Oncolytic viruses: cytolytic agents, replicating immunotherapeutics or both?

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Cancer cells have distinct hallmarks that promote growth and metastasis, but at the same time enhance their susceptibility to viral infection [1]. As a result, oncolytic viruses (OVs) possess an intrinsic ability or can be engineered to selectively target, replicate in and kill cancer cells, while leaving healthy cells unharmed. Oncolysis allows for amplification of the initial viral load due to viral replication and subsequent spread of infectious particles to neighboring cells upon cell lysis. In addition to this direct cytotoxic effect, OVs can be armed with therapeutic transgenes to take advantage of antigen release and presentation, as well as to potentiate immune responses and infiltration within the tumor microenvironment (TME) [2,3].

Selection of the right viral backbone

A significant challenge in OV design involves maximizing OV-mediated cancer cell death by increasing viral potency, while sparing nonmalignant cells. Though OVs are naturally selective to some extent toward cancer cells, additional viral attenuation is often necessary to engineer OVs that are both safe and effective. Common methods of attenuation involve the deletion of viral genes that are essential for infection of healthy cells [4] or genes that antagonize the host antiviral immune response [5]. Selecting an OV backbone with maximal oncolytic potency is not necessarily the optimal or only way of improving tumor clearance. Mounting evidence suggests that OV-mediated oncolysis, although an important first step, must be complemented by stimulation of a host antitumor immune response in order to maximize therapeutic benefit. As one example, targeted T-cell disruption before intratumor injection of oncolytic adenovirus (AdV) eliminated OV-mediated anti-tumor activity, even though OV replication and persistence was significantly increased [6].

Following viral mediated cell lysis, assorted tumor antigens, cytokines and chemokines are released into the extracellular space [2,3]. Certain OVs, such as AdV and coxsackievirus B3, initiate the release of danger-associated molecular patterns often resulting in immunogenic cell death (ICD) and a potent antitumor immune response [3]. These inherently immunogenic viral backbones are often associated with small genome size and a limited number of proteins to antagonize host antiviral immune responses. Larger DNA based viruses that are more genetically complex, such as Herpes simplex virus type-1 (HSV-1) and vaccinia virus (VV), have evolved to encode proteins involved in host immune evasion in order to facilitate the spread of infection [7,8]. However, these viral backbones can be engineered to initiate an inflammatory response simultaneously with the release of danger-associated molecular patterns initiating ICD programs [3,8]. Despite a robust viral infection, prevention of a therapeutic antitumor immune response may still occur due to the immunosuppressive nature of the TME [9]. Thus, selecting and/or engineering a therapeutic viral backbone that can initiate an immune response, while important, is likely not enough to break the immunological tolerance found in the TME in the majority of patients.

Arming viruses with immune modulators

Along with danger signals, cytokine release from infected cells is an important characteristic of OV-mediated ICD. This can lead to a localized inflammatory response and subsequent recruitment of lymphoid cells to the site of infection. As one might predict, the constellation of cytokines released during tumor infection varies depending on the OV platform being used. For instance, vesicular stomatitis virus and VV infections are known to promote the release and transcriptional activation of proinflammatory chemokines, CXCL1 and CXCL5, as well as cytokines like GM-CSF, promoting neutrophil infiltration [2]. Oncolytic reovirus infections locally increase the production of proinflammatory cytokines such as IL-6 and GM-CSF, allowing for increased lymphoid cell recruitment and MHC Class I molecule expression [3]. To augment the natural induction of cytokines, several groups have adopted the approach of encoding particular cytokines within OV backbones. Attractive candidates for this strategy include IL-12 [10] and TNF- α [11] which have promising therapeutic potential but unacceptable toxicities when administered systemically. In principle, OV-driven expression of this class of cytokine ensures localized, high dose treatment at the site of infection without systemic exposure. Many cytokines and co-stimulatory molecules have been encoded as transgenes into OVs, including CCL3 and CCL5 in AdV, IL-2 in HSV-1 and Newcastle Disease Virus, IL-12 in AdV and vesicular stomatitis virus, among others [2,3]. T-Vec, commercially known as Imlygic, is the first US FDA and EMEA approved OV built upon an HSV backbone that has been engineered to encode GM-CSF to stimulate the production/recruitment of granulocytes and monocytes within the TME and stimulate T-cell dependent immune responses against patient tumor antigens [12]. More recently, to stimulate and link innate and adaptive antitumor immune responses, assorted virally encoded immune modulators have been investigated. For instance OX40/OX40L, associated with increasing the effector to regulatory T-cell ratios, and the production and signaling of cytokines by T cells, antigen presenting cells, natural killer (NK) cells, and NK T-cells [13,14]; the Glucocorticoid-induced TNF receptor (GITR)/GITRL, with regulatory T-cell inhibitory effects and activating effects on CD8⁺ effector T-cells [15]; the inducible co-stimulator (ICOS)/ICOSL, which plays a key role in stimulating the proliferation, differentiation and function of various effector cells [16]; and CD137/CD137L, which inhibits apoptosis, and enhances the proliferation and effector functions of T- and NK cells [17].

A complementary strategy to initiating a natural T-cell mediated response against tumor antigens is to encode within the virus, Bi-specific T-cell Engagers or BiTEs [18]. These molecules consist of linked variable chain antibody fragments directed against the T-cell antigen CD3 and a specific tumor-associated antigen. BiTEs secreted from an infected cell will spread throughout the TME and lead to the formation of artificial immunological synapses between malignant cells and any T cell in the vicinity [18]. The net result of this strategy is to kill both infected and uninfected tumor cells with the goal of overall improved therapeutic outcomes.

When a robust T-cell response is initiated against a tumor, immunological resistance can emerge by cancer cell expression of immune checkpoints that lead to paralysis of invading tumor-specific T-cells [19]. To overcome this problem so-called 'immune checkpoint inhibitor' or ICI antibodies have been developed. For example, the ICI antibody directed against the CTLA-4, is effective in some melanoma patients, but often associated with severe immune-related toxicity and has generally lower efficacy in nonmelanoma tumors [19]. A second class of ICI antibodies targeting programmed PD-1 and PD-L1 have demonstrated clinical activity across a wider range of cancer types and in general have reduced toxicity when compared with anti-CTLA-4 [19]. Although these are both exciting therapeutics in their own right, they remain effective only in a minority of cancer patients and have off-target toxicities associated with systemic delivery. There is now considerable effort aimed at combining ICIs with OVs either as two stand-alone therapeutics or as a virus expressing an ICI transgene. In both preclinical and clinical settings combination of ICI antibodies with OVs have proven effective in a range of malignancies including refractory triple-negative breast cancer [20], melanoma [21] and gliomas [22]. Combining ICIs with OVs as a single therapeutic has been accomplished with a number of OV platforms including (but not limited to) VV [23], Influenza A virus [24], and Myxoma virus [25]. Often, these virally-encoded checkpoint inhibitors were equally or more effective than either therapy alone or in combination, and improved safety profiles [23–25].

The co-incident development of OVs and anticancer immune modulators has led to the convergence of the two classes of therapeutics. OVs seem destined to be not only cytolytic agents and immune adjuvants, but perhaps equally important, replicating cancer gene therapy vectors that selectively and locally express anticancer immune modulators.

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References

1. Pikor LA, Bell JC, Diallo JS. Oncolytic viruses: exploiting cancer's deal with the devil. *Trends Cancer* 1(4), 266–277 (2015).
2. Lichty BD, Breitbach CJ, Stojdl DF, Bell JC. Going viral with cancer immunotherapy. *Nat. Rev. Cancer* 14(8), 559–567 (2014).
3. Berkey SE, Thorne SH, Bartlett DL. Oncolytic virotherapy and the tumor microenvironment. *Adv. Exp. Med. Biol.* 1036, 157–172 (2017).
4. Deng L, Fan J, Ding Y *et al.* Oncolytic efficacy of thymidine kinase-deleted vaccinia virus strain Guang9. *Oncotarget* 8(25), 40533–40543 (2017).
5. Quan B, Seo HS, Blobel G, Ren Y. Vesiculoviral matrix (M) protein occupies nucleic acid binding site at nucleoporin pair (Rae1 * Nup98). *Proc. Natl Acad. Sci. USA* 111(25), 9127–9132 (2014).
6. Li X, Wang P, Li H *et al.* The efficacy of oncolytic adenovirus is mediated by T-cell responses against virus and tumor in Syrian Hamster Model. *Clin. Cancer Res.* 23(1), 239–249 (2017).
7. Su C, Zhan G, Zheng C. Evasion of host antiviral innate immunity by HSV-1, an update. *Virol. J.* 13, 38 (2016).
8. Heinrich B, Klein J, Delic M *et al.* Immunogenicity of oncolytic vaccinia viruses JX-GFP and TG6002 in a human melanoma *in vitro* model: studying immunogenic cell death, dendritic cell maturation and interaction with cytotoxic T lymphocytes. *Oncotargets Ther.* 10, 2389–2401 (2017).
9. Kouidhi S, Ben Ayed F, Benammar Elgaaid A. Targeting tumor metabolism: a new challenge to improve immunotherapy. *Front. Immunol.* 9, 353 (2018).
10. Wang P, Li X, Wang J *et al.* Re-designing Interleukin-12 to enhance its safety and potential as an anti-tumor immunotherapeutic agent. *Nat. Commun.* 8(1), 1395 (2017).
11. Martinez-Reza I, Diaz L, Garcia-Becerra R. Preclinical and clinical aspects of TNF-alpha and its receptors TNFR1 and TNFR2 in breast cancer. *J. Biomed. Sci.* 24(1), 90 (2017).
12. Bommareddy PK, Patel A, Hossain S, Kaufman HL. Talimogene Laherparepvec (T-VEC) and other oncolytic viruses for the treatment of melanoma. *Am. J. Clin. Dermatol.* 18(1), 1–15 (2017).
13. Jiang H, Rivera-Molina Y, Gomez-Manzano C *et al.* Oncolytic adenovirus and tumor-targeting immune modulatory therapy improve autologous cancer vaccination. *Cancer Res.* 77(14), 3894–3907 (2017).
14. Sagiv-Barfi I, Czerwinski DK, Levy S *et al.* Eradication of spontaneous malignancy by local immunotherapy. *Sci. Transl. Med.* 10(426), pii:eaan4488 (2018).
15. Knee DA, Hewes B, Brogdon JL. Rationale for anti-GITR cancer immunotherapy. *Eur. J. Cancer* 67, 1–10 (2016).
16. Zamarin D, Holmgard RB, Ricca J *et al.* Intratumoral modulation of the inducible co-stimulator ICOS by recombinant oncolytic virus promotes systemic anti-tumour immunity. *Nat. Commun.* 8, 14340 (2017).
17. Chen S, Lee LF, Fisher TS *et al.* Combination of 4–1BB agonist and PD-1 antagonist promotes antitumor effector/memory CD8 T cells in a poorly immunogenic tumor model. *Cancer Immunol. Res.* 3(2), 149–160 (2015).
18. Scott EM, Duffy MR, Freedman JD, Fisher KD, Seymour LW. Solid tumor immunotherapy with T Cell engager-armed oncolytic viruses. *Macromol. Biosci.* 18(1), doi:10.1002/mabi.201700187 (2018).
19. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* 27(4), 450–461 (2015).
20. Bourgeois-Daigneault MC, Roy DG, Aitken AS *et al.* Neoadjuvant oncolytic virotherapy before surgery sensitizes triple-negative breast cancer to immune checkpoint therapy. *Sci. Transl. Med.* 10(422), pii:eaao1641 (2018).
21. Ribas A, Dummer R, Puzanov I *et al.* Oncolytic virotherapy promotes intratumoral T Cell infiltration and improves anti-PD-1 immunotherapy. *Cell* 170(6), 1109e1110–1119e1110 (2017).
22. Samson A, Scott KJ, Taggart D *et al.* Intravenous delivery of oncolytic reovirus to brain tumor patients immunologically primes for subsequent checkpoint blockade. *Sci. Transl. Med.* 10(422), pii:eaam7577 (2018).
23. Kleinpeter P, Fend L, Thioudellet C *et al.* Vectorization in an oncolytic vaccinia virus of an antibody, a Fab and a scFv against programmed cell death -1 (PD-1) allows their intratumoral delivery and an improved tumor-growth inhibition. *Oncimmunology* 5(10), e1220467 (2016).

24. Hamilton JR, Vijayakumar G, Palese P. A recombinant antibody-expressing influenza virus delays tumor growth in a mouse model. *Cell Rep.* 22(1), 1–7 (2018).
25. Bartee MY, Dunlap KM, Bartee E. Tumor-localized secretion of soluble PD1 enhances oncolytic virotherapy. *Cancer Res.* 77(11), 2952–2963 (2017).