Oncolytic vaccinia virus: a silver bullet?

Expert Rev. Vaccines 9(12), 1353–1356 (2010)

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"The highly immunogenic nature of vaccinia infection ... was crucial in the eradication of smallpox. These observations have led to the exploration of various vaccinia virus strains in the immunotherapy of cancer and infectious diseases and in the exploration of oncolytic vaccinia virus for cancer therapy."

Currently, the most successful curative intent treatment for many cancers is surgery, yet many tumors remain inoperable. Radiotherapy can be effective and is often used in combination with surgery; for example, it is an effective treatment option for prostate cancer. However, metastatic disease is amenable to neither of these treatments and has the poorest outlook [1]. Chemotherapy is not often a curative treatment but can prolong a patient's life, sometimes with an acceptable impact on quality of life. Targeted therapies, such as monoclonal antibodies, are now being used to target tumors specifically, for example, to reduce growth factor-dependent growth stimulation or to inhibit tumor vascularization. Despite their good specificity, the toxicity profile of monclonal antibodies has proven to be as problematic as chemotherapy. Harnessing the power of the immune response is the most recently applied weapon in the fight against cancer and it has great potential. Many cancer immunotherapeutic vaccines are now in clinical trials [2] and are beginning to show encouraging results. A landmark in cancer immunotherapy, Dendreon's prostate cancer vaccine, sipuleucel-T (Provenge; Seattle, WA, USA) was the first antigen-specific therapeutic cancer vaccine to receive approval, in 2010, from the US FDA [101].

Early in the 20th Century, various infectious agents were tested for the treatment of cancer. The best known are Coley's toxins, derived from a mixture of bacterial agents, which achieved some remarkable results in the treatment of sarcoma [3]. At approximately the same time, crude preparations of viruses were also being tested for the treatment of cancer. Some successes were reported and, similarly to Coley's toxins, these were associated with fever, inflammation and innate immune responses. In the 1950s, Southam and colleagues attempted to treat cancers of various origins with better characterized viral strains, including vaccinia, mumps, West Nile, Ilheus and Bunyamwera viruses [4]. The history of oncolytic viruses is nicely described in a review by Kelly and Russell [5]. Purified viruses, in particular Newcastle Disease virus [6] and vaccinia virus (VV) [7], have been tested for their ability to 'antigenize' a tumor by injecting patients with viral oncolysates of tumor cells infected with a virus. Some clinical responses as a result of this treatment have been reported. Various viral constructs have also been used to target the expression of genes in tumors following various modes of administration. These include VV and other pox viruses (myxoma, racoonpox), herpes simplex virus, vesicular stomatitis virus (a rhabdovirus), Newcastle disease virus, adenovirus and α-virus. Many reports describe antitumor activity by the oncolytic mechanism of action of these viruses, exploiting the inherent capacity of oncolytic viruses to

EXPERT REVIEWS

KEYWORDS: cancer gene therapy • cancer immunotherapy • oncolytic virus • tumor-targeted suicide gene expression

 10.1586/ERV.10.137 ISSN 1476-0584

infect, replicate and propagate within and subsequently lyse cancer cells. In this article, we will focus on selective aspects of oncolytic VV as a novel modality in cancer treatment.

The highly immunogenic nature of vaccinia infection leading to a strong cytotoxic T-cell response and circulating neutralizing antibodies was crucial in the eradication of smallpox. These observations have led to the exploration of various VV strains in the immunotherapy of cancer and infectious diseases and in the exploration of oncolytic VV for cancer therapy [8]. The properties of oncolytic poxviruses as a novel class of cancer therapy have been recently reviewed [9]. Vaccinia is easy to produce and manipulate, can hold large DNA inserts and replication takes place exclusively in the cytoplasm, thereby eliminating any risk of integration [10]. An important development in the use of VV was the demonstration that the thymidine kinase (*TK*) gene could be exploited as a site of DNA insertion and subsequent selection of recombinant virus [11]. It was shown soon thereafter that TK-VV was attenuated such that it was much less likely to infect healthy organs and nervous tissue [12], an important observation, since nervous tissue infection was a toxicity issue during the smallpox campaign [13]. Interruption of the *TK* gene also restricts the virus's ability to propagate to areas with abundant free nucleotides, such as in tissue culture medium or in tumors. It was observed in the early 1990s that mice injected intravenously with TK-recombinant Copenhagen strain VV (Cop-VV) did not suffer grossly observable effects of viremia and cleared the virus easily. It was also noticed that if tumor-bearing mice were injected with TK- Cop-VV, the virus was quickly eliminated from healthy tissue; however, the virus not only remained in the tumor tissue, but replicated there. In these early experiments and in these tumor models, no deleterious effects of the TK-Cop-VV alone on tumor growth were noted. Nevertheless, if the virus was used as a vehicle to express cytokine genes in the tumor, growth of the tumor was significantly reduced and the tumor was often eliminated. The most effective cytokine in athymic mice bearing a human tumor was IL-6 [14] and in euthymic mice was IL-2 [14] and GM-CSF [15]. The cytokines IL-4, IL-5 and IL-7 were also tested but had little observed effect [15].

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Two recombinant oncolytic VV constructs are in clinical development. Intratumoral and intravenous administration of a targeted and armed oncolytic VV, JX594, is currently being explored in Phase I and Phase II trials by Jennerex Biotherapeutics, Inc. (San Francisco, CA, USA) and clinical benefit has been reported [8,9]. This construct is based on a TK-deleted vector derived from the Wyeth strain and expresses GM-CSF and β-galactosidase (β-Gal) [8]. The company Genelux Corp. (San Diego, CA, USA) is testing intravenous administration of the recombinant oncolytic VV construct GL-ONC1 in solid tumors. GL-ONC1 carries the gene sequences for green fluorescence protein, β-Gal and β-glucuronidase [102]. Interestingly, local expression of β-Gal in human lung tumors has been shown to generate strong anti-β-Gal immune responses associated with clinical benefit [16]. The role of the host immune response in augmenting the mechanism of activity of oncolytic viruses has recently been described [17].

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Recombinant VV have also been used to express suicide genes that convert a prodrug into a toxic drug within the tumor following intravenous administration. Suicide gene therapy results in the intracellular conversion of nontoxic prodrugs into potent chemotherapeutic drugs directly within the cancer cell. Bacterial and/or yeast cytosine deaminase (CDase) is a well-characterized enzyme–prodrug system that converts the relatively nontoxic anti-fungal agent 5-fluorocytosine (5-FC) to its highly toxic derivate 5-fluorouracil (5-FU). 5-FU, which is widely used in chemotherapy, is capable of diffusion into and out of cells resulting in significant bystander effect of CDase/ 5-FC [18]. Various mechanisms of action for 5-FU have been reported, including the inhibition of thymidylate synthase by 5-fluoro-2′-deoxyuridine-5′-monophosphate, incorporation of 5-fluorouridine-5′-triphosphate into RNA and incorporation of 5-fluoro-2′-deoxyuridine-5′-triphosphate into DNA. These reported mechanisms lead to the inhibition of DNA and RNA synthesis and interference with DNA repair [18,19]. Beyond its direct cytotoxic effect on tumor cells, 5-FU possesses immunogenic properties. This chemotherapeutic agent is a potent inducer of several Th1-type cytokines, such as IFN-γ, TNF-α, TNF-β and IL-12, and of effector cells carrying anticancer cytotoxicity mediated by natural killer cells and T cells. In addition, these abilities are closely associated with the *in vivo* anticancer effect of this agent [20]. A recent study demonstrated that 5-FU was able to reduce the number of myeloid-derived suppressor cells (MDSCs) [21]. MDSCs contribute to the immune tolerance of cancer, notably by inhibiting the function of $CD8^+$ T cells. Thus, their elimination may hamper tumor growth by enhancing anti-tumor T-cell functions. In this study, the authors observed that the elimination of MDSCs by 5-FU increased IFN-γ production by tumor-specific $CD8^+$ T cells infiltrating the tumor and promoted T-cell-dependent anti-tumor responses *in vivo* [21].

The antitumor effect of the CDase/5-FC combination on colon carcinoma has been demonstrated both *in vitro* and *in vivo*, and clinical trials have been reported showing safety of the CDase/5-FC combination [18]. Directed expression of the CDase enzyme in tumors has been accomplished using a TK-VV carrying the bacterial [22] or yeast [23] CDase genes. There have been several attempts to increase the efficiency of CDase/5-FC therapy. Construction of a bifunctional fusion gene CDase/uracil phosphoribosyltransferase (designated *FCU1*) was reported to shortcut rate-limiting enzymatic steps of the 5-FC/5-FU conversion, thus resulting in a greatly enhanced sensitivity of the cells to 5-FC compared with CDase alone when expressed in tumors by

gene delivery [24]. This prodrug expression system has now been expressed in a TK-VV and used to deliver and induce expression of FCU1 in human tumors growing in immunodeficient mice [25].

Since the early observation that cytokine genes can be delivered selectively to tumors by TK-recombinant VV, many developments have occurred. There are now clinical trials ongoing, testing the expression of a cytokine and/or antigenic bacterial proteins. The remaining toxicity of TK-VV is also being reduced by the deletion of other viral genes [26,27]. In addition, other genes that can be inserted into VV and delivered to tumors, notably prodrug-converting enzymes, are also in development. Clinical testing of these various constructs is planned and further refinements will probably lead to a viable treatment for cancer, in particular metastatic disease, in the not too distant future.

The term 'magic bullet' was originally coined by Paul Erlich in the late 19th Century. He used the term in reference to a treatment that is so exquisitely specific for a disease organism, or a cancer, that healthy tissues are left unharmed. His quest led to the discovery of two effective drugs for the treatment of syphilis and he was awarded the Nobel Prize in 1908 for his work in immunology. The terms 'magic bullet' and 'silver bullet' are now used interchangeably. The idea of a magic/silver bullet that can be administered to cancer patients, and is active only on the cells of the tumor, has been elusive so far; however, oncolytic viruses may well prove to be just what the doctor ordered.

Acknowledgements

The authors thank Marthe Bermond for her EndNote expertise and patience.

Financial & competing interests disclosure

The authors are all employees of Transgene SA and are beneficiaries of the Transgene employee stock option plan The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Ramalingam S, Belani C. Systemic chemotherapy for advanced non-small cell lung cancer: recent advances and future directions. *Oncologist* 13(Suppl. 1), 5–13 (2008).

2 Simmons O, Magee M, Nemunaitis J. Current vaccine updates for lung cancer. *Expert Rev. Vaccines* 9(3), 323–335 (2010).

3 Coley W. Late results of the treatment of inoperable sarcoma by the mixed toxins of Erysipelas and Bacillus prodigiosus. *Am. J. Med. Sci.* 131, 375–428 (1906).

4 Newman W, Southam CM. Virus treatment in advanced cancer. A pathological study of fifty seven cases. *Cancer* 7(1), 106–118 (1954).

5 Kelly E, Russell SJ. History of oncolytic viruses: genesis to genetic engineering. *Mol. Ther.* 15(4), 651–659 (2007).

6 Cassel WA, Murray DR. A ten-year follow-up on stage II malignant melanoma patients treated postsurgically with Newcastle disease virus oncolysate. *Med. Oncol. Tumor Pharmacother.* 9(4), 169–171 (1992).

7 Scoggin SD, Sivanandham M, Sperry RG, Wallack MK. Active specific adjuvant immunotherapy with vaccinia melanoma oncolysate. *Ann. Plast. Surg.* 28(1), 108–109 (1992).

8 Park BH, Hwang T, Liu TC *et al.* Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a Phase I trial. *Lancet Oncol.* 9(6), 533–542 (2008).

9 Kirn DH, Thorne SH. Targeted and armed oncolytic poxviruses: a novel multi-mechanistic therapeutic class for cancer. *Nat. Rev. Cancer* 9(1), 64–71 (2009).

10 Smith GL, Moss B. Infectious poxvirus vectors have capacity for at least 25,000 base pairs of foreign DNA. *Gene* 25(1), 21–28 (1983).

11 Mackett M, Smith GL, Moss B. General method for production and selection of infectious vaccinia virus recombinants expressing foreign genes. *J. Virol.* 49(3), 857–864 (1984).

12 Buller R, Smith GL, Cremer K, Notkins AL, Moss B. Decreased virulence of recombinant vaccinia virus expression vectors is associated with a thymidine kinase-negative phenotype. *Nature* 317, 813–815 (1985).

13 Kaplan C. Vaccinia virus: a suitable vehicle for recombinant vaccines? *Arch. Virol.* 106(1–2), 127–139 (1989).

14 Acres B, Dott K, Stefani L, Kieny MP. Directed cytokine expression in tumor cells *in vivo* using recombinant vaccinia virus. *Ther. Immunol.* 1(1), 17–23 (1994).

15 Acres B, Stefani L, Dott K, Kieny MP. Delivery of cytokine genes to tumor cells *in vivo* using recombinant vaccinia virus vectors – cytokine cDNA gene expression in vaccinia virus vector; tumor-bearing mouse injection for potential cancer cytokine-mediated gene therapy (conference abstract). *Cancer Gene Ther.* 1(4), 308 (1994).

16 Gahéry-Ségard H, Molinier-Frenkel V, Le Boulaire C *et al.* Phase I trial of recombinant adenovirus gene transfer in lung cancer. Longitudinal study of the immune responses to transgene and viral products. *J. Clin. Invest.* 100(9), 2218–2226 (1997).

17 Worschech A, Haddad D, Stroncek DF, Wang E, Marincola FM, Szalay AA. The immunologic aspects of poxvirus oncolytic therapy. *Cancer Immunol. Immunother.* 58(9), 1355–1362 (2009).

18 Grem JL. 5-Fluorouracil: forty-plus and still ticking. A review of its preclinical and clinical development. *Invest. New Drugs* 18(4), 299–313 (2000).

19 Noordhuis P, Holwerda U, van der Wilt CL *et al.* 5-Fluorouracil incorporation into RNA and DNA in relation to thymidylate synthase inhibition of human colorectal cancers. *Ann. Oncol.* 15(7), 1025–1032 (2004).

20 Okamoto M, Kasetani H, Kaji R *et al. cis*-Diamminedichloroplatinum and 5-fluorouracil are potent inducers of the cytokines and natural killer cell activity *in vivo* and *in vitro*. *Cancer Immunol. Immunother.* 47(4), 233–241 (1998).

21 Vincent J, Mignot G, Chalmin F *et al.* 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res.* 70(8), 3052–3061 (2010).

22 Gnant MF, Puhlmann M, Alexander HR Jr, Bartlett DL. Systemic administration of a recombinant vaccinia virus expressing the cytosine deaminase gene and subsequent treatment with 5-fluorocytosine leads to tumor-specific gene expression and prolongation of survival in mice. *Cancer Res.* 59(14), 3396–3403 (1999).

23 Chalikonda S, Kivlen MH, O'Malley ME *et al.* Oncolytic virotherapy for ovarian carcinomatosis using a replication-selective vaccinia virus armed with a yeast cytosine deaminase gene. *Cancer Gene Ther.* 15(2), 115–125 (2008).

24 Erbs P, Regulier E, Kintz J *et al. In vivo* cancer gene therapy by adenovirus-mediated transfer of a bifunctional yeast cytosine deaminase/uracil phosphoribosyltransferase fusion gene. *Cancer Res.* 60(14), 3813–3822 (2000).

25 Foloppe J, Kintz J, Futin N *et al.* Targeted delivery of a suicide gene to human colorectal tumors by a conditionally replicating vaccinia virus. *Gene Ther.* 15(20), 1361–1371 (2008).

26 McCart JA, Ward JM, Lee J *et al.* Systemic cancer therapy with a tumor-selective vaccinia virus mutant lacking thymidine kinase and vaccinia growth factor genes. *Cancer Res.* 61(24), 8751–8757 (2001).

27 Guo ZS, Naik A, O'Malley ME *et al.* The enhanced tumor selectivity of an oncolytic vaccinia lacking the host range and antiapoptosis genes *SPI-1* and *SPI-2*. *Cancer Res.* 65(21), 9991–9998 (2005).

Websites

101 Provenge (sipuleucel-T) www.provenge.com

102 Genelux C. Safety Study of GL-ONC1, an Oncolytic Virus, in Patients With Advanced Solid Tumors. ClinicalTrial.gov, April (2010). NCT00794131 http://clinicaltrials.gov/ct2/show/NCT00794131