Strategies of Vaccinia Virus in Tumor Virotherapy

Contributor: Xinjun Liu, Jian Zhao, Xiaopeng Li, Fengxue Lao, Min Fang

Oncolytic virotherapy has emerged as a novel form of cancer immunotherapy. Oncolytic viruses (OVs) can directly infect and lyse the tumor cells, and modulate the beneficial immune microenvironment. Vaccinia virus (VACV) is a promising oncolytic vector because of its high safety, easy gene editing, and tumor intrinsic selectivity. To further improve the safety, tumor-targeting ability, and OV-induced cancer-specific immune activation, various approaches have been used to modify OVs. The recombinant oncolytic VACVs with deleting viral virulence factors and/or arming various therapeutic genes have displayed better therapeutic effects in multiple tumor models. Moreover, the combination of OVs with other cancer immunotherapeutic approaches, such as immune checkpoint inhibitors and CAR-T cells, has the potential to improve the outcome in cancer patients. This will open up new possibilities for the application of OVs in cancer treatment, especially for personalized cancer therapies.

1. Vaccinia Virus Virulence Genes

In normal cells, a range of genetic products produced by wild type VACV can render cells to adapt to viral reproductions, including thymidine kinase (TK) and viral growth factor (VGF) [1][2]. TK is one of the key enzymes in DNA metabolism [3]. In the replication process of VACV, a high concentration of nucleic acid pool generated with TK is needed to complete the replications of virus progeny [4]. When the TK gene is deleted, the replication of VACV will be restricted and display obvious tumor selectivity since there is usually a high activity of TK in tumor cells [5][6]. Therefore, TK gene deletion becomes an effective method to improve tumor selectivity in oncolytic VACV therapy. Comparing the replication ability between wild type and TK gene knocking-down Wyeth strains VACV, both viruses replicated effectively in human PANC-1 pancreatic cancer cells and human MCF-7 breast cancer cells, and viral titers were almost the same. However, in non-proliferating or non-transformed cells, the replication capacity of TK knocking-down particles was significantly reduced about 10 times [6], indicating the preference to tumor cells of VACV after TK gene deletion. JX-594, a modified Wyeth strain VACV through insertion of GM-CSF into the TK gene for TK deletion, also displayed an obvious selectivity in tumor cells [7], further demonstrating an advantage of TK gene deletion in tumor selectivity.

VGF is a homologous gene to epidermal growth factor (EGF). It has an EGF-like activity in virus-infected cultures, which can compete with EGF for binding to EGF membrane receptors (EGFR) to mediate localized cellular proliferation [8]. VACV often promotes the proliferation of host and peripheral cells through VGF to achieve its own replication and proliferation [1], while the absence of that factor significantly reduces the viral virulence and damage to normal cells [9]. Thus, VGF-deleted VACV has also been broadly applied in tumor therapy. In proliferating Swiss 3T3 cells, the VGF gene is not necessary for VACV replication. However, in stationary and contact-inhibited Swiss 3T3 cells, the replication efficiency and lethality of VGF-deficient VACV are reduced compared with wide type VACV [9], indicating the enhanced tumor selectivity after VGF deletion. To further achieve tumor selectivity, TK and VGF genes were both deleted to form a double-deleted VACV (vvDD). vvDD displayed comparable replication ability in dividing cells as TK or VGF single knockout virus. However, in resting cells, the replication ability of vvDD was significantly reduced than TK or VGF single knockout VACV [2]. Thus, vvDD can be used as a good oncolytic vector for further enhancement of tumor selectivity. JX-963, formed by vvDD with the insertion of GM-CSF, shows a greater efficacy against the primary and metastasizing lung tumors either compared with vvDD or JX-594 (Wyeth single TK-deleted VACV expressing GM-CSF) both in vitro and in vivo [10].

In addition, A48R and B18R are also deleted for tumor selectivity [11]. A48R codes for thymidylate kinase which participates in nucleotide metabolism and the deletion of A48R attenuates the WR virus. The B18R gene codes for a soluble type I interferon (IFN) receptor protein, which interferes with anti-viral responses to protect from the antiviral effects of type I IFN [12]. Unlike normal cells, tumor cells usually show IFN deficiency [13]. Thus, VACV lacking the B18R gene also reduces virulence and tends to replicate in tumor cells [11]. C7L and K1L are two important genes in regulating

the virus-host range, including the infection capability of VACV in human cells. Deletions of C7L and K1L lead to deficient replication capability in mammalian cells and decreased virulence in mice. The deletions of C7L and K1L are also used for VACV attenuation to create a more safe and more effective HIV vaccine vector [14].

2. Cytokines and Chemokines

2.1. Cytokines

Cytokines are a class of proteins secreted by immune cells and tissue cells, which can regulate the immune response by binding to corresponding receptors. Cytokine therapy for tumor patients has been an important treatment modality. However, cytokine in monotherapy does not fully achieve the efficacy seen in preclinical trials [15], as it is often associated with severe dose-limiting toxicities because of its short half-life [15][16]. Therefore, new strategies to avoid its disadvantages for tumor treatment are needed. Reports showed that utilizing VACV to deliver cytokines not only makes cytokines persist but also enhances the antitumor immunity induced by VACV [10][17][18].

GM-CSF is associated with a variety of immune cell activation and differentiation. GM-CSF can increase the expression of class II major histocompatibility complex (MHC) molecules on the surface of macrophages [19][20], and recruit and activate dendritic cells (DC). When mice bearing established Lewis lung carcinoma were pretreated with docetaxel followed by GM-CSF-producing tumor vaccine, significant tumor regression, and prolonged survival were observed compared with chemotherapy alone, further studies revealed that augmentation of vaccine-induced antitumor immunity in docetaxel-treated mice primarily due to the enhanced survival of antigen-experienced T cells [21]. OVs generated with GM-CSF expression include adenovirus [22][23], herpes viruses [24], and VACV, all display positive tumor treatment outcomes. Recombinant VACV expressing GM-CSF, such as JX-594 and JX-963 [6][10][25][26], display potential therapeutic effects with safety and enhanced antitumor efficacy, including the destruction of tumor blood vessels [25][27]. JX-594 has shown exciting results in phase I clinical trials targeting a variety of tumors as local or systemic therapy, and a phase III clinical trial has also recently been completed targeting hepatocellular carcinoma.

Other cytokines, such as interferons (IFN) and interleukins (IL), have also displayed promising results in oncolytic viral therapy. IFNs have multiple antitumor mechanisms, including direct inhibition of tumor cell proliferation, anti-angiogenesis, and induction of tumor-specific cytotoxic T-cells [28]. IFN-β expressing oncolytic VACV ($TK^-/B18R^-/IFN\text{-}\beta^+$, JX-795) therapy significantly increased the survival of breast cancer-bearing mouse models compared to the control group [17][29]. IL-12 plays a key role in augmenting the cytotoxic activity of T cells and natural killer cells. A recombinant VACV vKT0334 containing the genes encoding murine IL-12 efficiently infected a variety of tumor cell lines and produced high amounts of biologically active mIL-12, and the antitumor activity of vKT0334 was also significantly enhanced compared with the backbone virus (vKT033, VACV do not express IL-12) in sarcoma tumor mice model [18]. Other interleukins, such as IL-15, also display beneficial antitumor effects after cooperating with VACV vector. A recombinant VACV expressing a superagonist IL-15 (a fusion protein of IL-15 and IL-15Rα) led to significant tumor regression and extended survival of the mice bearing colon or ovarian cancer [30].

Although cytokine expression obtains good results, it is at the cost of reduced oncolytic activity due to the cytokine-associated immunity resulting in less viral replication and earlier clearance [31]. Toxicity effects have also been reported with systemic cytokine release caused by OVs-driven cytokine expression [16][31][32]. Cytokines are potent and complex immune mediators, designing cytokine expression recombinant oncolytic virotherapy requires a profound knowledge of cytokine biology and contemporary biotechnology to exploit their anti-tumor activity while minimizing toxicity.

2.2. Chemokines

Chemokines are secreted chemotactic cytokines that mediate the migration and positioning of immune cells within various tissues. Chemokines influence the host immune response to tumors by directing leukocyte trafficking into tumor lesions to mediate both antitumor and protumor immune effects [18]. Among the chemokines, the type-1 chemokines CXCL9, CXCL10, and CXCL11 contribute to the inhibition of angiogenesis and tumor progression [33][34]. They recruit effector CD8$^+$ T cells and Th1 cells expressing CXC-chemokine receptor 3 (CXCR3), the common receptor of CXCL9, CXCL10, and CXCL11, to migrate into tumors for mediating antitumor immunity. Tumors with higher-level expressions of CXCL9, CXCL10, and CXCL11 displayed increased infiltration of antitumor T lymphocytes [17]. CXCL11-producing tumors were completely rejected in a T-cell lymphoma mice model [35]. VACV expressing CXCL11 increased CXCL11 expression levels in virus-infected cells and stimulated T cells trafficking to malignant tissues, resulting in prolonged survival of mice with TC1 murine lung tumors, and the proliferation ability of the recombinant virus was not affected by CXCL11 expression, demonstrating the good antitumor potential of oncolytic CXCL11 expressing VACV [17][36]. In another study, the expression of CXCL11 on VACV also showed an enhanced therapeutic efficacy without affecting virus infection and replication,

displaying increased local numbers of CD8^{+} CTLs as well as reduced expression of several suppressive molecules such as TGF-β, COX2, and CCL22 in the tumor microenvironment (TME), thus prolonged the survival of mice with a murine AB12 mesothelioma model [37].

CCL5 is also a potent chemoattractant that can attract NK cells, T cells, and DC expressing its receptor CCR5 [38]. CCL5 plays a crucial role in maintaining several functions of T cells such as survival, migration, and differentiation [39]. Reports showed that CCL5-armed VACV (vvDD-CCL5) enhanced immune cell infiltration into colorectal tumors in mice. Moreover, CCL5 expression also resulted in prolonged persistence of the virus exclusively within the tumor. Further enhancement of antitumor effectiveness was achieved when vvCCL5 was used in conjunction with DC1 vaccination, correlating with increased immune cell infiltration into the tumor and an apparent Th1 skewing of the infiltrating T-cells [40].

Hot tumors are typically characterized by the accumulation of pro-inflammatory cytokines, CD8^{+} T cells, and NK cells; these malignancies normally have a better response rate to immunotherapy treatment. Conversely, cold malignancies present immunosuppressive immune cells in the tumor, including Tregs, and tend to have a poor prognosis and response to immune checkpoint inhibitors [41]. Utilizing VACV vectors to deliver chemokines or VACV combined with chemokine therapy may alter the TME, recruit CD8^{+} T cell and NK cells into the tumor, and turn the cold tumor into a hot tumor to achieve better therapeutic antitumor efficacy.

3. Costimulators

T cells play a key role in controlling tumor growth. There is growing evidence that effective T cell activation can control tumor growth and prolong the survival of tumor patients [42][43][44][45]. For T cell activation, the second or costimulatory signal between T cell and costimulating molecules on antigen-presenting cells (APC) is needed and necessary under natural physiological conditions [46][47]. B7-1 (CD80) and B7-2 (CD86) are two important costimulatory molecules presenting on the surface of specialized APC and interact with CD28 or cytotoxic T-lymphocyte antigen (CTLA-4) expressed on T cells [48]. CD28 delivers a positive stimulatory signal to T cells [49], while CTLA-4 delivers a negatively regulating signal to T cells [50]. The affinity of CTLA-4 to B7 is much higher than CD28, thus the expression of CTLA-4 dampens CD28 co-stimulating signal of T cells by competing with CD28 to bind to B7, which leads to B7 consumption and T cells suppression [51]. Some studies found that B7-1 is also expressed in some tumors, the expression of B7-1 in metastatic lesions is much lower than in normal tissues, implying the correlations of B7-1 downregulation with tumor progression [52][53]. Moreover, most mouse and human tumors do not express B7-1 or B7-2. Thus, even though a potent antigen is expressed on the tumor cells, it is unlikely to activate antitumor T-cell responses due to the absence of costimulators [46]. Therefore, increasing the expression of B7 molecules on tumors can provide co-stimulation to elicit effective antitumor T cell immunity.

VACV vector is a good tool to deliver B7 molecules, which can turn tumor cells themselves into APC to provide a costimulatory signal for T cells after infection, thus triggering potent tumor-specific T cell responses [54]. Mouse melanoma cells infected with a set of recombinant VACV encoding the murine B7-1 or/and B7-2 provided effective costimulation, resulting in the proliferation of resting CD4^{+} T cells. Moreover, when B7-1 and B7-2 were delivered together on the same cell, the proliferative response of CD4^{+} T cells was further increased [55]. In another study, inoculation of rV-B7-1- or rV-B7-2 (recombinant vaccinia viruses containing the murine B7-1 or B7-2 genes)-infected tumor cells into immunocompetent animals resulted in no tumor growth [46]. In a melanoma study, VACV vectors expressing B7-1 effectively overcame the immunosuppressive TME [50][56] and enhanced tumor-specific T cell responses. Therefore, the utility of recombinant vaccinia viruses to deliver B7 molecules to tumor cells is a potential strategy to enhance antitumor T cell responses.

4. BiTE

Bispecific antibodies are engineered proteins that can simultaneously engage with two different antigens [57]. Bispecific T cell engager (BiTE) is generated by single-chain variable fragments (scFv) of two different monoclonal antibodies, one of which is usually a CD3 agonist to engage with CD3 on T cells, and the other can recognize a surface antigen on target such as tumor cells. They are connected through a short, flexible peptide chain [58]. Through co-engaging with CD3 and targeted cell antigens, BiTE-mediated T cell activation can occur without co-stimulating signals or in vitro prestimulation, which is also independent of the presentation of antigen peptides on surface MHC molecules [59][60]. Therefore, tumor cell killing mediated by BiTE is no longer limited to the expression of MHC molecules on targeted cells, even if MHC expression has been lost by tumor cells, which is usually an immune evasion strategy of tumors [61].

Though the advantages of BiTE bring promising in solid tumor patients treatment, challenges are still faced in the application of BiTE against common cancers, such as variable mutational burden in tumors, poor BiTE drug delivery into

tumors, an overwhelmingly immunosuppressive TME which may make BiTE hard to work, off-target toxicities against adjacent normal cells, dose-dependent severe systemic side effects, and so on [62].

Fibroblast activation protein (FAP) expression is closely related to the proliferation and apoptosis of tumor-related fibroblasts. Reports showed that the generated BiTE-VACV with an anti-mouse CD3 and an anti-FAP, mFAP-TEA-VV, mediated effective T cell killing of FAP expressing cells, which was accompanied by increased secretion of IFN-γ and IL-2. In vivo, mFAP-TEA-VV enhanced viral titer within the tumor and had potent antitumor activity in comparison to control VVs in an immunocompetent B16 melanoma mouse model [63]. EphA2-TEA-VV is a recombinant VV encoding a secretory bispecific T-cell engager consisting of two single-chain variable fragments specific for CD3 and the tumor cell surface antigen EphA2. Compared with the unmodified virus, the replication and oncolysis abilities of EphA2-TEA-VV were not changed. EphA2-TEA-VV not only killed infected tumor cells but also induced bystander killing of noninfected tumor cells. Furthermore, EphA2-TEA-VV had potent antitumor activity in comparison with control VV in a lung cancer xenograft model [42].

5. Tumor Antigens

Tumor antigens (TAs) can be generally categorized as tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) [64]. Though many studies have employed TAA in virotherapy, including oncolytic VACV [65][66][67][68][69], since TAAs express not only in tumor cells but also in healthy tissues [64][70], they may also lead to off-target effects. Therefore, targeting TSAs is a good way to treat tumors. TSAs are either oncogenic viral antigens or tumor-specific neoantigens [71]. Oncogenic viral antigens are identified in virus-induced tumors. Clinical antitumor efficacy of VACV vectors armed with oncogenic viral antigens is confirmed in oncogenic virus clearance and tumors regression, such as oncolytic VACV containing E6 and E7 antigens from human papillomavirus 16 (HPV16) in HPV eradication and against cervical cancer [72] [73]. HER2 is overexpressed and/or amplified in several types of tumors, such as breast cancer and gastric cancer [74]. Previous studies demonstrated enhanced efficacy of oncolytic VACV recombinants encoding combined HER2 and GM-CSF in modulating the microenvironment of myeloid-derived suppressor cells (MDSCs)-rich tumors [75].

Tumor-specific neoantigens are generated by tumors through multiple mutation events [76][77]. High mutation burden neoantigens (high authentic neoantigens) might induce successful antitumor immunity as potent immunogens [78][79][80]. Many tumor vaccines are generated with neoantigens [81]. However, when the tumors are with intermediate to low mutation burdens, the application of neoantigen-targeted vaccines is highly limited since the detection of neoantigens is hard due to the technical limitations of detection [82]. Excitingly, with the development of deep-sequencing technologies, it becomes possible to identify the mutations and predict potential neoantigens [83]. Bioinformatic analysis of The Cancer Genome Atlas (TCGA) data revealed that increased point mutations and neoantigen burdens of tumors are correlated with increased cytotoxic T cells infiltration [82], implying that combining neoantigen with oncolytic VACV might increase the expression of low burden neoantigens in tumors, thus eliciting anti-tumor neoantigen-specific T cells, and expanding the antitumor efficacy. In addition, the anti-tumor immunity can be further amplified by the newly released neoantigens taken up by APC after tumor cell lysis, thus producing a more direct and effective antitumor immunity. Since the majority of neoantigens are exclusive to tumors [76][77] and mutated unique to individual patients [84], the therapy of oncolytic VACV armed with neoantigens also means personalized therapy [85]. Accordingly, oncolytic VACV armed with neoantigens is a personalized oncolytic agent, which can be tailor-made for tumor improving and curing.

6. Combinations

6.1. Combination with Pharmaceutical Drugs

Oncolytic viral therapy faces the limitation of viral clearance due to the generation of neutralizing antibodies, the presence of Abs specific to VACV can prohibit the booster effect of recombinant VACV vectors [86]. Therefore, combination strategies are elicited to circumvent these problems, thus achieving a successful vaccination and even boosting the viral oncolytic effects.

Cyclooxygenase-2 (COX-2) is a key enzyme in fatty acid metabolism, which is upregulated during cancer. Enhanced COX-2 activity promotes angiogenesis, inhibits apoptosis, and increases the metastatic potential of carcinogenesis [87]. The COX-2 inhibitor is a class of nonsteroidal anti-inflammatory drugs. It inhibits the activity of the COX-2 enzyme and reduced antibody production specific to VACV by inhibiting antibody induction [88]. One study found that COX-2 inhibitors prevented the generation of VACV-specific neutralizing antibodies, thus circumventing the limitation of viral clearance without losing infectivity in mice vaccinated with VACV previously [89], indicating the assistant of COX-2 inhibitors in protecting VACV clearance. Further, the treatment of VACV in combination with COX-2 inhibitors regressed ovarian

tumors and enhanced the survival of tumor-bearing mice, the efficacy was more effective than either administration of VACV or COX-2 inhibitors alone [89]. The product of COX-2 activity, prostaglandin E2 (PGE-2), is a key mediator of resistance to immunotherapies [90]. The elevation expression of PGE-2 couples with an increase in the suppressive chemokine profile and a high level of granulocytic myeloid-derived suppressor cells (MDSC), which can result in a loss of immunotherapeutic potential [91]. Therefore, COX-2 inhibitors can block PGE-2 activity and, thus, reverse the PGE-2-created tumor immunosuppressive microenvironment, synergizing with oncolytic VACV for TME remolding [92], further confirming the benefit of COX-2 inhibitors and oncolytic VACV in combinations against tumors. Indeed, studies showed that vaccinia vectors engineered to target PGE-2 overcame localized immunosuppression and sensitized established tumors resistant to immunotherapy [91].

Sunitinib is a receptor tyrosine kinase inhibitor. It has also been used to combine with VACV to assist the antitumor activity as sunitinib can decrease Treg and MDSC in some tumors [93][94][95]. In a carcinoembryonic antigen transgenic (CEA-Tg) mice model, the combined treatment of sunitinib and modified VACV reduced tumor volumes and increased survival of CEA-Tg mice, concomitantly with increased intratumoral antigen-specific T lymphocytes infiltration and decreased immunosuppressive immune cells [96]. In a renal tumor mice model, oncolytic VACV in combination with sunitinib also significantly increased the overall survival benefit of tumor mice more than VACV or sunitinib treatment alone [97]. In addition, other pharmaceutical drugs, such as histone deacetylase inhibitor trichostatin A [98], chemokine modulating drug cocktail [99], and deoxyadenosine analog cytarabine [100] are also employed to combine with oncolytic VACV to explore better tumor treatment.

6.2. Combination with Anti-Angiogenesis Strategies

Angiogenesis is an important part of tumor growth and development [101]. In the period of malignancy, tumor cells start to secrete angiogenic growth factors which bind to receptors on endothelial cells of nearby blood vessels, initiating new vessel formation to support further tumor growth [101]. Factors, such as vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR), play crucial roles in tumor angiogenesis to support tumor initiation and development [102], and the expression levels of VEGF and EGFR are highly correlated with the incidence of tumor growth and poor prognosis [103][104][105][106][107][108]. Thus, VEGF and EGFR have been proposed as key targets to combine with oncolytic viral therapy for exploring a better regimen against tumors.

Oncolytic VACV has been shown to induce a profound, rapid, and tumor-specific vascular collapse in both preclinical models and clinical studies. It was observed that revascularization after viral therapy was dramatically delayed and did not occur until after viral clearance, and VEGF levels in the tumor were suppressed throughout the period of active viral infection [27][97][109]. In other studies, VACV vectors armed with single-chain antibodies against VEGF also presented a significant decrease in neo-angiogenesis compared with VACV vectors treated alone, followed by reduced tumor volumes, decreased tumor neo-angiogenesis, and increased survival of mice bearing the tumors [110][111][112]. Moreover, when VACV was simultaneously armed with the two anti-angiogenesis factors namely anti-VEGF and anti-EGFR (GLV-1h444), it was found that only minimal tumors were grown after GLV-1h444 injection in mice carrying A549 tumor xenografts, which displayed a better therapeutic efficacy than either of the two single antibodies alone expressing VACV injected ones [113]. Thus, the combination of anti-angiogenesis therapy and oncolytic VACV might be an ideal path to enhance the anti-angiogenesis effects for preventing tumor development.

6.3. Combination with Immune Checkpoint Blockade or CAR-T Cell Therapy

Immune checkpoint blockade (ICB) of PD-1/PD-L1 has led to significant tumor regressions and improved survival in a subset of patients with many reported tumor entities [114][115][116][117][118]. Reports showed checkpoint blockage produced rapid and durable immune responses, especially in PD-1-expressing tumor patients [115][119][120]. As a negative signal regulatory protein, PD-1 is expressed on activated T cells to inhibit T cell activation. The ligand PD-L1 is expressed on tumor cells, which binds to T cells expressing PD-1, blocking T cell functions as a tumor evading mechanism, resulting in decreased productions of effector cytokines and lower cytolytic activity [120][121][122]. Therefore, the blockade of the PD-1/PD-L1 pathway can promote the activation of tumor-specific T cells, which leads to enhanced anti-tumor T cell responses [123]. However, the majority of tumor patients are still refractory to immune checkpoint inhibition, which might be because of the lack of pre-existing immune responses [118][123][124], an important condition for successful ICB therapy [125].

OVs infection of tumors might overcome resistance to ICB by improving the magnitude and quality of anti-tumor T cell responses [118][126]. Reports showed dramatic tumor regression and metastatic inhibition after combined usage of anti-PD-1 antibody and oncolytic VACV in a previously treatment-refractory carcinoma model [127], with an increased CD8^{+} T cells infiltration and DC activation [30]. The monotherapy of metastatic sarcoma with ICB showed little activity [127], demonstrating the excellent benefits to enhance T cells activity after oncolytic VACV in combination with ICB. In a

pancreatic ductal adenocarcinoma treatment study, oncolytic VACV and anti-PD-L1 combination also displayed good tumor attacking ability [128], and the complementary benefit of oncolytic VACV in ICB treatment was further confirmed. A clinical phase II trial in patients with advanced NSCLC is underway to evaluate the anti-tumor efficacy of the combination therapy with modified VACV and nivolumab, an agent of anti-PD-1 monoclonal antibody (NCT02823990) [120].

Chimeric antigen receptor (CAR) T-cell therapy has been one of the most promising immunotherapies in the past decade. Despite the recent success of CAR T-cell therapy in hematologic malignancies, especially in B-cell lymphoma and acute lymphoblastic leukemia, the application of this treatment approach in solid tumors has faced several obstacles: the heterogeneous expression of antigens and the induction of effective immune responses in the immunosuppressive TME [129][130]. Previous studies showed that VACV (expressing a non-signaling truncated CD19 (CD19t) protein for tumor-selective delivery) enabled targeting by CD19-CAR T cells. OV19t induced local immunity characterized by tumor infiltration of endogenous and adoptively-transferred CAR T cells in several mouse tumor models [131]. A combination immunotherapy approach using oncolytic viruses might promote de novo CAR T cell targeting of solid tumors.

References

1. Kirn, D.H.; Thorne, S.H. Targeted and armed oncolytic poxviruses: A novel multi-mechanistic therapeutic class for cancer. Nat. Rev. Cancer 2009, 9, 64–71.

2. McCart, J.A.; Ward, J.M.; Lee, J.; Hu, Y.; Alexander, H.R.; Libutti, S.K.; Moss, B.; Bartlett, D.L. Systemic cancer therapy with a tumor-selective vaccinia virus mutant lacking thymidine kinase and vaccinia growth factor genes. Cancer Res. 2001, 61, 8751–8757.

3. He, Q.; Zou, L.; Zhang, P.A.; Lui, J.X.; Skog, S.; Fornande, T. The clinical significance of thymidine kinase I measurement in serum of breast cancer patients using the anti-TK1 antibody. Int. J. Biol. Markers 2001, 15, 139–146.

4. Puhlmann, M.; Gnant, M.; Brown, C.K.; Alexander, H.R.; Bartlett, D.L. Thymidine kinase-deleted vaccinia virus expressing purine nucleoside phosphorylase as a vector for tumor-directed gene therapy. Hum. Gene Ther. 1999, 10, 649–657.

5. Puhlmann, M.; Brown, C.K.; Gnant, M.; Huang, J.; Libutti, S.K.; Alexander, H.R.; Bartlett, D.L. Vaccinia as a vector for tumor-directed gene therapy: Biodistribution of a thymidine kinase-deleted mutant. Cancer Gene Ther. 2000, 7, 66–73.

6. Kim, J.H.; Oh, J.Y.; Park, B.H.; Lee, D.E.; Kim, J.S.; Park, H.E.; Roh, M.S.; Je, J.E.; Yoon, J.H.; Thorne, S.H.; et al. Systemic armed oncolytic and immunologic therapy for cancer with JX-594, a targeted poxvirus expressing GM-CSF. Mol. Ther. 2006, 14, 361–370.

7. Parato, K.A.; Breitbach, C.J.; Le Boeuf, F.; Wang, J.; Storbeck, C.; Ilkow, C.; Diallo, J.S.; Falls, T.; Burns, J.; Garcia, V.; et al. The oncolytic poxvirus JX-594 selectively replicates in and destroys cancer cells driven by genetic pathways commonly activated in cancers. Mol. Ther. 2012, 20, 749–758.

8. Buller, R.M.L.; Chakrabarti, S.; Moss, B.; Fredrickson, T. Cell Proliferative Response to Vaccinia Virus Is Mediated by Vgf. Virology 1988, 164, 182–192.

9. Buller, R.M.; Chakrabarti, S.; Cooper, J.A.; Twardzik, D.R.; Moss, B. Deletion of the vaccinia virus growth factor gene reduces virus virulence. J. Virol. 1988, 62, 866–874.

10. Thorne, S.H.; Hwang, T.H.H.; O'Gorman, W.E.; Bartlett, D.L.; Sei, S.; Kanji, F.; Brown, C.; Werier, J.; Cho, J.H.; Lee, D.E.; et al. Rational strain selection and engineering creates a broad-spectrum, systemically effective oncolytic poxvirus, JX-963. J. Clin. Investig. 2007, 117, 3350–3358.

11. Mejías-Pérez, E.; CarreO-Fuentes, L.; Esteban, M. Development of a Safe and Effective Vaccinia Virus Oncolytic Vector WR-Δ4 with a Set of Gene Deletions on Several Viral Pathways. Mol. Ther. Oncolytics 2017, 8, 27–40.

12. Alcami, A.; Symons, J.A.; Smith, G.L. The vaccinia virus soluble alpha/beta interferon (IFN) receptor binds to the cell surface and protects cells from the antiviral effects of IFN. J. Virol. 2000, 74, 11230–11239.

13. Katsoulidis, E.; Kaur, S.; Platanias, L.C. Deregulation of Interferon Signaling in Malignant Cells. Pharmaceuticals 2010, 3, 406–418.

14. Liu, Z. Deletion of C7L and K1L Genes Leads to Significantly Decreased Virulence of Recombinant Vaccinia Virus Tian Tan. Aids Res. Hum. Retrov. 2013, 29, A153.

15. Conlon, K.C.; Miljkovic, M.D.; Waldmann, T.A. Cytokines in the Treatment of Cancer. J. Interferon Cytokine Res. 2019, 39, 6–21.

16. Berraondo, P.; Sanmamed, M.F.; Ochoa, M.C.; Etxeberria, I.; Aznar, M.A.; Pérez-Gracia, J.L.; Rodríguez-Ruiz, M.E.; Ponz-Sarvise, M.; Castañón, E.; Melero, I. Cytokines in clinical cancer immunotherapy. Br. J. Cancer 2019, 120, 6–15.

17. Pol, J.G.; Workenhe, S.T.; Konda, P.; Gujar, S.; Kroemer, G. Cytokines in oncolytic virotherapy. Cytokine Growth Factor Rev. 2020, 56, 4–27.

18. Meko, J.B.; Yim, J.H.; Tsung, K.; Norton, J.A. High cytokine production and effective antitumor activity of a recombinant vaccinia virus encoding murine interleukin 12. Cancer Res. 1995, 55, 4765–4770.

19. Mastrangelo, M.J.; Maguire, H.C.; Eisenlohr, L.C.; Laughlin, C.E.; Monken, C.E.; McCue, P.A.; Kovatich, A.J.; Lattime, E.C. Intratumoral recombinant GM-CSF-encoding virus as gene therapy in patients with cutaneous melanoma. Cancer Gene Ther. 1999, 6, 409–422.

20. van de Laar, L.; Coffer, P.J.; Woltman, A.M. Regulation of dendritic cell development by GM-CSF: Molecular control and implications for immune homeostasis and therapy. Blood 2012, 119, 3383–3393.

21. Chu, Y.; Wang, L.X.; Yang, G.; Ross, H.J.; Urba, W.J.; Prell, R.; Jooss, K.; Xiong, S.; Hu, H.M. Efficacy of GM-CSF-pro ducing tumor vaccine after docetaxel chemotherapy in mice bearing established Lewis lung carcinoma. J. Immunother. 2006, 29, 367–380.

22. Malhotra, S.; Kim, T.; Zager, J.; Bennett, J.; Ebright, M.; D'Angelica, M.; Fong, Y. Use of an oncolytic virus secreting GM -CSF as combined oncolytic and immunotherapy for treatment of colorectal and hepatic adenocarcinomas. Surgery 20 07, 141, 520–529.

23. Ramesh, N.; Ge, Y.; Ennist, D.L.; Zhu, M.; Mina, M.; Ganesh, S.; Reddy, P.S.; Yu, D.C. CG0070, a conditionally replicati ng granulocyte macrophage colony-stimulating factor-armed oncolytic adenovirus for the treatment of bladder cancer. Clin. Cancer Res. 2006, 12, 305–313.

24. Kohno, S.I.; Luo, C.; Nawa, A.; Fujimoto, Y.; Watanabe, D.; Goshima, F.; Tsurumi, T.; Nishiyama, Y. Oncolytic virotherap y with an HSV amplicon vector expressing granulocyte-macrophage colony-stimulating factor using the replication-com petent HSV type 1 mutant HF10 as a helper virus. Cancer Gene Ther. 2007, 14, 918–926.

25. Liu, T.C.; Hwang, T.; Park, B.H.; Bell, J.; Kirn, D.H. The targeted oncolytic poxvirus JX-594 demonstrates antitumoral, a ntivascular, and anti-HBV activities in patients with hepatocellular carcinoma. Mol. Ther. 2008, 16, 1637–1642.

26. Lee, J.H.; Roh, M.S.; Lee, Y.K.; Kim, M.K.; Han, J.Y.; Park, B.H.; Trown, P.; Kirn, D.H.; Hwang, T.H. Oncolytic and immu nostimulatory efficacy of a targeted oncolytic poxvirus expressing human GM-CSF following intravenous administration in a rabbit tumor model. Cancer Gene Ther. 2010, 17, 73–79.

27. Breitbach, C.J.; Arulanandam, R.; De Silva, N.; Thorne, S.H.; Patt, R.; Daneshmand, M.; Moon, A.; Ilkow, C.; Burke, J.; Hwang, T.H.; et al. Oncolytic Vaccinia Virus Disrupts Tumor-Associated Vasculature in Humans. Cancer Res. 2013, 73, 1265–1275.

28. Wang, L.; Lynn, R.C.; Cheng, G.; Alexander, E.; Kapoor, V.; Moon, E.K.; Sun, J.; Fridlender, Z.G.; Isaacs, S.N.; Thorne, S.H.; et al. Treating tumors with a vaccinia virus expressing IFNβ illustrates the complex relationships between oncolyti c ability and immunogenicity. Mol. Ther. J. Am. Soc. Gene Ther. 2012, 20, 736.

29. Kirn, D.H.; Wang, Y.; Le Boeuf, F.; Bell, J.; Thorne, S.H. Targeting of interferon-beta to produce a specific, multi-mechan istic oncolytic vaccinia virus. PLoS Med. 2007, 4, e353.

30. Kowalsky, S.J.; Liu, Z.Q.; Feist, M.; Berkey, S.E.; Ma, C.; Ravindranathan, R.; Dai, E.; Roy, E.J.; Guo, Z.S.; Bartlett, D. L. Superagonist IL-15-Armed Oncolytic Virus Elicits Potent Antitumor Immunity and Therapy That Are Enhanced with P D-1 Blockade. Mol. Ther. 2018, 26, 2476–2486.

31. Li, J.; O'Malley, M.; Sampath, P.; Kalinski, P.; Bartlett, D.L.; Thorne, S.H. Expression of CCL19 from Oncolytic Vaccinia Enhances Immunotherapeutic Potential while Maintaining Oncolytic Activity. Neoplasia 2012, 14, 1115–1121.

32. Leonard, J.P.; Sherman, M.L.; Fisher, G.L.; Buchanan, L.J.; Larsen, G.; Atkins, M.B.; Sosman, J.A.; Dutcher, J.P.; Vogel zang, N.J.; Ryan, J.L. Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon -gamma production. Blood 1997, 90, 2541–2548.

33. Nazari, A.; Ahmadi, Z.; Hassanshahi, G.; Abbasifard, M.; Taghipour, Z.; Falahati-Pour, S.K.; Khorramdelazad, H. Effecti ve Treatments for Bladder Cancer Affecting CXCL9/CXCL10/CXCL11/CXCR3 Axis: A Review. Oman. Med. J. 2020, 35, e103.

34. Kistner, L.; Doll, D.; Holtorf, A.; Nitsche, U.; Janssen, K.P. Interferon-inducible CXC-chemokines are crucial immune mo dulators and survival predictors in colorectal cancer. Oncotarget 2017, 8, 89998–90012.

35. Hensbergen, P.J.; Wijnands, P.; Schreurs, M.; Scheper, R.J.; Willemze, R.; Tensen, C.P. The CXCR3 targeting chemoki ne CXCL11 has potent antitumor activity in vivo involving attraction of CD8+ T lymphocytes but not inhibition of angioge nesis. J. Immunother. 2005, 28, 343.

36. Moon, E.; Wang, L.C.S.; Bekdache, K.; Lynn, R.C.; Lo, A.; Thorne, S.H.; Albelda, S.M. Intra-Tumoral Delivery of CXCL1 1 via a Vaccinia Virus, but not by Modified T Cells, Enhances the Efficacy of Adoptive T Cell Therapy and Vaccines. On coimmunology 2017, 7, e1395997.

37. Liu, Z.Q.; Ravindranathan, R.; Li, J.; Kalinski, P.; Guo, Z.S.; Bartlett, D.L. CXCL11-Armed oncolytic poxvirus elicits potent antitumor immunity and shows enhanced therapeutic efficacy. Oncoimmunology 2016, 5, e1091554.

38. Lavergne, E.; Combadière, C.; Iga, M.; Boissonnas, A.; Bonduelle, O.; Maho, M.; Debré, P.; Combadiere, B. Intratumoral CC Chemokine Ligand 5 Overexpression Delays Tumor Growth and Increases Tumor Cell Infiltration. J. Immunol. 2004, 173, 3755.

39. Bhat, H.; Zaun, G.; Hamdan, T.A.; Lang, J.; Adomati, T.; Schmitz, R.; Friedrich, S.K.; Bergerhausen, M.; Cham, L.B.; Li, F.; et al. Arenavirus Induced CCL5 Expression Causes NK Cell-Mediated Melanoma Regression. Front. Immunol. 2020, 11, 1849.

40. Li, J.; O'Malley, M.; Urban, J.; Sampath, P.; Guo, Z.S.; Kalinski, P.; Thorne, S.H.; Bartlett, D.L. Chemokine expression from oncolytic vaccinia virus enhances vaccine therapies of cancer. Mol. Ther. 2011, 19, 650–657.

41. Rizzo, A.; Ricci, A.D. PD-L1, TMB, and other potential predictors of response to immunotherapy for hepatocellular carcinoma: How can they assist drug clinical trials? Expert Opin. Investig. Drugs 2022, 31, 415–423.

42. Yu, F.; Wang, X.; Bartlett, D.L.; Gottschalk, S.M.; Song, X.T. T-cell Engager-armed Oncolytic Vaccinia Virus Significantly Enhances Antitumor Therapy. Mol. Ther. 2014, 22, 102–111.

43. Pule, M.A.; Savoldo, B.; Myers, G.D.; Rossig, C.; Russell, H.V.; Dotti, G.; Huls, M.H.; Liu, E.; Gee, A.P.; Mei, Z.; et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: Persistence and antitumor activity in individuals with neuroblastoma. Nat. Med. 2008, 14, 1264–1270.

44. Restifo, N.P.; Dudley, M.E.; Rosenberg, S.A. Adoptive immunotherapy for cancer: Harnessing the T cell response. Nat. Rev. Immunol. 2012, 12, 269–281.

45. Bollard, C.M.; Gottschalk, S.; Leen, A.M.; Weiss, H.; Straathof, K.C.; Carrum, G.; Khalil, M.; Wu, M.F.; Huls, M.H.; Chang, C.C.; et al. Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer. Blood 2007, 110, 2838–2845.

46. Hodge, J.W.; Abrams, S.; Schlom, J.; Kantor, J.A. Induction of antitumor immunity by recombinant vaccinia virus expressing B7-1 or B7-2 co-stimulatory molecules. Cancer Res. 1994, 54, 5552–5555.

47. Zajac, P.; Schutz, A.; Oertli, D.; Noppen, C.; Schaefer, C.; Heberer, M.; Spagnoli, G.C.; Marti, W.R. Enhanced generation of cytotoxic T lymphocytes using recombinant vaccinia virus expressing human tumor-associated antigens and B7 costimulatory molecules. Cancer Res. 1998, 58, 4567–4571.

48. Carroll, M.W.; Overwijk, W.W.; Surman, D.R.; Tsung, K.; Moss, B.; Restifo, N.P. Construction and characterization of a triple-recombinant vaccinia virus encoding B7-1, interleukin 12, and a model tumor antigen. J. Natl. Cancer Inst. 1998, 90, 1881–1887.

49. Chen, L. Co-inhibitory molecules of the B7–CD28 family in the control of T-cell immunity. Nat. Rev. Immunol. 2004, 4, 336–347.

50. Kaufman, H.L.; Deraffele, G.; Mitcham, J.; Moroziewicz, D.; Cohen, S.M.; Hurst-Wicker, K.S.; Cheung, K.; Lee, D.S.; Divito, J.; Voulo, M.; et al. Targeting the local tumor microenvironment with vaccinia virus expressing B7.1 for the treatment of melanoma. J. Clin. Invest. 2005, 115, 1903–1912.

51. Topalian, S.L.; Drake, C.G.; Pardoll, D.M. Immune checkpoint blockade: A common denominator approach to cancer therapy. Cancer Cell 2015, 27, 450–461.

52. Koyama, S.; Maruyama, T.; Adachi, S.; Nozue, M. Expression of costimulatory molecules, B7-1 and B7-2 on human gastric carcinoma. J. Cancer Res. Clin. Oncol. 1998, 124, 383–388.

53. Feng, X.Y.; Lu, L.; Wang, K.F.; Zhu, B.Y.; Wen, X.Z.; Peng, R.Q.; Ding, Y.; Li, D.D.; Li, J.J.; Li, Y.; et al. Low expression of CD80 predicts for poor prognosis in patients with gastric adenocarcinoma. Future Oncol. 2019, 15, 473–483.

54. Leach, D.R.; Krummel, M.F.; Allison, J.P. Enhancement of antitumor immunity by CTLA-4 blockade. Science 1996, 271, 1734–1736.

55. Oertli, D.; Marti, W.R.; Norton, J.A.; Tsung, K. Non-replicating recombinant vaccinia virus encoding murine B-7 molecules elicits effective costimulation of naive CD4+ splenocytes in vitro. J. Gen. Virol. 1996, 77 Pt 12, 3121–3125.

56. Haddad, D. Genetically engineered vaccinia viruses As Agents for Cancer Treatment, Imaging, and Transgene Delivery. Front. Oncol. 2017, 23, 7.

57. Runcie, K.; Budman, D.R.; John, V.; Seetharamu, N. Bi-specific and tri-specific antibodies- the next big thing in solid tumor therapeutics. Mol. Med. 2018, 24, 50.

58. Baeuerle, P.A.; Reinhardt, C. Bispecific T-Cell Engaging Antibodies for Cancer Therapy. Cancer Res. 2009, 69, 4941–4944.

59. Scott, E.M.; Duffy, M.R.; Freedman, J.D.; Fisher, K.D.; Seymour, L.W. Solid Tumor Immunotherapy with T Cell Engager-Armed Oncolytic Viruses. Macromol. Biosci. 2018, 18, 1700187.

60. Offner, S.; Hofmeister, R.; Romaniuk, A.; Kufer, P.; Baeuerle, P.A. Induction of regular cytolytic T cell synapses by bispecific single-chain antibody constructs on MHC class I-negative tumor cells. Mol. Immunol. 2006, 43, 763–771.

61. Garrido, F.; Aptsiauri, N.; Doorduijn, E.M.; Garcia Lora, A.M.; van Hall, T. The urgent need to recover MHC class I in cancers for effective immunotherapy. Curr. Opin. Immunol. 2016, 39, 44–51.

62. Guo, Z.S.; Lotze, M.T.; Zhu, Z.; Storkus, W.J.; Song, X.T. Bi- and Tri-Specific T Cell Engager-Armed Oncolytic Viruses: Next-Generation Cancer Immunotherapy. Biomedicines 2020, 8, 204.

63. Yu, F.; Hong, B.; Song, X. A T-cell engager-armed oncolytic vaccinia virus to target the tumor stroma. Cancer Transl. Med. 2017, 3, 122–132.

64. Hu, Z.; Ott, P.A.; Wu, C.J. Towards personalized, tumour-specific, therapeutic vaccines for cancer. Nat. Rev. Immunol. 2018, 18, 168–182.

65. Tagliamonte, M.; Petrizzo, A.; Tornesello, M.L.; Buonaguro, F.M.; Buonaguro, L. Antigen-specific vaccines for cancer treatment. Hum. Vaccines Immunother. 2014, 10, 3332–3346.

66. Masuelli, L.; Fantini, M.; Benvenuto, M.; Sacchetti, P.; Giganti, M.G.; Tresoldi, I.; Lido, P.; Lista, F.; Cavallo, F.; Nanni, P.; et al. Intratumoral delivery of recombinant vaccinia virus encoding for ErbB2/Neu inhibits the growth of salivary gland carcinoma cells. J. Transl. Med. 2014, 10, 12.

67. Nesslinger, N.J.; Ng, A.; Tsang, K.-Y.; Ferrara, T.; Schlom, J.; Gulley, J.L.; Nelson, B.H. A viral vaccine encoding PSA induces antigen spreading to a common set of self proteins in prostate cancer patients. Clin. Cancer Res. 2010, 16, 4046.

68. Gulley, J.L.; Arlen, P.M.; Madan, R.A.; Tsang, K.Y.; Pazdur, M.P.; Skarupa, L.; Jones, J.L.; Poole, D.J.; Higgins, J.P.; Hodge, J.W.; et al. Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. Cancer Immunol. Immun. 2010, 59, 663–674.

69. Gulley, J.; Chen, A.P.; Dahut, W.; Arlen, P.M.; Bastian, A.; Steinberg, S.M.; Tsang, K.; Panicali, D.; Poole, D.; Schlom, J.; et al. Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. Prostate 2010, 53, 109–117.

70. Dudley, M.E.; Wunderlich, J.R.; Robbins, P.F.; Yang, J.C.; Hwu, P.; Schwartzentruber, D.J.; Topalian, S.L.; Sherry, R.; Restifo, N.P.; Hubicki, A.M.; et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002, 298, 850–854.

71. Bendjama, K.; Quemeneur, E. Modified Vaccinia virus Ankara-based vaccines in the era of personalized immunotherapy of cancer. Hum. Vaccines Immunother. 2017, 13, 1997–2003.

72. Brun, J.L.; Dalstein, V.; Leveque, J.; Mathevet, P.; Raulic, P.; Baldauf, J.J.; Scholl, S.; Huynh, B.; Douvier, S.; Riethmuller, D.; et al. Regression of high-grade cervical intraepithelial neoplasia with TG4001 targeted immunotherapy. Am. J. Obstet. Gynecol. 2011, 204, 169.e1–169.e8.

73. Zhao, L.; Liu, B.; Ren, J.; Feng, J.; Pang, Z.; Gao, J.; Zhang, H.; Tan, W.; Tian, H.; Ruan, L. Immunogenicity in mice and rhesus monkeys vaccinated with recombinant vaccinia virus expressing bivalent E7E6 fusion proteins from human papillomavirus types 16 and 18. Virol. J. 2011, 8, 302.

74. Ricci, A.D.; Rizzo, A.; Rojas Llimpe, F.L.; Di Fabio, F.; De Biase, D.; Rihawi, K. Novel HER2-Directed Treatments in Advanced Gastric Carcinoma: AnotHER Paradigm Shift? Cancers 2021, 13, 1664.

75. de Vries, C.R.; Monken, C.E.; Lattime, E.C. The addition of recombinant vaccinia HER2/neu to oncolytic vaccinia-GMCSF given into the tumor microenvironment overcomes MDSC-mediated immune escape and systemic anergy. Cancer Gene Ther. 2015, 22, 154–162.

76. Benvenuto, M.; Focaccetti, C.; Izzi, V.; Masuelli, L.; Modesti, A.; Bei, R. Tumor antigens heterogeneity and immune response-targeting neoantigens in breast cancer. Semin. Cancer Biol. 2019, 72, 65–75.

77. Heemskerk, B.; Kvistborg, P.; Schumacher, T.N. The cancer antigenome. EMBO J. 2013, 32, 194–203.

78. Brown, S.D.; Warren, R.L.; Gibb, E.A.; Martin, S.D.; Spinelli, J.J.; Nelson, B.H.; Holt, R.A. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. Genome Res. 2014, 24, 743–750.

79. Rooney, M.S.; Shukla, S.A.; Wu, C.J.; Getz, G.; Hacohen, N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 2015, 160, 48–61.

80. Gubin, M.M.; Zhang, X.; Schuster, H.; Caron, E.; Ward, J.P.; Noguchi, T.; Ivanova, Y.; Hundal, J.; Arthur, C.D.; Krebber, W.J.; et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014, 515, 577–581.

81. Aldous, A.R.; Dong, J.Z. Personalized neoantigen vaccines: A new approach to cancer immunotherapy. Bioorg. Med. Chem. 2018, 26, 2842–2849.

82. Martin, S.D.; Brown, S.D.; Wick, D.A.; Nielsen, J.S.; Kroeger, D.R.; Twumasi-Boateng, K.; Holt, R.A.; Nelson, B.H. Low Mutation Burden in Ovarian Cancer May Limit the Utility of Neoantigen-Targeted Vaccines. PLoS ONE 2016, 11, e0155189.

83. Schumacher, T.N.; Schreiber, R.D. Neoantigens in cancer immunotherapy. Science 2015, 348, 69–74.

84. Yarchoan, M.; Johnson, B.A.; Lutz, E.R., 3rd; Laheru, D.A.; Jaffee, E.M. Targeting neoantigens to augment antitumour imunity. Nat. Rev. Cancer 2017, 17, 209–222.

85. Martin, S.D.; Coukos, G.; Holt, R.A.; Nelson, B.H. Targeting the undruggable: Immunotherapy meets personalized oncology in the genomic era. Ann. Oncol. 2015, 26, 2367–2374.

86. Kirn, D.H.; Wang, Y.H.; Liang, W.C.; Contag, C.H.; Thorne, S.H. Enhancing poxvirus oncolytic effects through increased spread and immune evasion. Cancer Res. 2008, 68, 2071–2075.

87. Desai, S.J.; Prickril, B.; Rasooly, A. Mechanisms of Phytonutrient Modulation of Cyclooxygenase-2 (COX-2) and Inflammation Related to Cancer. Nutr. Cancer 2018, 70, 350–375.

88. Ryan, E.P.; Malboeuf, C.M.; Bernard, M.; Rose, R.C.; Phipps, R.P. Cyclooxygenase-2 inhibition attenuates antibody responses against human papillomavirus-like particles. J. Immunol. 2006, 177, 7811–7819.

89. Chang, C.L.; Ma, B.; Pang, X.W.; Wu, T.C.; Hung, C.F. Treatment With Cyclooxygenase-2 Inhibitors Enables Repeated Administration of Vaccinia Virus for Control of Ovarian Cancer. Mol. Ther. 2009, 17, 1365–1372.

90. Tang, B.; Guo, Z.S.; Bartlett, D.L.; Yan, D.Z.; Schane, C.P.; Thomas, D.L.; Liu, J.; McFadden, G.; Shisler, J.L.; Roy, E.J. Synergistic Combination of Oncolytic Virotherapy and Immunotherapy for Glioma. Clin. Cancer Res. 2020, 26, 2216–2230.

91. Hou, W.Z.; Sampath, P.; Rojas, J.J.; Thorne, S.H. Oncolytic Virus-Mediated Targeting of PGE2 in the Tumor Alters the Immune Status and Sensitizes Established and Resistant Tumors to Immunotherapy. Cancer Cell 2016, 30, 108–119.

92. Chon, H.J.; Lee, W.S.; Yang, H.; Kong, S.J.; Lee, N.K.; Moon, E.S.; Choi, J.; Han, E.C.; Kim, J.H.; Ahn, J.B.; et al. Tumor Microenvironment Remodeling by Intratumoral Oncolytic Vaccinia Virus Enhances the Efficacy of Immune-Checkpoint Blockade. Clin. Cancer Res. 2019, 25, 1612–1623.

93. Draghiciu, O.; Nijman, H.W.; Hoogeboom, B.N.; Meijerhof, T.; Daemen, T. Sunitinib depletes myeloid-derived suppressor cells and synergizes with a cancer vaccine to enhance antigen-specific immune responses and tumor eradication. Oncoimmunology 2015, 4, e989764.

94. Finke, J.H.; Rini, B.; Ireland, J.; Rayman, P.; Richmond, A.; Golshayan, A.; Wood, L.; Elson, P.; Garcia, J.; Dreicer, R.; et al. Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients. Clin. Cancer Res. 2008, 14, 6674–6682.

95. Kim, M.; Nitschke, M.; Sennino, B.; Murer, P.; Schriver, B.J.; Bell, A.; Subramanian, A.; McDonald, C.E.; Wang, J.; Cha, H.; et al. Amplification of Oncolytic Vaccinia Virus Widespread Tumor Cell Killing by Sunitinib through Multiple Mechanisms. Cancer Res. 2018, 78, 922–937.

96. Farsaci, B.; Higgins, J.P.; Hodge, J.W. Consequence of dose scheduling of sunitinib on host immune response elements and vaccine combination therapy. Int. J. Cancer 2012, 130, 1948–1959.

97. Hou, W.; Chen, H.; Rojas, J.; Sampath, P.; Thorne, S.H. Oncolytic vaccinia virus demonstrates antiangiogenic effects mediated by targeting of VEGF. Int. J. Cancer 2014, 135, 1238–1246.

98. MacTavish, H.; Diallo, J.S.; Huang, B.; Stanford, M.; Le Boeuf, F.; De Silva, N.; Cox, J.; Simmons, J.G.; Guimond, T.; Falls, T.; et al. Enhancement of vaccinia virus based oncolysis with histone deacetylase inhibitors. PLoS ONE 2010, 5, e14462.

99. Francis, L.; Guo, Z.S.; Liu, Z.; Ravindranathan, R.; Urban, J.A.; Sathaiah, M.; Magge, D.; Kalinski, P.; Bartlett, D.L. Modulation of chemokines in the tumor microenvironment enhances oncolytic virotherapy for colorectal cancer. Oncotarget 2016, 7, 22174–22185.

100. Peng, J.; Wang, S.; Fan, W.; Li, S.; Wu, Y.; Mou, X.; Wang, J.; Tong, X. Synergistic suppression effect on tumor growth of acute myeloid leukemia by combining cytarabine with an engineered oncolytic vaccinia virus. Onco Targets Ther. 2018, 11, 6887–6900.

101. Marme, D. Tumor Angiogenesis: A Key Target for Cancer Therapy. Oncol. Res. Treat. 2018, 41, 164.

102. Nicholson, R.I.; Gee, J.M.; Harper, M.E. EGFR and cancer prognosis. Eur. J. Cancer 2001, 37 (Suppl. S4), S9–S15.

103. Serrano, M.J.; Alvarez-Cubero, M.J.; De Miguel Perez, D.; Rodríguez-Martínez, A.; Gonzalez-Herrera, L.; Robles-Fernandez, I.; Hernandez, J.E.; Puche, J.L.G.; Lorente, J.A. Significance of EGFR Expression in Circulating Tumor Cells. Ad

v. Exp. Med. Biol. 2017, 994, 285–296.

104. De Luca, A.; Carotenuto, A.; Rachiglio, A.; Gallo, M.; Maiello, M.R.; Aldinucci, D.; Pinto, A.; Normanno, N. The role of the EGFR signaling in tumor microenvironment. J. Cell Physiol. 2008, 214, 559–567.

105. Giltnane, J.M.; Moeder, C.B.; Camp, R.L.; Rimm, D.L. Quantitative multiplexed analysis of ErbB family coexpression for primary breast cancer prognosis in a large retrospective cohort. Cancer 2009, 115, 2400–2409.

106. Siveen, K.S.; Prabhu, K.; Krishnankutty, R.; Kuttikrishnan, S.; Tsakou, M.; Alali, F.Q.; Dermime, S.; Mohammad, R.M.; Uddin, S. Vascular Endothelial Growth Factor (VEGF) Signaling in Tumour Vascularization: Potential and Challenges. Curr. Vasc. Pharmacol. 2017, 15, 339–351.

107. Matsumoto, K.; Ema, M. Roles of VEGF-A signalling in development, regeneration, and tumours. J. Biochem. 2014, 156, 1–10.

108. Carmeliet, P. VEGF as a key mediator of angiogenesis in cancer. Oncology 2005, 69, 4–10.

109. Breitbach, C.J.; Paterson, J.M.; Lemay, C.G.; Falls, T.J.; McGuire, A.; Parato, K.A.; Stojdl, D.F.; Daneshmand, M.; Speth, K.; Kirn, D.; et al. Targeted inflammation during oncolytic virus therapy severely compromises tumor blood flow. Mol. Ther. 2007, 15, 1686–1693.

110. 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. A novel vaccinia virus with dual oncolytic and anti-angiogenic therapeutic effects against triple-negative breast cancer. Breast Cancer Res. Treat. 2014, 148, 489–499.

111. Adelfinger, M.; Bessler, S.; Frentzen, A.; Cecil, A.; Langbein-Laugwitz, J.; Gentschev, I.; Szalay, A.A. Preclinical Testing Oncolytic Vaccinia Virus Strain GLV-5b451 Expressing an Anti-VEGF Single-Chain Antibody for Canine Cancer Therapy. Viruses 2015, 7, 4075–4092.

112. Patil, S.S.; Gentschev, I.; Adelfinger, M.; Donat, U.; Hess, M.; Weibel, S.; Nolte, I.; Frentzen, A.; Szalay, A.A. Virotherapy of canine tumors with oncolytic vaccinia virus GLV-1h109 expressing an anti-VEGF single-chain antibody. PLoS ONE 2012, 7, e47472.

113. Huang, T.; Wang, H.Q.; Chen, N.G.; Frentzen, A.; Minev, B.; Szalay, A.A. Expression of anti-VEGF antibody together with anti-EGFR or anti-FAP enhances tumor regression as a result of vaccinia virotherapy. Mol. Ther.Oncolytics 2015, 2, 15003.

114. Topalian, S.L.; Hodi, F.S.; Brahmer, J.R.; Gettinger, S.N.; Smith, D.C.; McDermott, D.F.; Powderly, J.D.; Carvajal, R.D.; Sosman, J.A.; Atkins, M.B.; et al. Safety, Activity, and Immune Correlates of Anti-PD-1 Antibody in Cancer. N. Engl. J. Med. 2012, 366, 2443–2454.

115. Johnson, D.B.; Rioth, M.J.; Horn, L. Immune Checkpoint Inhibitors in NSCLC. Curr. Treat Option. Oncol. 2014, 15, 658–669.

116. Lynch, T.J.; Bondarenko, I.; Luft, A. Ipilimumab in Combination With Paclitaxel and Carboplatin As First-Line Treatment in Stage IIIB/IV Non-Small-Cell Lung Cancer: Results From a Randomized, Double-Blind, Multicenter Phase II Study. J. Clin. Oncol. 2012, 30, 3654.

117. Hodi, F.S. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. N. Engl. J. Med. 2010, 363, 1290.

118. Woller, N.; Gurlevik, E.; Fleischmann-Mundt, B.; Schumacher, A.; Knocke, S.; Kloos, A.M.; Saborowski, M.; Geffers, R.; Manns, M.P.; Wirth, T.C.; et al. Viral Infection of Tumors Overcomes Resistance to PD-1-immunotherapy by Broadening Neoantigenome-directed T-cell Responses. Mol. Ther. 2015, 23, 1630–1640.

119. Zhu, L.C.; Jing, S.S.; Wang, B.; Wu, K.; Shenglin, M.A.; Zhang, S. Anti-PD-1/PD-L1 Therapy as a Promising Option for Non-Small Cell Lung Cancer: A Single arm Meta-Analysis. Pathol. Oncol. Res. 2016, 22, 331–339.

120. Remy-Ziller, C.; Thioudellet, C.; Hortelano, J.; Gantzer, M.; Nourtier, V.; Claudepierre, M.C.; Sansas, B.; Préville, X.; Bendjama, K.; Quemeneur, E.; et al. Sequential administration of MVA-based vaccines and PD-1/PD-L1-blocking antibodies confers measurable benefits on tumor growth and survival: Preclinical studies with MVA-Gal and MVA-MUC1 (TG4010) in a murine tumor model. Hum. Vaccinia Immunother. 2018, 14, 140–145.

121. Dong, H.; Strome, S.E.; Salomao, D.R.; Tamura, H.; Hirano, F.; Flies, D.B.; Roche, P.C.; Lu, J.; Zhu, G.; Tamada, K.; et al. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. Nat. Med. 2002, 8, 793–800.

122. Blank, C.; Brown, I.; Peterson, A.C.; Spiotto, M.; Iwai, Y.; Honjo, T.; Gajewski, T.F. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. Cancer Res. 2004, 64, 1140–1145.

123. Tumeh, P.C.; Harview, C.L.; Yearley, J.H.; Shintaku, I.P.; Taylor, E.J.; Robert, L.; Chmielowski, B.; Spasic, M.; Henry, G.; Ciobanu, V.; et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014, 515, 568–571.

124. Yuan, J.; Adamow, M.; Ginsberg, B.A.; Rasalan, T.S.; Ritter, E.; Gallardo, H.F.; Xu, Y.; Pogoriler, E.; Terzulli, S.L.; Kuk, D.; et al. Integrated NY-ESO-1 antibody and CD8+ T-cell responses correlate with clinical benefit in advanced melanoma patients treated with ipilimumab. Proc. Natl. Acad. Sci. USA 2011, 108, 16723–16728.

125. Chen, D.S.; Mellman, I. Elements of cancer immunity and the cancer-immune set point. Nature 2017, 541, 321–330.

126. Foy, S.P.; Mandl, S.J.; dela Cruz, T.; Cote, J.J.; Gordon, E.J.; Trent, E.; Delcayre, A.; Breitmeyer, J.; Franzusoff, A.; Rountree, R.B. Poxvirus-based active immunotherapy synergizes with CTLA-4 blockade to increase survival in a murine tumor model by improving the magnitude and quality of cytotoxic T cells. Cancer Immunol. Immunother. 2016, 65, 537–549.

127. Smith, H.G.; Mansfield, D.; Roulstone, V.; Kyula-Currie, J.N.; McLaughlin, M.; Patel, R.R.; Bergerhoff, K.F.; Paget, J.T.; Dillon, M.T.; Khan, A.; et al. PD-1 Blockade Following Isolated Limb Perfusion with Vaccinia Virus Prevents Local and Distant Relapse of Soft-tissue Sarcoma. Clin. Cancer Res. 2019, 25, 3443–3454.

128. Woo, Y.; Zhang, Z.; Yang, A.; Chaurasiya, S.; Park, A.K.; Lu, J.; Kim, S.I.; Warner, S.G.; Von Hoff, D.; Fong, Y. Novel Chimeric Immuno-Oncolytic Virus CF33-hNIS-antiPDL1 for the Treatment of Pancreatic Cancer. J. Am. Coll Surg. 2020, 230, 709–717.

129. Rezaei, R.; Esmaeili Gouvarchin Ghaleh, H.; Farzanehpour, M.; Dorostkar, R.; Ranjbar, R.; Bolandian, M.; Mirzaei Nodooshan, M.; Ghorbani Alvanegh, A. Combination therapy with CAR T cells and oncolytic viruses: A new era in cancer immunotherapy. Cancer Gene Ther. 2022, 29, 647–660.

130. Watanabe, N.; McKenna, M.K.; Rosewell Shaw, A.; Suzuki, M. Clinical CAR-T Cell and Oncolytic Virotherapy for Cancer Treatment. Mol. Ther. 2021, 29, 505–520.

131. Park, A.K.; Fong, Y.; Kim, S.I.; Yang, J.; Murad, J.P.; Lu, J.; Jeang, B.; Chang, W.C.; Chen, N.G.; Thomas, S.H.; et al. Effective combination immunotherapy using oncolytic viruses to deliver CAR targets to solid tumors. Sci. Transl. Med. 2020, 12, eaaz1863.