperscript{139} particularly since the neoantigens are generally thought to comprise relatively weak immunogens. Considerable attention in the field is focused toward this goal, building on the other powerful attributes of the oncolytic approach.\textsuperscript{140}
### Table 3. Preclinical studies of antibody-armed oncolytic viruses targeting tumor immune cells

| Target | Virus (species) | Antibody form (promoter) | Effect ex vivo/in vivo | Delivery route (frequency) | Ref. |
|--------|-----------------|--------------------------|------------------------|---------------------------|------|
| FRβ    | EnAd-3FR (Ad11p/Ad3) | anti-FRβ/CD3 BiTE (MLP) | patient-derived malignant ascites; ∆ ascitic CD11b⁺ CD64⁺ macrophages (FC); ↑ T cell activation (FC); ↑ TAM repolarization (FC, CD80, CD86); ↑ IFNγ (ELISA) | N/A (ex vivo) | 115 |
| PD-1/PD-L1 | WR-mAb1 (VV) | anti-PD-1 IgG | fibrosarcoma/s.c. in C57BL/6 mice; ↓ tumor mass; ↑ survival; ↑ intratumoral CD4⁺, IFNγ⁺TNF-α⁺CD8⁺ T cell ratio, ↓ Tregs (FC, 7 dpi) | i.t. (2; 0, dpi) | 116 |
| PD-1/PD-L1 | MV-aPD-L1 (MV) | anti-PD-L1 scFv-Fc | melanoma/s.c. in C57BL/6 mice: ↓ tumor mass; ↑ survival; ↑ IFNγ⁺CD8 T cells, CD8⁺/Treg ratio (FC, 5 dpi) primary melanoma biopsy; ↓ tumor mass | i.t. (5; 0, 1, 2, 3, 4 dpi) N/A (ex vivo) | 117 |
| PD-1/PD-L1 | OVH-aMPD-1 (HSV) | anti-PD-1 scFv (CMV promoter) | hepatoma/s.c. in C57BL/6 mice: ↓ local, distant tumor mass; ↓ survival; ↓ s.c. rechallenged tumor; ↑ local and distant intratumoral CD4⁺CD69, CD4⁺ICOS⁺, CD8⁺CD69, CD8⁺ICOS⁺, MDSCs (FC; 10 dpi) | i.t. (2; 0, 3 dpi) | 118 |
| CTLA-4 | MV-aCTLA-4 (MV) | anti-CTLA-4 scFv-Fc | melanoma/s.c. in C57BL/6 mice: ↓ tumor mass; CD8⁺/Treg ratio (FC, 5 dpi) | i.t. (5; 0, 1, 2, 3, 4 dpi) | 117 |
| CTLA-4 | SKL002 (Ad) | anti-CTLA-4 IgG (E3 promoter) | melanoma/s.c. in C57BL/6 mice: ↓ tumor mass, metastasis | i.v. (3; every 3 days) i.t. (4; every other day) | 119 |
| CTLA-4 | Ad5/3-Δ24aCTLA4 (Ad) | anti-CTLA-4 IgG (CMV promoter) | prostate and lung cancer/s.c. in nude mice: ↓ tumor mass; ↑ tumor apoptosis (ICHC, active caspase 3, 5 dpi) PBMCs from patients with solid tumor refractory to chemotherapy; ↑ IL-2, IFNγ (FC) | i.t. (3; 0, 2, 4 dpi) | 120 |
| CTLA-4 | IAV-CTLA4 (orthomyxovirus) | anti-CTLA-4 scFv | Melanoma/s.d. in C57BL/6 mice: ↓ local, distant tumor mass; ↑ survival | i.t. (4; 0, 2, 4, 6 dpi) | 121 |
| CTLA-4 | virus 27 (HSV) | anti-CTLA-4 Ab-like molecule | lymphoma/s.c. in BALB/c mice: ↓ local, distant tumor mass | i.t. (3; every 2 days) | 122 |
| CTLA-4 | rNDV-CTLA-4 (NDV) + radiation | anti-CTLA-4 scFv | melanoma/s.d. in C57BL/6 mice: ↓ tumor mass; ↑ survival | i.t. (5; 0, 2, 4, 6, 8 dpi) | 123 |
| TGF-β/TGF-βR | rAd.sT (Ad) | soluble TGFβRII-Fc (TERT promoter) | breast, renal cancer/s.c. in BALB/c mice: ↓ tumor mass, metastasis; ↓ metastasis-related genes (PTHrP, CXCXR4), angiogenesis-related genes (VEGFA, VEGFR) (qPCR, 5 dpi); ↑ intratumoral IFNγ, TNF-α, IL-2 (qPCR, 5 dpi); ↑ CD8⁺/CD4⁺ T cells ratio, CD44⁺CD62L⁺ memory T cells in PBMCs (FC, 5 dpi); ↓ MDSCs (FC, 5 dpi); ↓ splenic Tregs (FC, 24 dpi) | i.p. (2; 0, 3 dpi) | 124 |
| TGF-β/TGF-βR | mAd.sTβRIIFc (Ad5/48) | soluble TGFβRII-Fc | prostate cancer injected into left heart ventricle of nude mice (bone metastasis model); ↓ tumor mass, bone metastasis; ↑ survival; ↓ tumor-induced osteolytic bone destructions (microCT, TRAP, 43 dpi) | i.v. (2; 0, 3 dpi) | 125,126 |

(Continued on next page)
Immunosuppressive populations suppress efficacy of oncolytic virotherapy

Despite the pro-inflammation responses during oncolysis, anti-tumor activity is still partly impaired by peripheral tolerance. Sobol et al.\textsuperscript{139} reported that the ability of OV to induce CD8\textsuperscript{+} T cell responses against tumor-associated antigens (TAAs) was attenuated in a tolerized antigen model, potentially reflecting central tolerance or peripheral tolerance mediated by Tregs, TGF-β, and IL-10. In addition, TAMS can secrete macrophage-derived IFNβ and confer upon tumors a constitutive antiviral state with resistance to virotherapy by upregulating IFN-stimulated genes.\textsuperscript{141} In support of this, systemic depletion of CD11b\textsuperscript{+} myeloid cells has been shown to enhance oncolytic HSV anti-tumor responses, suggesting that targeted depletion of immunosuppressive macrophages may restore OV-mediated anti-tumor efficacy.\textsuperscript{142}

Strategies for targeting immunosuppressive cells using OVs

**Immunotherapy targeted to folate receptor β (FRβ)**

FRβ was identified as a differential expressed marker on M2-polarized macrophages \textit{in vitro}.\textsuperscript{143} Although a strong association of FRβ with anti-inflammation and tumor progression has been observed, only a few therapeutic approaches targeting FRβ have been reported. An earlier study reported the use of chimeric antigen receptor (CAR) T cells expressing anti-FRβ scFv to regress acute myeloid leukemia (AML) in a human xenograft mouse model, and the antitumor effect was augmented by FRβ-inducing agent all-trans retinoic acid (ATRA).\textsuperscript{144} Importantly, the CAR T cells were not toxic to normal healthy hematopoietic stem and progenitor cells (HSCs) or monocytes expressing low levels of FRβ. An anti-FRβ/CD3 BiTE was later reported and encoded in EnAd for targeting M2 macrophages. The armed OV triggered endogenous T cell activation and depleted the M2 macrophages within primary samples of malignant ascites from cancer patients, sparing the more pro-inflammatory M1 macrophages.\textsuperscript{115}

**Targeting PD-1/PD-L1**

PD-1 is an inhibitory receptor expressed on activated T cells, B cells, natural killer (NK) cells, and some myeloid populations, yet its immunological functions are best characterized in conventional T cells. Persistent antigen stimulation (cancer or chronic infection) drives T cells to an exhausted state as indicated by PD-1 expression. Tumors express PD-L1 and PD-L2, which act as inhibitory signals to impair T cell effector functions and assist in immune evasion.\textsuperscript{145} Many OVs armed with anti-PD-1/PD-L1 antibodies have shown promising results in syngeneic melanoma mouse models. For example, measles virus (MV) encoding anti-PD-L1 scFv fused with Fc domain (MV-aPD-L1) demonstrated a greater cytotoxic potential than did the unarmed MV by increasing tumor-infiltrating IFNγ-producing CD8\textsuperscript{+} T cells and the ratio of CD8\textsuperscript{+} T cells to Tregs.\textsuperscript{147} This armed MV also killed primary melanoma \textit{ex vivo}. In addition, Kleinpeter et al.\textsuperscript{148} reported that VV encoding anti-PD-1 immunoglobulin (Ig) G or scFv showed better fibrosarcoma regression and overall survival than did the unarmed virus in a syngeneic mouse model, although no significant difference was observed in CD4\textsuperscript{+}, CD8\textsuperscript{+}, and Treg populations. In addition, unarmed HSV was reported to induce a type I IFN response, which upregulated PD-L1 expression in the TME.\textsuperscript{116} An anti-PD-1 scFv-armed HSV enhanced phagocytosis of infected cancer cells and improved antigen presentation by DCs as compared to unarmed HSV. It regressed primary and distant tumor formation apparently by enhancing the activation of intratumoral CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells in a syngeneic liver cancer model, although an infiltration of CD155\textsuperscript{+} MDSCs was observed. Long-term surviving mice were also protected in tumor rechallenge studies. Blockade of T cell immunoreceptor with Ig and ITIM domains (TIGIT), a receptor of CD155, further augmented the therapeutic efficacy. All three aforementioned studies, i.e., the armed MV, VV, HSV, showed similar therapeutic responses to the combined therapy of unarmed OVs with antibodies, confirming that the therapeutic effect is not virus type- or antibody form-dependent.

**Targeting CTLA-4**

CTLA-4 is an immune checkpoint that is closely associated with the modulation of T cell activity. CTLA-4 dampens TCR signaling by binding to B7-1 (CD80) and B7-2 (CD86) with higher affinity and avidity than CD28. Secondary lymphoid organs, primarily lymph nodes, are the sites of T cell priming, where CTLA-4 primarily regulates T cell activity.\textsuperscript{146} Anti-CTLA-4 monoclonal antibody (mAb)-armed Ads Ad5/3-Δ24ΔCTLAT4\textsuperscript{A} and SKL002\textsuperscript{119} enhanced anti-tumor activity without affecting antiviral immunity or the viral titers in a xenograft mouse model of lung and prostate cancer. The combined
therapy of SKL002 with GM-CSF-armed Ad further regressed the formation of lung tumor in the syngeneic melanoma mouse model. Nonetheless, Ad5 does not replicate productively in mice and may not fully represent the immune responses in humans. Recently, Hamilton et al.\textsuperscript{121} engineered the replication-competent influenza A virus (IAV) and inserted the anti-CTLA-4 scFv gene segment downstream of PB1 and PA viral polymerase, restricting the antibody expression during the viral life cycle. The armed IAV not only inhibited the growth of treated tumor but also demonstrated abscopal effects to regress distal tumor formation and prolong survival in a syngeneic melanoma mouse model. In addition, Engeland et al.\textsuperscript{122} reported that anti-CTLA-4 scFv-armed MV encoding (MV-aCTLA-4) increased the ratio of tumor-infiltrating CD8$^+$ T cells to Tregs and the IFN$\gamma$ response. Although the armed MV significantly regressed tumor formation in the syngeneic melanoma model, mice receiving armed MV injections showed inferior overall survival than did those receiving combination treatments of unarmed MV and systemic CTLA-4 antibody. Interestingly, Newcastle disease virus (NDV) expressing anti-CTLA-4 scFv induced potent anti-tumor responses comparable to systemic anti-CTLA-4 in combination with ionizing irradiation.\textsuperscript{12,123} Systemic anti-tumor responses were also observed in an armed HSV-1, which expressed anti-CTLA-4 antibody-like molecule, GM-CSF, and a fusogenic form of the envelope glycoprotein of gibbon ape leukemia virus (GALV-GP-R$^+\) ).\textsuperscript{122}

**FUTURE PERSPECTIVES**

Targeting the TME is still a relatively new concept in gene and virotherapy, and novel strategies continue to emerge. Of all of the challenges faced, those provided by CAFs and other immunosuppressive cells are perhaps the greatest since their many facets affect many different aspects of cancer biology as well as limiting the effectiveness of different therapeutic approaches.

Essentially, how to deplete CAFs and other immunosuppressive cells effectively and specifically comprises a major research direction, but one in which molecular approaches are uniquely able to address. Encouragingly, worldwide attention is increasingly being focused on these aspects of the TME, and particularly on reversing its local immunosuppressive effects. Depletion of CAFs is being explored in clinical trials using OV$s$ expressing CAF-specific BiTEs for targeted T cell cytotoxicity, and it is encouraging that BiTEs recognizing FR$\beta$\textsuperscript{124} have also been reported to selectively deplete M2 macrophages. There are also additional potential targets that have not been received much attention, for instance, fibroblast specific-protein-1 (FSP1) is a CAF marker that is present in multiple cancer stroma.\textsuperscript{147} Another CAF population expresses a different set of markers, including $\alpha$-smooth muscle actin ($\alpha$-SMA), neuron-glial antigen-2 chondroitin sulfate proteoglycan (NG2), and platelet-derived growth factor receptor-$\beta$ (PDGFR$\beta$).\textsuperscript{148} Targeting multiple CAF markers using locally expressed BiTEs could allow simultaneously depletion of different CAF populations and may facilitate reversal of their deleterious effects.

Exploration of more sophisticated tri- or quadri-constructs, aiming at improving the efficacy of the T cell engager, is an important research area. One approach is to incorporate multiple domains targeting the antigens or CD3 to increase the avidity of the BiTE to the target cells or T cells and enhance the BiTE-mediated cytotoxicity. Another strategy is to incorporate an additional domain targeting the costimulatory signals such as CD28 and 4-1BB. Anti-CD28 bispecific antibody has been reported to improve the antitumor activity of anti-CD3 BiTE by enhancing the formation of “pseudo” immune synapse.\textsuperscript{149}

Furthermore, exploring NK cells and macrophages as effector cells to which bispecific antibodies can crosslink the target cells may exploit new mechanisms of innate antitumor immunity. For example, NK cell-mediated cytotoxicity was enhanced by anti-CD16/CD33 bispecific killer cell engager (BiKE) against primary myelodysplastic syndromes (MDSs) and CD33$^+$ MDSC populations.\textsuperscript{150} In 2019, a TriKE targeting CD16 and NKp46 on NK cell exhibited more potent immune responses with similar pharmacokinetics and improved unwanted effects compared with an IgG antibody targeting the same antigen.\textsuperscript{151} It follows that identifying gene and virotherapy strategies that can fully utilize the therapeutic power of NK cells and macrophages could unleash still more potent antitumor and anti-TME effects.

**ACKNOWLEDGMENTS**

This work was supported by Cancer Research UK (grant no. C552/A29106).

**DECLARATION OF INTERESTS**

L.W.S. owns equity or share options in PsiOxus Therapeutics, which is leading the clinical development of EnAd and its derivatives. The remaining authors declare no competing interests.

**REFERENCES**

1. Balkwill, F.R., Capasso, M., and Hagemann, T. (2012). The tumor microenvironment at a glance. J. Cell Sci. 125, 5591–5596.
2. Lamagna, C., and Bergers, G. (2006). The bone marrow constitutes a reservoir of pericyte progenitors. J. Leukoc. Biol. 80, 677–681.
3. Motz, G.T., Santoro, S.P., Wang, L.-P., Garrassan, T., Lastra, R.R., Hagemann, I.S., Lal, P., Feldman, M.D., Benencia, F., and Coukos, G. (2014). Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. Nat. Med. 20, 607–615.
4. Quail, D.F., and Joyce, J.A. (2013). Microenvironmental regulation of tumor progression and metastasis. Nat. Med. 19, 1423–1437.
5. Roma-Rodrigues, C., Mendes, R., Baptista, P.V., and Fernandes, A.R. (2019). Targeting tumor microenvironment for cancer therapy. Int. J. Mol. Sci. 20, 840.
6. Zhang, W.-W., Li, L., Li, D., Liu, J., Li, X., Li, W., Xu, X., Zhang, M.J., Chandler, L.A., Lin, H., et al. (2018). The first approved gene therapy product for cancer Ad-p53 (Gendicine): 12 years in the clinic. Hum. Gene Ther. 29, 160–179.
7. McGranahan, N., and Swanton, C. (2017). Clonal heterogeneity and tumor evolution: Past, present, and the future. Cell 168, 613–628.
8. Kakarla, S., Song, X.-T., and Gottschalk, S. (2012). Cancer-associated fibroblasts as targets for immunotherapy. Immunotherapy 4, 1129–1138.
9. Toro Bejarano, M., and Merchán, J.R. (2015). Targeting tumor vasculature through oncolytic virotherapy: recent advances. Oncolytic Virother. 4, 169–181.
10. Marchand, J.-B., Semmrich, M., Fend, L., Rehm, M., Silvestre, N., Teige, I., Follope, F., Mårtensson, L., Quéméneur, E., and Frendeus, B. (2020). BT-001, an oncolytic vaccinia virus armed with a Treg-depletion-optimized recombinant human anti-
CTLA4 antibody and GM-CSF to target the tumor microenvironment. Cancer Res 80 (16 Suppl), 5602.

11. Seymour, L.W., and Fisher, K.D. (2016). Oncolytic viruses: Finally delivering. Br. J. Cancer 114, 357–361.

12. Dhar, D., Toth, K., and Wold, W.S. (2012). Syrian hamster tumor model to study oncolytic Ad5-based vectors. Methods Mol. Biol. 797, 53–63.

13. Goerin, M.V., Finiguerra, V., Van den Eynde, B.J., Bercovici, N., and Trautmann, A. (2020). Preclinical murine tumor models: A structural and functional perspective. eLife 9, e50740.

14. Yamaguchi, R., and Perkins, G. (2018). Animal models for studying tumor microenvironment (TME) and resistance to lymphatic infiltration. Cancer Biol. Ther. 19, 745–754.

15. Yoshida, G.I. (2020). Applications of patient-derived tumor xenograft models and tumor organoids. J. Hematol. Oncol. 13, 4.

16. Scott, E.M., Frost, S., Khalique, H., Freedman, J.D., Seymour, L.W., and Leitkühnel, F. (2020). Use of liquid patient ascites fluids as a preclinical model for oncolytic virus activity. Methods Mol. Biol. 2058, 261–270.

17. Hutt, M., Färber-Schwarz, A., Unverdorben, F., Richter, F., and Kontermann, R.E. (2012). Plasma half-life extension of small recombinant antibodies by fusion to immunoglobulin-binding domains. J. Biol. Chem. 287, 4462–4469.

18. Hamers-Casterman, C., Atarhouch, T., Muyldermans, S., Robinson, G., Hamers, C., Songa, E.B., Bendahman, N., and Hamers, R. (1993). Naturally occurring antibodies devoid of light chains. Nature 363, 446–448.

19. Herrmiston, T.W., and Kuhn, I. (2002). Armed therapeutic viruses: strategies and challenges to arming oncolytic viruses with therapeutic genes. Cancer Gene Ther. 9, 1022–1035.

20. Robinson, M., Ge, Y., Ko, D., Yenduri, S., Laflamme, G., Hawkins, L., and Jooss, K. (2008). Comparison of the E3 and L3 regions for arming oncolytic adenoviruses to achieve a high level of tumor-specific transgene expression. Cancer Gene Ther. 15, 9–17.

21. Wirth, T., Zender, L., Schulte, B., Mundt, B., Plentz, R., Rudolph, K.L., Manns, M., Kubicka, S., and Kuhnel, F. (2003). A telomerase-dependent conditionally replicating adenovirus for selective treatment of cancer. Cancer Res. 63, 3181–3188.

22. DeWeese, T.L., van der Poel, H., Li, S., Mikhak, B., Drew, R., Goemann, M., Hamper, U., Dejong, R., Detorie, N., Rodriguez, R., et al. (2001). A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. Cancer Res. 61, 7464–7472.

23. Chen, X., and Song, E. (2019). Turning foes to friends: Targeting cancer-associated fibroblasts. Nat. Rev. Drug Discov. 18, 99–115.

24. LAKINS, M.A., Ghorani, E., Munir, H., Martins, C.P., and Shields, J.D. (2018). Cancer-associated fibroblasts induce antigen-specific deletion of CD8 T cells to protect tumour cells. Nat. Commun. 9, 948.

25. McKee, T.D., Grandi, P., Mok, W., Alexandrakis, G., Insin, N., Zimmer, J.P., Baswendi, M.G., Boucher, Y., Breakfield, X.O., and Jain, R.K. (2006). Degradation of fibrillar collagen in a human melanoma xenograft improves the efficacy of an oncolytic herpes simplex virus vector. Cancer Res. 66, 2509–2513.

26. Erdogan, B., and Webb, D.J. (2017). Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. Biochem. Soc. Trans. 45, 229–236.

27. Henke, E., Nandigama, R., and Ergin, S. (2020). Extracellular matrix in the tumor microenvironment and its impact on cancer therapy. Front. Mol. Biosci. 6, 160.

28. Vähä-Koskela, M.J., Kallio, J.P., Jansson, L.C., Heikkilä, J.E., Zakhartchenko, V.A., Kallajo, M.A., Kahari, V.M., and Hinkkanen, A.E. (2006). Oncolytic capacity of attenuated replicative Semliki Forest virus in human melanoma xenografts in severe combined immunodeficient mice. Cancer Res. 66, 7185–7194.

29. Boire, A., Covic, L., Agarwal, A., Jacques, S., Sherif, S., and Kuliopulos, A. (2005). PARI is a matrix metalloproteinase-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. Cell 120, 303–313.

30. Hanahan, D., and Coussens, L.M. (2012). Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 21, 309–322.

31. Sottile, J. (2004). Regulation of angiogenesis by extracellular matrix. Biochim. Biophys. Acta 1654, 13–22.

32. Sakai, E., Astaturov, I., Cukierman, E., DeNardo, D.G., Egelblad, M., Evans, R.M., Fearon, D., Greten, F.R., Ingrionale, S.R., Hunter, T., et al. (2020). A framework for advancing our understanding of cancer-associated fibroblasts. Nat. Rev. Cancer 20, 174–186.

33. Lopez, M.V., Rivera, A.A., Viale, D.L., Benedetti, L., Cuneo, N., Kimball, K.J., Wang, M., Douglas, J.T., Zhu, Z.B., Bravo, A.L., et al. (2012). A tumor-stroma targeted oncolytic adenovirus replicated in human ovary cancer samples and inhibited growth of disseminated solid tumors in mice. Mol. Ther. 20, 2222–2233.

34. Viale, D.L., Cafaratta, E.G., Gould, D., Rotondaro, C., Chernajovsky, Y., Coriel, D.T., Podhajcer, O.L., and Veronica Lopez, M. (2013). Therapeutic improvement of a stroma-targeted CRAd by incorporating motifs responsive to the melanoma microenvironment. J. Invest. Dermatol. 133, 2576–2584.

35. Jing, Y., Chavez, V., Ban, Y., Acquavella, N., El-Asiry, D., Pronin, A., Chen, X., and Merchán, J.R. (2017). Molecular effects of stromal-selective targeting by uPAR-retargeted oncolytic virus in breast cancer. Mol. Cancer Res 15, 1410–1420.
49. Garin-Chesa, P., Old, L.J., and Retting, W.J. (1990). Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. Proc. Natl. Acad. Sci. USA 87, 7233–7238.

50. Wikberg, M.L., Edin, S., Lundberg, I.V., Van Gulpen, B., Dahlin, A.M., Rutegard, J., Stenling, R., Oberg, A., and Palmqvist, R. (2013). High intratumoral expression of fibroblast activation protein (FAP) in colon cancer is associated with poorer patient prognosis. Tumour Biol. 34, 1013–1020.

51. Sandberg, T.P., Stuart, M.P.M.E., Oosting, J., Tollenaar, R.A.E.M., Sier, C.F.M., and Mesker, W.E. (2019). Increased expression of cancer-associated fibroblasts markers at the invasive front and its association with tumor-stroma ratio in colorectal target. BMC Cancer 19, 284.

52. Brennen, W.N., Isaacs, J.T., and Denmeade, S.R. (2012). Rationale behind targeting fibroblast activation protein-expressing carcinoma-associated fibroblasts as a novel chemotherapeutic strategy. Mol. Cancer Ther. 11, 257–266.

53. Holheinz, R.D., al-Batran, S.E., Hartmann, F., Hartung, G., Jäger, D., Renner, C., Tanswell, P., Kunz, U., Amelberg, A., Kuthan, H., and Stehle, G. (2003). Stromal antigen targeting by a humanised monoclonal antibody: An early phase II trial of sibutramin in patients with metastatic colorectal cancer. Onkologie 26, 44–48.

54. Narra, K., Mullins, S.R., Lee, H.O., Strzemkowski-Brun, B., Magalón, K., Christiansen, V.J., McKee, P.A., Egleston, B., Cohen, S.J., Weiner, L.M., et al. (2007). Phase II trial of single agent Val-boroPro (talismabot) inhibiting fibroblast activation protein in patients with metastatic colorectal cancer. Cancer Biol. Ther. 6, 1691–1699.

55. Roberts, E.W., Deonarine, A., Jones, J.O., Denton, A.E., Feig, C., Lyons, S.K., Espeli, M., Kraman, M., McKenna, B., Wells, R.J., et al. (2013). Depletion of stromal cells expressing fibroblast activation protein-z from skeletal muscle and bone marrow results in cachexia and anemia. J. Exp. Med. 210, 1137–1151.

56. Angell, T.E., Lechner, M.G., Jang, J.K., Lo Presti, J.S., and Epstein, A.L. (2014). MHC class I loss is a frequent mechanism of immune escape in papillary thyroid cancer that is reversed by interferon and selumetinib treatment in vitro. Clin. Cancer Res. 20, 6034–6043.

57. Freedman, J.D., Duffy, M.R., Lei-Rossmann, J., Muntzner, A., Scott, E.M., Hagel, J., Campo, L., Bryant, R.J., Verrill, C., Lambert, A., et al. (2018). An oncolytic virus expressing a T-cell engager simultaneously targets cancer and immunosuppressive stromal cells. Cancer Res. 78, 6852–6865.

58. de Sostoa, J., Fajardo, C.A., Moreno, R., Ramos, M.D., Farrera-Sal, M., and Alemany, F., et al. (2016). Anti-tumor effects of DNA vaccine targeting human broblastic activation protein-expressing carcinoma-associated stromal cells explain tumor vessel leakiness. Am. J. Pathol. 189, 219–230.

59. Hou, W., Chen, H., Rojas, J., Sampath, P., and Thorne, S.H. (2014). Oncolytic vaccinia virus demonstrates antiangiogenic effects mediated by targeting of the antitumor activity of antiangiogenic proteins delivered by gene transfer. Proc. Natl. Acad. Sci. USA 106, 12915–12920.

60. Weibel, S., Hofmann, E., Basse-Luesebrink, T.C., Donat, U., Seubert, C., Advani, S.J., Frentzen, A., Chen, N.G., Ehrig, K., et al. (2013). Combination of fractionated irradiation with anti-VEGF expressing vaccinia virus therapy enhances tumor control by simultaneous radiosensitization of tumor associated endothelium. Int. J. Cancer 133, 2989–2999.

61. Kuo, C.J., Farnebo, F., Yu, E.Y., Christofferson, R., Swearingen, R.A., Carter, R., von Recum, H.A., Yuan, J., Kamihara, J., Flynn, E., et al. (2001). Comparative evaluation of the antitumor activity of antiangiogenic proteins delivered by oncolytic vaccinia virus. Mol. Ther. 2, 1769–1776.

62. Buckel, L., Advani, S.J., Frentzen, A., Zhang, Q., Yu, Y.A., Chen, N.G., Ehrig, K., Stritzker, J., Mundt, A.J., and Sclater, A.A. (2013). Attenuation of the hypoxia inducible factor pathway after oncolytic adenovirus infection coincides with decreased vessel perfusion. Cancers (Basel) 2, 851.

63. Xia, Q., Zhang, F.-F., Geng, F., Liu, C.-L., Xu, P., Lu, Z.-Z., Wu, H., et al. (2016). Anti-tumor effects of DNA vaccine targeting human broblast activation protein-expressing carcinoma-associated stromal cells as a novel antibody target in human epithelial cancers. Mol. Ther. Oncolytics 4, 382–4391.
84. Gil, M., Komorowski, M.P., Sehahdi, M., Rokita, H., McGray, A.J.R., Opyrchal, M., Oduslu, K.O., and Kozbor, D. (2014). CXCL12/CXCR4 blockade by oncolytic virotherapy inhibits ovarian cancer growth by decreasing immunosuppression and targeting cancer-initiating cells. J. Immunol 193, 5327–5337.

85. Patil, S.S., Gentschew, I., Adelfinger, M., Donat, U., Hess, M., Weibel, S., Nolte, I., Frentzen, A., and Szalay, A.A. (2012). Virotherapy of canine tumors with oncolytic vaccinia virus GLV-1h109 expressing an anti-VEGFR single-chain antibody. PLoS ONE 7, e47472.

86. Gholemí, S., Marano, A., Chen, N.G., Aguilar, R.J., Frentzen, A., Chen, C.H., Lou, E., Fujisawa, S., Eveno, C., Belin, L., et al. (2014). A novel vaccinia virus with dual oncolytic and anti-angiogenic therapeutic effects against triple-negative breast cancer. Breast Cancer Res. Treat. 148, 489–499.

87. Adelfinger, M., Bessler, S., Frentzen, A., Cecil, A., Langbein-Lauquitz, J., Gentschew, I., and Szalay, A.A. (2015). Preclinical testing oncolytic vaccinia virus strain GLV-5b51 expressing an anti-VEGFR single-chain antibody for canine cancer therapy. Viruses 7, 4075–4092.

88. Adelfinger, M., Gentschew, I., Grimm de Guibert, J., Weibel, S., Langbein-Lauquitz, J., Harit, B., Murua Escobar, H., Nolte, I., Chen, N.G., Aguilar, R.J., et al. (2014). Evaluation of a new recombinant oncolytic vaccinia virus strain GLV-5b451 for feline mammary carcinoma therapy. PLoS ONE 9, e104337.

89. Xie, Y., Hicks, M.J., Kaminsky, S.M., Moore, M.A., Crystal, R.G., and Rafii, S. (2014). AAV-mediated persistent bevacizumab therapy suppresses tumor growth of ovarian cancer. Gynecol. Oncol. 135, 325–332.

90. Guse, K., Diaconu, I., Rajeczi, M., Slonecka, M., Hakkarainen, T., Ristimäki, A., Kanerva, A., Pesonen, S., and Hemminki, A. (2009). Ad5/3-9HIF-Delta24-VEGFR-1-Ig, an infectivity enhanced, dual-targeted and antiangiogenic oncolytic adenovirus for kidney cancer treatment. Gene Ther. 16, 1009–1020.

91. Niethammer, A.G., Xiang, R., Becker, J.C., Wodrich, H., Perul, I., Karsten, G., Elicitr, B.F., and Resfeld, R.A. (2002). A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. Nat. Med. 8, 1369–1375.

92. Moreira, Y., Bequet-Romero, M., Ayala, M., Velazco, J.C., Pérez, P.P., Alba, J.S., Ancizar, J., Rodríguez, M., Cosme, K., and Gavilondo, J.V. (2010). Immunogenicity and some safety features of a VEGF-based cancer therapeutic vaccine in rats, rabbits and non-human primates. Vaccine 28, 3453–3461.

93. Jarosz, M., Jazwiencka-Rakus, J., Cichosi, T., Głowala-Kosińska, M., Smolarczyk, R., Smagur, A., Malina, S., Sochanik, A., and Szala, S. (2013). Therapeutic antitumor potential of endoglin-based DNA vaccine combined with immunomodulatory agents. Gene Ther. 20, 262–273.

94. Haller, B.K., Bräve, A., Wallgard, E., Roswall, P., Sunkari, V.G., Mattson, U., Hellström, M., and Pietras, K. (2010). Therapeutic efficacy of the recombinant disintegrin ADAMTS-9 in murine mammary adenocarcinoma. OncoImmunology 9, 4276–4286.

95. Wagner, S.C., Riordan, N.H., Ichim, T.E., Szymanski, J., Ma, H., Perez, J.A., Lopez, J., Plata-Munoz, J.J., Silva, F., Patel, A.N., and Kesari, S. (2016). Safety of targeting tumor endothelial cell antigens. J. Transl. Med. 14, 90.

96. Ichim, T.E., Li, S., Ma, H., Yurova, Y.V., Szymanski, J.S., Patel, A.N., Kesari, S., Min, W.P., and Wagner, S.C. (2015). Induction of tumor inhibitory anti-angiogenic response through immunization with interferon Gamma primed placental endothelial cells: ValloVax®. J. Transl. Med. 13, 90.

97. Kuo, C.-H., Chang, B.-I., Lee, F.-T., Chen, P.-K., Lee, J.-S., Shi, G.-Y., and Wu, H.L. (2015). Development of recombinant adenovirus-associated virus serotype 2/8 carrying kringle domains of human plasminogen for sustained expression and cancer therapy. Hum. Gene Ther. 26, 603–613.

98. Chu, Y., Liu, H., Lou, G., Zhang, Q., and Wu, C. (2014). Human placenta mesenchymal stem cells expressing exogenous kringle-1 protein by fiber-modified adenovirus suppress angiogenesis. Cancer Gene Ther. 21, 200–208.

99. Cao, R., Wu, H.-L., Veitomäki, N., Linden, P., Farnebo, J., Shi, G.-Y., and Cao, Y. (1999). Suppression of angiogenesis and tumor growth by the inhibitor Ki-5 generated by plasmid-mediated proteolysis. Proc. Natl. Acad. Sci. USA 96, 5728–5733.

100. Zhang, X., Xu, W., Qian, H., Zhu, W., and Zhang, R. (2011). Mesenchymal stem cells modified to express lentivirus TNF-α Tumstatin45,132 inhibit the growth of prostate cancer. J. Cell. Mol. Med. 15, 433–444.

101. Daugimont, L., Vandermeulen, G., Defresne, F., Bouzin, C., Mir, L.M., Bouquet, C., Feron, O., and Préat, V. (2011). Antitumoral and antiangiogenic effect of antiangiogenic plasmids in B16 melanoma: Higher efficiency of the recombinant disintegrin domain of ADAM 15. Eur. J. Pharm. Biopharm. 78, 314–319.

102. Matsumoto, K., and Nakamura, T. (2005). Mechanisms and significance of bifunctional NK4 in cancer treatment. Biochem. Biophys. Res. Commun. 333, 316–327.

103. Gholami, S., Marano, A., Chen, N.G., Aguilar, R.J., Frentzen, A., Chen, C.H., Lou, E., Fujisawa, S., Eveno, C., Belin, L., et al. (2014). A novel vaccinia virus with dual oncolytic and anti-angiogenic therapeutic effects against triple-negative breast cancer. Breast Cancer Res. Treat. 148, 489–499.

104. Kishi, Y., Kuba, K., Nakamura, T., Watanabe, K., Suzuki, Y., Mizuno, S., Nukiwa, T., Matsumoto, K., and Nakamura, T. (2009). Systemic NK4 gene therapy inhibits tumor growth and metastasis of melanoma and lung carcinoma in syngeneic mouse tumor models. Cancer Sci. 100, 1351–1358.

105. Tomioka, D., Maehara, N., Kuba, K., Mizumoto, K., Tanaka, M., Matsumoto, K., and Nakamura, T. (2001). Inhibition of growth, invasion, and metastasis of human pancreatic cancer cells by NK4 in an orthotopic mouse model. Cancer Res. 61, 7518–7524.

106. Tomioka, D., Maehara, N., Kuba, K., Mizumoto, K., Tanaka, M., Matsumoto, K., and Nakamura, T. (2001). Inhibition of growth, invasion, and metastasis of human pancreatic cancer cells by NK4 in an orthotopic mouse model. Cancer Res. 61, 7518–7524.

107. Zhu, Y., Cheng, M., Yang, Z., Zeng, C.Y., Chen, J., Xia, Y., Luo, S.W., Zhang, K.H., Zhou, S.F., and Lu, N.H. (2014). Mesenchymal stem cell-based NK4 gene therapy in nude mice bearing gastric cancer xenografts. Drug Des. Devel. Ther. 8, 2449–2462.

108. Gholami, S., Marano, A., Chen, N.G., Aguilar, R.J., Frentzen, A., Chen, C.H., Lou, E., Fujisawa, S., Eveno, C., Belin, L., et al. (2014). A novel vaccinia virus with dual oncolytic and anti-angiogenic therapeutic effects against triple-negative breast cancer. Breast Cancer Res. Treat. 148, 489–499.
HSV-1 promotes antitumor immunity and synergizes with TIGIT blockade. Cancer Immunol. Res. 8, 632–647.

119. Du, T., Shi, G., Li, Y.M., Zhang, J.F., Tian, H.W., Wei, Y.Q., Deng, H., and Yu, D.C. (2014). Tumor-specific oncolytic adenoviruses expressing granulocyte macrophage colony-stimulating factor or anti-CTLA4 antibody for the treatment of cancers. Cancer Gene Ther. 21, 340–348.

120. Diao, J.D., Hemminki, O., Diaconu, I., Hirвинen, M., Bonetti, A., Guse, K., Escutenaire, S., Kanerva, A., Posenen, S., Löskog, A., et al. (2012). Targeted cancer immunotherapy with oncolytic adenovirus coding for a fully human monoclonal antibody specific for CTLA-4. Gene Ther. 19, 988–998.

121. Hamilton, J.R., Vijayakumar, G., and Palese, P. (2018). A recombinant antibody-expressing influenza virus delays tumor growth in a mouse model. Cell Rep. 22, 1–7.

122. Thomas, S., Kunchoria, L., Rould, V., Kysyla, J.N., Mansfield, D., Borroweddy, P.K., Smith, H., Kaufman, H.L., Harrington, K.I., and Coffin, R.S. (2019). Development of a new fusion-enhanced oncolytic immunotherapy platform based on herpes simplex virus type 1. J. Immunother. Cancer. 7, 214.

123. Vijayakumar, G., Palese, P., and Goff, P.H. (2019). Oncolytic Newcastle disease virus expressing a checkpoint inhibitor as a radioenhancing agent for murine melanoma. EBioMedicine 49, 96–105.

124. Yang, Y., Xu, W., Peng, D., Wang, H., Zhang, X., Wang, H., Xiao, F., Zhu, Y., Ji, Y., Gulskota, K., et al. (2019). An oncolytic adenovirus targeting transforming growth factor β inhibits protumorigenic signals and produces immune activation: A novel approach to enhance anti-PD-1 and anti-CTLA4 therapy. Hum. Gene Ther. 30, 1117–1132.

125. Xu, W., Zhang, Z., Yang, Y., Hu, Z., Wang, C.H., Morgan, M., Wu, Y., Hutton, R., Xiao, S., Stock, S., et al. (2014). AdS5/84 hexon oncolytic virus expressing sTGFβRIIFc produces reduced hepatic and systemic toxicities and inhibits prostate cancer bone metastasis. Mol. Ther. 22, 1504–1517.

126. Hu, Z., Gupta, I., Zhang, Z., Gerseny, H., Berg, A., Chen, Y.J., Zhang, Z., Du, H., Brendler, C.B., Xiao, X., et al. (2012). Systemic delivery of oncolytic adenoviruses targeting transforming growth factor-β inhibits established bone metastasis in a prostate cancer mouse model. Hum. Gene Ther. 23, 871–882.

127. Hu, Z., Gerseny, H., Zhang, Z., Chen, Y.J., Berg, A., Zhang, Z., Stock, S., and Seth, P. (2011). Oncolytic adenovirus expressing soluble TGFβRIIIFc inhibits established bone metastases: A safe and effective systemic therapeutic approach for breast cancer. Mol. Ther. 19, 1609–1618.

128. Gujar, S.A., and Lee, P.W.K. (2014). Oncolytic virus-mediated reversal of impaired tumor antigen presentation. Front. Oncol. 4, 77.

129. Hervas-Stubbs, S., Perez-Gracia, J.L., Rouzaut, A., Sanmamed, M.F., Le Bon, A., and Melero, I. (2011). Direct effects of type I interferons on cells of the immune system. Immunol. Rev. 241, 69–93.

130. Kawai, T., and Akira, S. (2006). Innate immune recognition of viral infection. Nat. Rev. Immunol. 6, 441–453.

131. Nesslinger, N.J., Ng, A., Tsang, K.-Y., Ferrara, T., Schlom, J., Gulley, J.L., and Nelson, B.H. (2010). A viral vaccine encoding prostate-specific antigen enhances antitumor immunity. Mol. Ther. 18, 1646–1656.

132. Brossart, P. (2020). The role of antigen spreading in the effectiveness of oncolytic virotherapy. Mol. Cancer Ther. 19, 335–344.

133. Sobol, P.T., Boudreau, J.E., Stephenson, K., Wan, Y., Lichty, B.D., and Mosman, K.L. (2011). Adaptive antiviral immunity is a determinant of the therapeutic success of oncolytic vireothery. Mol. Ther. 19, 335–344.

134. Gujar, S.A., Dielschneider, R., Clements, D., Helson, E., Shmulevitz, M., Marcato, P., P.K., Smith, H., Kaufman, H.L., Harrington, K.I., and Coffin, R.S. (2019). Development of a new fusion-enhanced oncolytic immunotherapy platform based on herpes simplex virus type 1. J. Immunother. Cancer. 7, 214.

135. Gujar, S.A., Marcato, P., Pan, D., and Lee, P.W.K. (2010). Reovirus virotherapy overrides tumor antigen presentation evasion and promotes protective antitumor immunity. Mol. Cancer Ther 9, 2924–2933.

136. Curtisinger, J.M., and Mescher, M.F. (2010). Inflammatory cytokines as a third signal for T cell activation. Curr. Opin. Immunol. 22, 333–340.

137. Hervas-Stubbs, S., Riezu-Boj, J.L., Gonzalez, I., Mancheño, U., Dubrot, J., Arpílcuceta, A., Gabari, I., Palazon, A., Aranguren, A., Ruiz, J., et al. (2010). Effects of IFN-γ as a signal-3 cytokine on human naive and antigen-experienced CD8(+) T cells. Eur. J. Immunol. 40, 3389–3402.

138. Lapteva, N., Aldrich, M., Rollins, L., Ren, W., Goltsova, T., Chen, S.-Y., and Huang, X.F. (2009). Attraction and activation of dendritic cells at the site of tumor elicits potent antitumor immunity. Mol. Ther 17, 1626–1636.

139. Du, T., Shi, G., Li, Y.M., Zhang, J.F., Tian, H.W., Wei, Y.Q., Deng, H., and Yu, D.C. (2014). Tumor-specific oncolytic adenoviruses expressing granulocyte macrophage colony-stimulating factor or anti-CTLA4 antibody for the treatment of cancers. Cancer Gene Ther. 21, 340–348.