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Oncolytic Viruses as Antigen-Agnostic Cancer Vaccines

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Selective destruction of neoplastic tissues by oncolytic viruses (OVs) leads to antigen-agnostic boosting of neoantigen-specific cytotoxic T lymphocyte (CTL) responses, making OVs ideal companions for checkpoint blockade therapy. Here we discuss the mechanisms whereby OVs modulate both adjuvanticity and antigenicity of tumor cells. Suppression of antitumor immunity after OV therapy has not been observed, possibly because viral antigen expression diminishes as the antiviral response matures, thereby progressively honing the CTL response to tumor neoantigens. By combining direct *in situ* tumor destruction with the ability to boost antitumor immunity, OVs also have the potential to be powerful standalone cancer therapies.

Oncolytic Viruses

Oncolytic viruses (OVs) are replication competent viruses that selectively propagate in tumor cells and/or in the immunosuppressive tumor microenvironment. Because of their intrinsic antigenicity, tumors are *a priori* evolved to evade immune detection, and have sluggish or defective pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) responses, which make them especially susceptible to virus infections (Galluzzi et al., 2017; Xia et al., 2016). However, to increase their utility as anticancer agents, OVs are generally engineered or adapted to further increase their antitumor specificity, safety, immunogenicity, oncolytic potency, and druggability, and the list of virus platforms adapted for oncolytic applications is rapidly growing (Maroun et al., 2017). Irrespective of the status of the molecular signaling machinery in a tumor and the degree to which it reprograms an immune privileged microenvironment, introducing an OV does cause cellular damage, eventually inducing pro-inflammatory DAMP and PAMP responses, and promoting phagocytosis of dead or injured virus-infected tumor cells (Chiocca and Rabkin, 2014). The weaker the innate and adaptive immune responses to the intratumoral virus infection, the more extensive its spread and the more damage it causes (Liu et al., 2014; Ruotsalainen et al., 2015). Thus, a virus infection in a tumor typically ends up breaking tolerance and eliciting innate and adaptive immune responses that result in its ultimate elimination.

Although there is enormous diversity in the structures and replication strategies of known viruses, they have all evolved to keep their infected cell substrates alive long enough to manufacture virus progeny and, therefore, encode combat proteins that control cell death and limit the emission of danger signals from infected cells (Finlay and McFadden, 2006). Speed of replication and stealth in the face of innate and adaptive immune responses also help viruses to complete their life cycle before the infected cell has time to mount an effective response. But these are only temporary holding measures that serve to slow, but not stop, the crescendo of innate and adaptive host immune responses. Thus, depending on its immunogenicity, genome complexity, speed of intratumoral propagation, and capacity for controlling host responses, a given OV may spread more or less extensively in a tumor before it is contained. Sometimes, where a profoundly unresponsive and immunosuppressive tumor is invaded by a rapidly propagating OV, the infection can spread sufficiently to destroy the entire tumor (Naik et al., 2012; Russell et al., 2014). However, partial tumor damage is more typical and the recent surge of interest in oncolytic virotherapy is on account of its potential ability to reprogram the tumor microenvironment during this destructive phase in such a way as to boost systemic antitumor immunity, thereby providing an ideal accompaniment to immune checkpoint blockade (Guo et al., 2017; Lichty et al., 2014).

Clinical Experience with OVs

Recent clinical experience with first generation OVs has confirmed their druggability and anticancer potential (Russell and Peng, 2017). T-VEC, an attenuated herpes simplex virus incorporating a granulocyte-macrophage colony-stimulating factor (GM-CSF) transgene, was granted US and European marketing approvals in 2015 for intratumoral therapy in patients with unresectable stage 3 and 4 melanoma. Approval was based on a 16% durable remission rate and modest survival prolongation in the phase 3 registration trial (Andtbacka et al., 2015a), with complete resolution rate of 47% for injected skin lesions versus only 9% for deep visceral lesions (Andtbacka et al., 2016). Virus did not spread from injected to uninjected lesions, and visceral lesion responses were attributed to boosting of systemic antitumor immunity. T-VEC was subsequently combined with ipilimumab (anti-CTLA4) and increased the overall response rate from 18% with ipilimumab alone to 39% with combination therapy in a 200-patient randomized phase 2 melanoma trial (Chesney et al., 2017). An even higher overall response rate of 68% was reported when T-VEC was combined with the anti-PD1 antibody pembrolizumab, with 33% of patients achieving complete disease remission (Ribas et al., 2017).

Aside from T-VEC, several additional non-herpes virus oncolytics are showing early clinical promise. Tumor responses have been achieved following intratumoral or locoregional administration of oncolytic strains of vaccinia virus, coxsackievirus

A21 (CVA21), vesicular stomatitis virus, measles virus, poliovirus, C-type retrovirus, adenovirus, and other viruses in early stage clinical trials in a range of cancer types, and, in many cases, there is evidence that these responses are at least partially immune mediated (Guo et al., 2017; Russell and Peng, 2017). For example, an intraperitoneally administered measles virus boosted the immune response to known ovarian tumor antigens in patients with refractory ovarian cancer (Galanis et al., 2015) and strong evidence of synergy was observed using intratumoral CVA21 in combination with checkpoint antibody therapy for melanoma therapy (Andtbacka et al., 2015b).

In light of the observation that T-VEC injected tumors are more likely to respond than distant metastases, emphasis is rapidly shifting from intratumoral to intravenous OV delivery. For a variety of reasons (size, manufacturing, high seroprevalence), intravenous use of HSV-based oncolytics may be problematic. However, feasibility for the systemic approach has been well documented for several OV families in preclinical cancer models and is gaining traction in the clinic. In one compelling demonstration of the potential power of the approach, complete resolution of multiple plasmacytomas and clearance of diffuse bone marrow infiltration were documented in a patient with treatment-refractory multiple myeloma following a single intravenous infusion of a recombinant measles virus (Russell et al., 2014). Systemic antitumor activity has also been documented for reovirus, adenovirus, CVA21, VSV, vaccinia, and Newcastle disease virus oncolytics (Russell et al., 2012), and some of these agents have already advanced to combination studies with checkpoint antibody therapy.

Using OVs with Immune Checkpoint Blockade

Cancers displaying immunogenic peptide-MHC complexes (neoantigens, oncofetal antigens, or other tumor-associated antigens, hereafter referred to as TAA) are potentially vulnerable to TAA-reactive cytotoxic T lymphocyte (CTL), but are often protected from CTL-mediated lysis through upregulation of inhibitory immune checkpoints (Topalian et al., 2015). This protection can be reversed by immune checkpoint blockade therapy using antibodies reactive with PD-1, PD-L1, or CTLA-4, which have recently gained FDA approvals for therapy of melanoma, lung cancer, kidney, bladder, and head and neck cancers, Hodgkin's lymphoma, and microsatellite unstable malignancies (Alexander, 2016; Salama and Moschos, 2017). In general, cancers responsive to checkpoint blockade have a higher mutational burden, and hence more neoantigens, than those that are nonresponsive. Unfortunately, many patients have a low precursor frequency of TAA-reactive T cells and respond poorly to checkpoint blockade due to paucity of antigen presentation on tumor cells and/or weak adjuvanticity of tumor cell death in an immunosuppressive tumor microenvironment (Schumacher and Schreiber, 2015).

Simply restating the above, the mode of action of immune checkpoint blockade is to remove the inhibitory signals that tumor cells present to their would-be executioner CTLs. The potency of checkpoint inhibitor antibodies is therefore limited by the number of TAA-reactive CTLs available to attack the tumor. Thus, if OV therapy were to increase the number of available tumor-reactive CTLs, it should also boost the response to checkpoint antibody therapy. Available preclinical and clinical evidence support this mechanism (Bartee and Li, 2017; Chesney et al., 2017; Durham et al., 2017; Engeland et al., 2014; Gao et al., 2009; Puzanov et al., 2016; Ribas et al., 2017; Shen et al., 2016; Woller et al., 2015) and further demonstrate that OV-mediated upregulation of the PD-1/PD-L1 axis in virus-infected tumors can be overridden by checkpoint antibodies (Samson et al., 2018). Below we discuss our current understanding of the impact of OV infection both on adjuvanticity and antigenicity of dead or dying tumor cells and how this primes and amplifies the available pool of tumor-killing TAA-reactive CTLs.

Whether priming a new antitumor response or boosting a preexisting response, to amplify TAA-reactive CTL tumor-resident dendritic cells (DCs) must phagocytose, process, and cross-present tumor antigens, migrate to lymphoid follicles, and coordinate the engagement, activation, and amplification of helper T cells along with CTLs (Vyas et al., 2008). The helper T cells interact with MHC class 2-peptide complexes on the DC surface and release cytokines that drive the proliferation of CTLs interacting with MHC class 1-peptide complexes also presented by the DC. In a tumor responding to conventional therapy, this entire process may fail due to lack of adjuvanticity at the site of tumor cell death, or due to lack of antigenicity of the dying tumor cells (Galluzzi et al., 2017).

Tumor cells typically die by apoptosis, which is relatively noninflammatory and lacks adjuvanticity, such that tumor-resident DCs are not sufficiently activated to phagocytose dying cells, process and present peptides, or migrate to regional lymph nodes, and new DC progenitors are not recruited (Woo et al., 2015). Also, tumor-resident macrophages may have been irreversibly programmed by long association with the tumor cells to actively promote the generation of antigen-specific suppressor T cells capable of damping down the antitumor CTL response (Dehne et al., 2017; Gordon et al., 2017; Hou et al., 2016; Nagata and Tanaka, 2017). In the following sections we discuss the impact of OV infection on these two critical parameters of adjuvanticity and antigenicity.

OVs, Inflammatory Cell Death, and Adjuvanticity

Unlike their uninfected counterparts, OV-infected tumor cells undergo inflammatory death, their PAMPs and DAMPs having been activated as a consequence of virus inflicted damage, their apoptotic cell death pathways blocked, and their necroptotic cell death machinery activated (Kaminskyy and Zhivotovsky, 2010; Schock et al., 2017). Considerable evidence has recently emerged to support the superiority of necroptotic death as a driver of anticancer immunity, and the mechanisms by which the various known DAMPs boost tumor cell adjuvanticity have been comprehensively reviewed elsewhere (Galluzzi et al., 2017; Krysko et al., 2017). In brief, released ATP enhances the recruitment of DCs and their activation; annexin A1 guides the final approach of DCs to dying tumor cells; calreticulin and phosphatidylserine exposed on the cell surface act as "eat me" signals promoting phagocytosis; HMGB1 drives DC maturation; type I interferons (IFNs) increase the expression of MHC-peptide complexes and promote the intratumoral release of CXCL10, a T cell chemokine. Although beyond the scope of this review, it should be noted that, not only do apoptosis and necroptosis come in many (more or less immunogenic) forms, but there are in addition several alternative cell death pathways

that can be activated in a virus-infected tumor cell, adding further complication to the analysis of forces sculpting the antitumor immune response (Galluzzi et al., 2018).

Irrespective of their propensity to cause necroptosis, OVs activate PAMPs in infected cancer cells, which drives adjuvanticity independent of the mode of cell death (Schock et al., 2017). A further consideration is that many virus-infected cancer cells in the tumor microenvironment are likely eaten alive, and die not by necroptosis but by phagoptosis inside the phagocytosing macrophage or DC (Brown and Neher, 2012). Thus, as discussed below, in oncolytic virotherapy, tumor cell PAMPs may be more important drivers of adjuvanticity and effective antigen-presenting cell (APC) engagement than the mode of cell death.

How OV-Infected Tumor Cells Activate Phagocytosing APCs

When tumor cells are killed by viruses, both the adjuvanticity and antigenicity of the phagocytosed tumor cells are driven by their cellular contents. The body has evolved processes to efficiently eliminate apoptotic cells via phagocytosis while at the same time ensuring that there are no inflammatory counter reactions (Nagata and Tanaka, 2017). This is impressive, since up to 50 billion apoptotic cells are efficiently processed by phagocytes on a daily basis (Toda et al., 2015). Tumor cells almost certainly mimic this non-innate immune provoking death pathway, after engulfment, which helps them to become immunologically indolent (Mohme et al., 2017; Nagata and Tanaka, 2017; Toda et al., 2015). After phagocytosis, tumor cell nucleic acid is proficiently digested by DNases before it can robustly activate innate immune pathways and trigger cytokine production, including type I IFNs, which are required to stimulate cross-priming events and facilitate antitumor T cell activity (Nagata and Tanaka, 2017; Schiavoni et al., 2013). A key challenge has therefore been to convert such "cold tumors" into "hot" or immunologically reactive ones. OVs are able to do this in a number of ways. For example, first the infection of the tumor cell itself may trigger innate immune signaling and alert the immunosurveillance system to the infected tumor microenvironment (Barber, 2011; Franz and Kagan, 2017; Takeuchi and Akira, 2009). Second, tumor cells infected with viruses are full of cytosolic PAMPs in the form of microbial nucleic acid. Following engulfment, the phagocyte degradation machinery likely gets overwhelmed by the "eaten" microbial-specific molecules/nucleic acid, which interact with extrinsic innate immune sensors to generate cytokines required for cross-priming and adaptive immunity (Nagata and Tanaka, 2017; Schiavoni et al., 2013). The key innate immune pathways and sensors have now been largely uncovered. For example, Toll-like receptor (TLR) TLR3, which recognizes viral double-stranded RNA (dsRNA) or TLR7, which recognizes viral ssRNA, may facilitate the intrinsic (in the tumor cell) and extrinsic (in the phagocyte) production of cytokines following the infection of tumor cells with RNA-based OVs (Moresco et al., 2011; Takeuchi and Akira, 2009). TLR9, which recognizes single-stranded microbial DNA of approximately 21 nucleotides may perform a similar function in relation to DNA-based OVs, such as HSV1 (Moresco et al., 2011; Takeuchi and Akira, 2009). The Rig-I-like helicase (RIG-I and MDA5) family may also play a key role in recognizing the RNA from OVs, such as VSV and measles, which activate cytokine production through the adaptor MAVS (Takeuchi and Akira, 2009). Third, it has become clear that innate immune STING signaling plays a significant role in facilitating T cell responses to dying tumor cells (Barber, 2015). After phagocytosis, the partially degraded genomic DNA compartmentalized in the nucleus of tumor cells is efficiently processed by DNaseII in the lysosomal compartment (Barber, 2015; Nagata and Tanaka, 2017). However, a small fraction of nucleic acid appears capable of leaking out and binding to a cellular synthase called cGAS, which generates self-DAMPS referred to as cyclic dinucleotides (Chen et al., 2016). These bind to the sensor STING, which triggers type I IFN production required for cross-priming and the generation of T cells to tumor antigens. Mice lacking STING have a reduced capacity to generate antitumor T cell responses (Corrales et al., 2017). Conversely, the use of STING therapeutic agonists, introduced into the tumor microenvironment, are able to augment antitumor T cell activity, presumably by activating APCs (Corrales et al., 2017). Such STING agonists may also help overcome checkpoint inhibition, by increasing the production of antitumor T lymphocytes (Corrales et al., 2017). Innate immune agonists may also be useful in helping to boost OV-mediated cross-priming. STING signaling has also been shown to be important in the immunogenic effectiveness of radiation treatment. That is, radiation facilitates the development of cytosolic micronuclei comprising pieces of the host genome. These micronuclei activate the cGAS/STING axis in the cytosol to generate the production of cytokines, which alert the immunosurveillance system (Corrales et al., 2017; Deng et al., 2014; Harding et al., 2017; Mackenzie et al., 2017). The same micronuclei may mimic cytosolic OV PAMPs/nucleic acid and play a key role in stimulating APCs in *trans*, to facilitate cross-priming through extrinsic STING or alternate innate immune signaling. Of note is that STING signaling, rather than the RIG-MDA5 pathway appears recurrently defective in numerous tumor types (Xia et al., 2016). Ironically, this may help explain mechanisms of oncolysis, especially by OVs such as HSV1, since such microbes will be able to productively replicate in such cells (Alvarez-Breckenridge et al., 2015; Xia et al., 2016). Nevertheless, the mechanisms underlining oncolysis still remain to be fully clarified (Barber, 2005). The further elucidation of innate immune processes may help explain mechanisms of oncolysis, the antigenicity and immunogenicity of dying cells, and the control of antitumor T cell production. Shaping the same processes using agonists or OVs that manipulate innate immune signaling may lead to a new generation of therapies that potently boost antitumor immune regimes.

Antigenicity of OV-Infected Cells: Impact of Virus-Encoded T Cell Epitopes

Aside from adjuvanticity, the other key parameter driving the generation of tumor-reactive CTLs is the antigenicity of dying tumor cells (Galluzzi et al., 2017). Mutational burden, and hence the number of available neoantigenic targets, varies greatly between tumors (Alexandrov et al., 2013). Thus, the neoantigen repertoire for some tumors may be so small as to preclude the possibility of generating a sufficient diversity of tumor-reactive CTLs for therapeutic efficacy. However, where the problem is an insufficiency of MHC class 2 epitopes being presented on the surface of the lymph node-resident DC, and hence a failure to recruit

sufficient T helper cells to support the amplification of tumor-reactive CTLs, the OV-encoded antigens may be of major importance (Ichikawa et al., 2012; Knutson and Disis, 2005). Thus, a DC-resident APC displaying a mix of MHC class 1 cross-presented tumor epitopes and MHC class 2 cross-presented OV epitopes should robustly amplify the tumor-reactive CTLs.

Epitope Focusing

Although there is a clear potential advantage to the co-presentation of virally encoded T-helper epitopes with TAA epitopes for amplification of tumor-reactive CTLs, there is a related concern as to whether the parallel cross-presentation of viral antigens and tumor antigens in class 1 MHC molecules on the DC surface will lead to epitope interference, with the abundance of viral epitopes overshadowing and diminishing the amplification of tumor-reactive CTLs. T cells are known to compete with each other during the genesis and maturation of the T cell response to a complex antigenic challenge leading to epitope dominance and epitope interference (Kedl et al., 2000, 2003). Epitope interference could in theory negate the potential immunological benefits of OV infection, but this has not to date been documented. On the contrary, available experimental evidence, albeit from a small number of studies, shows that OV infection usually boosts the antitumor CTL response (Ribas et al., 2017; Shen et al., 2016; Woller et al., 2015).

We postulate that the lack of interference is due to epitope focusing, wherein progressive honing of the CTL response to tumor antigens occurs throughout the course of an OV infection, driven by a progressively increasing ratio of tumor neoantigen to viral antigen. Thus, the abundance of viral antigens is highest in infected tumor cells, which die and/or are phagocytosed at early time points post OV administration, but is lower in cells being phagocytosed at later time points when antiviral immune responses are suppressing the expression of viral genes and sensitizing the cells to apoptosis/necroptosis. In concert with the declining representation of viral antigens in dying tumor cells, there should be no parallel reduction in the expression of tumor antigens. The ratio of viral to tumor antigens delivered to phagocytosing APCs is therefore predicted to become progressively more favorable to the tumor antigens as the infection progresses. Thus, while interference has been well documented between distinct viral epitopes during virus infection of normal tissues (Farrington et al., 2013; Kenney et al., 2015), interference between viral and tumor antigens during an OV infection presents a fundamentally different scenario.

Aside from the progressive attenuation of viral antigen expression in phagocytosed OV-infected tumor cells, additional factors may disfavor the suppression of TAA-reactive CTLs during an OV infection. For example, epitope interference only arises when there is cross-competition between antigens that are simultaneously expressed on the surface of a single APC (Kedl et al., 2003). But in an OV-treated cancer patient uninfected tumor cells may continue to die and/or be phagocytosed (albeit less efficiently than virus-infected cells), ensuring the continued presence of APC presenting only tumor antigens. Also, epitope interference in models of virus infection and tissue rejection is readily overcome by increasing the number of antigen-bearing APCs or the precursor frequency of T cells reacting with the non-dominant antigen (Kedl et al., 2003). As discussed previously, OV infection provides a powerful stimulus to the generation of antigen-bearing APCs so the number of these cells is not expected to be limiting. Also, since cancer patients are not tumor-naive, precursor frequencies for TAA-reactive CTLs are expected to be favorable prior to the administration of oncolytic virotherapy. From the above model, it can be predicted that sequential administration of immunologically unrelated OVs may be a superior therapeutic strategy compared with repeat administration of a single OV species. Experiments are underway to test this concept.

Host Protein Shutoff

Certain aspects of OV biology may disfavor the amplification of neoantigen-reactive CTLs. To fully subvert the macromolecular synthetic machinery of the host cell toward the production of progeny virus particles, and to prevent the production of antiviral proteins, many viruses have evolved to suppress or shut off host cell protein synthesis (Clemens, 2005; Rivas et al., 2016). Since this may reduce the abundance of tumor neoantigen in the infected tumor cell, viruses that efficiently shut down host cell translation may be less attractive candidates for combination therapy with checkpoint inhibitor antibodies. Mechanisms of host shutoff include suppression of RNA splicing by the HSV immediate-early protein ICP27 (Hardy and Sandri-Goldin, 1994), suppression of nucleo-cytoplasmic mRNA transport by the VSV matrix protein, which targets the nuclear pore complex (Gustin, 2003), and cleavage by picornavirus proteases of eukaryotic initiation factor 4G (eIF4G) (Castello et al., 2011), a key component of the eIF4F cap-binding complex (picornaviral internal ribosomal entry sites allow them to bypass the need for cap-dependent translation). In addition to these general strategies, certain viruses specifically inhibit the formation and surface display of MHC-peptide complexes on infected target cells (Hewitt, 2003), thereby interfering with T cell-mediated killing. One example is the 88 amino acid ICP47 protein of HSV-1; ICP47 acts as a competitive inhibitor of peptide binding to TAP (Hill et al., 1995), the transporter associated with antigen processing, which guides the assembly of peptide sequences into nascent MHC class 1 molecules in the endoplasmic reticulum (ER) before they are transported to the surface of the cell. A second example is the adenovirus E3/19K gene product, an integral type 1 membrane protein that binds MHC class 1 molecules in the ER, preventing their transport to the plasma membrane (Bennett et al., 1999).

In parallel with the targeted suppression of host cell protein synthesis by the invading virus, one of the key defensive strategies of a virus-infected host cell is to globally suppress its own macromolecular synthetic machinery, thereby interfering with virus progeny production. This is most notably achieved through phosphorylation of the Met-tRNA$_f$-binding factor eIF2α by the IFN-inducible, dsRNA-activated protein PKR (Fernandes, 2016; Gale and Katze, 1998). Not surprisingly, virally encoded proteins often interfere with this pathway, for example, via the HSV-1-encoded protein ICP34.5, which recruits protein phosphatase 1 to dephosphorylate eIF2α (Mohr, 2004). Another well-characterized pathway that works via RNAse L involves the IFN-inducible 2′,5′-oligoadenylate synthases. These enzymes synthesize 2′,5′-oligoadenylates, powerful allosteric activators of RNAse L, an enzyme that efficiently cleaves both viral and cellular RNAs as well as triggering apoptosis (Drappier and Michiels, 2015).

From the foregoing discussion, it is clear that suppression of host cell protein synthesis in virus-infected cells has the potential

to negatively affect the expression and presentation of tumor-associated antigens. Indeed this will be a very interesting area for further study to determine the importance of preformed tumor antigens already synthesized prior to viral invasion, the relative impact of different mechanisms of host cell protein synthesis shutoff, and the numerous engineering strategies that might be deployed to modulate this activity in different OV platforms.

Engineering OVs for Cancer Immunotherapy

Aside from their selective intratumoral propagation and inflammatory tumor cell killing, OVs can be engineered to further modulate their impact on tumor-immune system interactions (Elsedawy and Russell, 2013). For example, the GM-CSF cistron inserted into T-VEC (HSV) and JX594 (vaccinia) OVs drives the release of high concentrations of this cytokine at sites of infection, which is considered beneficial for enhancement of DC recruitment, activation, and function (Liu et al., 2003; Kim et al., 2006). Likewise, insertion of the IFN-β transgene in an oncolytic VSV has been shown to enhance its ability to drive the recruitment, activation, and function of antitumor T cells (Obuchi et al., 2003). These examples are merely the tip of the iceberg. Numerous preclinical studies have been performed using a variety of OV platforms engineered to encode additional cytokines, chemokines, and checkpoint inhibitor antibodies or intracellular proteins that promote the induction of innate immune signaling or inflammatory cell death (Keller and Bell, 2016). An alternative OV engineering strategy has been to inactivate the molecular mechanisms used by certain viruses to suppress or evade immune detection. Examples include the disruption or removal of HSV ICP47 or adenovirus E3/19K gene sequences whose normal function is to suppress MHC class 1 expression, or of the VSV M, measles V, and HSV γ34.5 proteins, which suppress the release of and/or response to type I IFNs by infected cells (Maroun et al., 2017).

Summary

To summarize, OVs offer a highly versatile array of novel therapeutic reagents with which to mediate *in situ* killing of tumor cells, at the same time creating the local pro-inflammatory conditions required to boost the host antitumor immune response. A major advantage of the approach is that it requires no *a priori* knowledge of the identity of the tumor neoantigens unique to a given patient/tumor and is therefore considered "antigen agnostic," as opposed to the use of personalized nucleic acid vaccines whose efficacy depends upon the accurate prediction of tumor neoepitopes in each cancer patient. The potential of the approach as a means to enhance the antitumor potency of immune checkpoint blockade is currently being evaluated in several ongoing clinical trials, but the optimal virus designs, doses, schedules, and routes of administration for this application have yet to be determined. Finally, it is worth noting that ongoing preclinical studies are seeking to develop a newer generation of OV therapies that can be used as standalone anticancer agents capable of inducing complete disease remission after a single administration.

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DECLARATION OF INTERESTS

Drs Russell and Barber, Mayo Clinic and University of Miami, have a financial interest in Vyriad, an oncolytic virotherapy company. Drs Russell and Barber serve on the Board of Vyriad. Dr Russell is an officer of Vyriad.

REFERENCES

Alexander, W. (2016). The checkpoint immunotherapy revolution: what started as a trickle has become a flood, despite some daunting adverse effects; new drugs, indications, and combinations continue to emerge. P T *41*, 185–191.

Alexandrov, L.B., Nik-Zainal, S., Wedge, D.C., Aparicio, S.A., Behjati, S., Biankin, A.V., Bignell, G.R., Bolli, N., Borg, A., Borresen-Dale, A.L., et al. (2013). Signatures of mutational processes in human cancer. Nature *500*, 415–421.

Alvarez-Breckenridge, C.A., Choi, B.D., Suryadevara, C.M., and Chiocca, E.A. (2015). Potentiating oncolytic viral therapy through an understanding of the initial immune responses to oncolytic viral infection. Curr. Opin. Virol. *13*, 25–32.

Andtbacka, R.H., Kaufman, H.L., Collichio, F., Amatruda, T., Senzer, N., Chesney, J., Delman, K.A., Spitler, L.E., Puzanov, I., Agarwala, S.S., et al. (2015a). Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J. Clin. Oncol. *33*, 2780–2788.

Andtbacka, R.H., Ross, M., Puzanov, I., Milhem, M., Collichio, F., Delman, K.A., Amatruda, T., Zager, J.S., Cranmer, L., Hsueh, E., et al. (2016). Patterns of clinical response with talimogene laherparepvec (T-VEC) in patients with melanoma treated in the OPTiM phase III clinical trial. Ann. Surg. Oncol. *23*, 4169–4177.

Andtbacka, R.H.I., Curti, B.D., Kaufman, H., Daniels, G.A., Nemunaitis, J.J., Spitler, L.E., Hallmeyer, S., Lutzky, J., Schultz, S.M., Whitman, E.D., et al. (2015b). Final data from CALM: a phase II study of Coxsackievirus A21 (CVA21) oncolytic virus immunotherapy in patients with advanced melanoma. J. Clin. Oncol. *33*, https://doi.org/10.1200/JCO.2014.58.3377.

Barber, G.N. (2005). VSV-tumor selective replication and protein translation. Oncogene *24*, 7710–7719.

Barber, G.N. (2011). Innate immune DNA sensing pathways: STING, AIMII and the regulation of interferon production and inflammatory responses. Curr. Opin. Immunol. *23*, 10–20.

Barber, G.N. (2015). STING: infection, inflammation and cancer. Nat. Rev. Immunol. *15*, 760–770.

Bartee, E., and Li, Z. (2017). In vivo and in situ programming of tumor immunity by combining oncolytics and PD-1 immune checkpoint blockade. Exp. Hematol. Oncol. *6*, 15.

Bennett, E.M., Bennink, J.R., Yewdell, J.W., and Brodsky, F.M. (1999). Cutting edge: adenovirus E19 has two mechanisms for affecting class I MHC expression. J. Immunol. *162*, 5049–5052.

Brown, G.C., and Neher, J.J. (2012). Eaten alive! Cell death by primary phagocytosis: 'phagoptosis'. Trends Biochem. Sci. *37*, 325–332.

Castello, A., Alvarez, E., and Carrasco, L. (2011). The multifaceted poliovirus 2A protease: regulation of gene expression by picornavirus proteases. J. Biomed. Biotechnol. *2011*, 369648.

Chen, Q., Sun, L., and Chen, Z.J. (2016). Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. Nat. Immunol. *17*, 1142–1149.

Chesney, J., Puzanov, I., Collichio, F., Singh, P., Milhem, M.M., Glaspy, J., Hamid, O., Ross, M., Friedlander, P., Garbe, C., et al. (2017). Randomized, open-label phase II study evaluating the efficacy and safety of talimogene laherparepvec in combination with ipilimumab versus ipilimumab alone in patients with advanced, unresectable melanoma. J. Clin. Oncol. https://doi.org/10.1200/JCO.2017.73.7379.

Chiocca, E.A., and Rabkin, S.D. (2014). Oncolytic viruses and their application to cancer immunotherapy. Cancer Immunol. Res. *2*, 295–300.

Clemens, M.J. (2005). Translational control in virus-infected cells: models for cellular stress responses. Semin. Cell Dev. Biol. *16*, 13–20.

Corrales, L., Matson, V., Flood, B., Spranger, S., and Gajewski, T.F. (2017). Innate immune signaling and regulation in cancer immunotherapy. Cell Res. *27*, 96–108.

Dehne, N., Mora, J., Namgaladze, D., Weigert, A., and Brune, B. (2017). Cancer cell and macrophage cross-talk in the tumor microenvironment. Curr. Opin. Pharmacol. *35*, 12–19.

Deng, L., Liang, H., Xu, M., Yang, X., Burnette, B., Arina, A., Li, X.D., Mauceri, H., Beckett, M., Darga, T., et al. (2014). STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity *41*, 843–852.

Drappier, M., and Michiels, T. (2015). Inhibition of the OAS/RNase L pathway by viruses. Curr. Opin. Virol. *15*, 19–26.

Durham, N.M., Mulgrew, K., McGlinchey, K., Monks, N.R., Ji, H., Herbst, R., Suzich, J., Hammond, S.A., and Kelly, E.J. (2017). Oncolytic VSV primes differential responses to immuno-oncology therapy. Mol. Ther. *25*, 1917–1932.

Elsedawy, N.B., and Russell, S.J. (2013). Oncolytic vaccines. Expert Rev. Vaccines *12*, 1155–1172.

Engeland, C.E., Grossardt, C., Veinalde, R., Bossow, S., Lutz, D., Kaufmann, J.K., Shevchenko, I., Umansky, V., Nettelbeck, D.M., Weichert, W., et al. (2014). CTLA-4 and PD-L1 checkpoint blockade enhances oncolytic measles virus therapy. Mol. Ther. *22*, 1949–1959.

Farrington, L.A., Smith, T.A., Grey, F., Hill, A.B., and Snyder, C.M. (2013). Competition for antigen at the level of the APC is a major determinant of immunodominance during memory inflation in murine cytomegalovirus infection. J. Immunol. *190*, 3410–3416.

Fernandes, J. (2016). Oncogenes: the passport for viral oncolysis through PKR inhibition. Biomark. Cancer *8*, 101–110.

Finlay, B.B., and McFadden, G. (2006). Anti-immunology: evasion of the host immune system by bacterial and viral pathogens. Cell *124*, 767–782.

Franz, K.M., and Kagan, J.C. (2017). Innate immune receptors as competitive determinants of cell fate. Mol. Cell *66*, 750–760.

Galanis, E., Atherton, P.J., Maurer, M.J., Knutson, K.L., Dowdy, S.C., Cliby, W.A., Haluska, P., Jr., Long, H.J., Oberg, A., Aderca, I., et al. (2015). Oncolytic measles virus expressing the sodium iodide symporter to treat drug-resistant ovarian cancer. Cancer Res. *75*, 22–30.

Gale, M., Jr., and Katze, M.G. (1998). Molecular mechanisms of interferon resistance mediated by viral-directed inhibition of PKR, the interferon-induced protein kinase. Pharmacol. Ther. *78*, 29–46.

Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L., and Kroemer, G. (2017). Immunogenic cell death in cancer and infectious disease. Nat. Rev. Immunol. *17*, 97–111.

Galluzzi, L., Vitale, I., Aaronson, S.A., Abrams, J.M., Adam, D., Agostinis, P., Alnemri, E.S., Altucci, L., Amelio, I., Andrews, D.W., et al. (2018). Molecular mechanisms of cell death: recommendations of the nomenclature committee on cell death 2018. Cell Death Differ. *25*, 486–541.

Gao, Y., Whitaker-Dowling, P., Griffin, J.A., Barmada, M.A., and Bergman, I. (2009). Recombinant vesicular stomatitis virus targeted to Her2/neu combined with anti-CTLA4 antibody eliminates implanted mammary tumors. Cancer Gene Ther. *16*, 44–52.

Gordon, S.R., Maute, R.L., Dulken, B.W., Hutter, G., George, B.M., McCracken, M.N., Gupta, R., Tsai, J.M., Sinha, R., Corey, D., et al. (2017). PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature *545*, 495–499.

Guo, Z.S., Liu, Z., Kowalsky, S., Feist, M., Kalinski, P., Lu, B., Storkus, W.J., and Bartlett, D.L. (2017). Oncolytic immunotherapy: conceptual evolution, current strategies, and future perspectives. Front. Immunol. *8*, 555.

Gustin, K.E. (2003). Inhibition of nucleo-cytoplasmic trafficking by RNA viruses: targeting the nuclear pore complex. Virus Res. *95*, 35–44.

Harding, S.M., Benci, J.L., Irianto, J., Discher, D.E., Minn, A.J., and Greenberg, R.A. (2017). Mitotic progression following DNA damage enables pattern recognition within micronuclei. Nature *548*, 466–470.

Hardy, W.R., and Sandri-Goldin, R.M. (1994). Herpes simplex virus inhibits host cell splicing, and regulatory protein ICP27 is required for this effect. J. Virol. *68*, 7790–7799.

Hewitt, E.W. (2003). The MHC class I antigen presentation pathway: strategies for viral immune evasion. Immunology *110*, 163–169.

Hill, A., Jugovic, P., York, I., Russ, G., Bennink, J., Yewdell, J., Ploegh, H., and Johnson, D. (1995). Herpes simplex virus turns off the TAP to evade host immunity. Nature *375*, 411–415.

Hou, W., Sampath, P., Rojas, J.J., and Thorne, S.H. (2016). Oncolytic virus-mediated targeting of PGE2 in the tumor alters the immune status and sensitizes established and resistant tumors to immunotherapy. Cancer Cell *30*, 108–119.

Ichikawa, K., Kagamu, H., Koyama, K., Miyabayashi, T., Koshio, J., Miura, S., Watanabe, S., Yoshizawa, H., and Narita, I. (2012). Epitope diversification driven by non-tumor epitope-specific Th1 and Th17 mediates potent antitumor reactivity. Vaccine *30*, 6190–6197.

Kaminskyy, V., and Zhivotovsky, B. (2010). To kill or be killed: how viruses interact with the cell death machinery. J. Intern. Med. *267*, 473–482.

Kedl, R.M., Kappler, J.W., and Marrack, P. (2003). Epitope dominance, competition and T cell affinity maturation. Curr. Opin. Immunol. *15*, 120–127.

Kedl, R.M., Rees, W.A., Hildeman, D.A., Schaefer, B., Mitchell, T., Kappler, J., and Marrack, P. (2000). T cells compete for access to antigen-bearing antigen-presenting cells. J. Exp. Med. *192*, 1105–1113.

Keller, B.A., and Bell, J.C. (2016). Oncolytic viruses-immunotherapeutics on the rise. J. Mol. Med. (Berl.) *94*, 979–991.

Kenney, L.L., Cornberg, M., Chen, A.T., Emonet, S., de la Torre, J.C., and Selin, L.K. (2015). Increased immune response variability during simultaneous viral coinfection leads to unpredictability in CD8 T cell immunity and pathogenesis. J. Virol. *89*, 10786–10801.

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. (2006). Systemic armed oncolytic and immunologic therapy for cancer with JX-594, a targeted poxvirus expressing GM-CSF. Mol. Ther. *14*, 361–370.

Knutson, K.L., and Disis, M.L. (2005). Augmenting T helper cell immunity in cancer. Curr. Drug Targets Immune Endocr. Metabol. Disord. *5*, 365–371.

Krysko, O., Aaes, T.L., Kagan, V.E., D'Herde, K., Bachert, C., Leybaert, L., Vandenabeele, P., and Krysko, D.V. (2017). Necroptotic cell death in anti-cancer therapy. Immunol. Rev. *280*, 207–219.

Lichty, B.D., Breitbach, C.J., Stojdl, D.F., and Bell, J.C. (2014). Going viral with cancer immunotherapy. Nat. Rev. Cancer *14*, 559–567.

Liu, B.L., Robinson, M., Han, Z.Q., Branston, R.H., English, C., Reay, P., McGrath, Y., Thomas, S.K., Thornton, M., Bullock, P., et al. (2003). ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Ther. *10*, 292–303.

Liu, Y.P., Wang, J., Avanzato, V.A., Bakkum-Gamez, J.N., Russell, S.J., Bell, J.C., and Peng, K.W. (2014). Oncolytic vaccinia virotherapy for endometrial cancer. Gynecol. Oncol. *132*, 722–729.

Mackenzie, K.J., Carroll, P., Martin, C.A., Murina, O., Fluteau, A., Simpson, D.J., Olova, N., Sutcliffe, H., Rainger, J.K., Leitch, A., et al. (2017). cGAS surveillance of micronuclei links genome instability to innate immunity. Nature *548*, 461–465.

Maroun, J., Munoz-Alia, M., Ammayappan, A., Schulze, A., Peng, K.-W., and Russell, S. (2017). Designing and building oncolytic viruses. Future Virol. *12*, 193–213.

Mohme, M., Riethdorf, S., and Pantel, K. (2017). Circulating and disseminated tumour cells - mechanisms of immune surveillance and escape. Nat. Rev. Clin. Oncol. *14*, 155–167.

Mohr, I. (2004). Neutralizing innate host defenses to control viral translation in HSV-1 infected cells. Int. Rev. Immunol. *23*, 199–220.

Moresco, E.M., LaVine, D., and Beutler, B. (2011). Toll-like receptors. Curr. Biol. *21*, R488–R493.

Nagata, S., and Tanaka, M. (2017). Programmed cell death and the immune system. Nat. Rev. Immunol. *17*, 333–340.

Naik, S., Nace, R., Federspiel, M.J., Barber, G.N., Peng, K.W., and Russell, S.J. (2012). Curative one-shot systemic virotherapy in murine myeloma. Leukemia *26*, 1870–1878.

Obuchi, M., Fernandez, M., and Barber, G.N. (2003). Development of recombinant vesicular stomatitis viruses that exploit defects in host defense to augment specific oncolytic activity. J. Virol. *77*, 8843–8856.

Puzanov, I., Milhem, M.M., Minor, D., Hamid, O., Li, A., Chen, L., Chastain, M., Gorski, K.S., Anderson, A., Chou, J., et al. (2016). Talimogene laherparepvec in combination with ipilimumab in previously untreated, unresectable stage IIIB-IV melanoma. J. Clin. Oncol. *34*, 2619–2626.

Ribas, A., Dummer, R., Puzanov, I., VanderWalde, A., Andtbacka, R.H.I., Michielin, O., Olszanski, A.J., Malvehy, J., Cebon, J., Fernandez, E., et al. (2017). Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. Cell *170*, 1109–1119 e1110.

Rivas, H.G., Schmaling, S.K., and Gaglia, M.M. (2016). Shutoff of host gene expression in influenza a virus and herpesviruses: similar mechanisms and common themes. Viruses *8*, 102.

Ruotsalainen, J.J., Kaikkonen, M.U., Niittykoski, M., Martikainen, M.W., Lemay, C.G., Cox, J., De Silva, N.S., Kus, A., Falls, T.J., et al. (2015). Clonal variation in interferon response determines the outcome of oncolytic virotherapy in mouse CT26 colon carcinoma model. Gene Ther. *22*, 65–75.

Russell, S.J., Federspiel, M.J., Peng, K.W., Tong, C., Dingli, D., Morice, W.G., Lowe, V., O'Connor, M.K., Kyle, R.A., Leung, N., et al. (2014). Remission of disseminated cancer after systemic oncolytic virotherapy. Mayo Clin. Proc. *89*, 926–933.

Russell, S.J., and Peng, K.W. (2017). Oncolytic virotherapy: a contest between apples and oranges. Mol. Ther. *25*, 1107–1116.

Russell, S.J., Peng, K.W., and Bell, J.C. (2012). Oncolytic virotherapy. Nat. Biotechnol. *30*, 658–670.

Salama, A.K., and Moschos, S.J. (2017). Next steps in immuno-oncology: enhancing antitumor effects through appropriate patient selection and rationally designed combination strategies. Ann. Oncol. *28*, 57–74.

Samson, A., Scott, K.J., Taggart, D., West, E.J., Wilson, E., Nuovo, G.J., Thomson, S., Corns, R., Mathew, R.K., Fuller, M.J., et al. (2018). Intravenous delivery of oncolytic reovirus to brain tumor patients immunologically primes for subsequent checkpoint blockade. Sci. Transl. Med. *10*, https://doi.org/10.1126/scitranslmed.aam7577.

Schiavoni, G., Mattei, F., and Gabriele, L. (2013). Type I interferons as stimulators of DC-mediated cross-priming: impact on anti-tumor response. Front. Immunol. *4*, 483.

Schock, S.N., Chandra, N.V., Sun, Y., Irie, T., Kitagawa, Y., Gotoh, B., Coscoy, L., and Winoto, A. (2017). Induction of necroptotic cell death by viral activation of the RIG-I or STING pathway. Cell Death Differ. *24*, 615–625.

Schumacher, T.N., and Schreiber, R.D. (2015). Neoantigens in cancer immunotherapy. Science *348*, 69–74.

Shen, W., Patnaik, M.M., Ruiz, A., Russell, S.J., and Peng, K.W. (2016). Immunovirotherapy with vesicular stomatitis virus and PD-L1 blockade enhances therapeutic outcome in murine acute myeloid leukemia. Blood *127*, 1449–1458.

Takeuchi, O., and Akira, S. (2009). Innate immunity to virus infection. Immunol. Rev. *227*, 75–86.

Toda, S., Nishi, C., Yanagihashi, Y., Segawa, K., and Nagata, S. (2015). Clearance of apoptotic cells and pyrenocytes. Curr. Top. Dev. Biol. *114*, 267–295.

Topalian, S.L., Drake, C.G., and Pardoll, D.M. (2015). Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell *27*, 450–461.

Vyas, J.M., Van der Veen, A.G., and Ploegh, H.L. (2008). The known unknowns of antigen processing and presentation. Nat. Rev. Immunol. *8*, 607–618.

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. (2015). Viral infection of tumors overcomes resistance to PD-1-immunotherapy by broadening neoantigenome-directed T-cell responses. Mol. Ther. *23*, 1630–1640.

Woo, S.R., Corrales, L., and Gajewski, T.F. (2015). Innate immune recognition of cancer. Annu. Rev. Immunol. *33*, 445–474.

Xia, T., Konno, H., and Barber, G.N. (2016). Recurrent loss of STING signaling in melanoma correlates with susceptibility to viral oncolysis. Cancer Res. *76*, 6747–6759.