Keywords: cancer gene therapy • cancer immunotherapy • oncolytic virus • tumor-targeted suicide gene expression
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].

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].

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 produgs 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 anti-tumor cytotoxicity mediated by natural killer cells and T cells. In addition, these abilities are closely associated with the in vivo antitumor 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 FCUI) 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 TKVV and used to deliver and induce expression of FCU1 in human tumors growing in immunodecient 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 TKVV 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 refections 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.

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Lusky, Erbs, Foloppe & Acres

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