Vaccinia virus has been studied extensively since its discovery as a smallpox vaccine in 1798. Its use as a smallpox vaccine documented its safety profile. It was later found that its large size and ability to accept large fragments of DNA combined with its natural tumor affinity make it an attractive agent for cancer therapy.

This chapter discusses the history of the vaccinia, the various strains available, the biology of the virus as well as the steps in creating recombinants. The various clinical and safety considerations will be addressed. We will also discuss the various methods used to treat cancer using the vaccinia virus and will review the recent clinical trials using vaccinia in the treatment of cancer.

**Key Words:** Vaccinia; pox viruses; oncolytic virus; extracellular enveloped virus; intracellular mature virus.

1. **INTRODUCTION**

The concept of tumor directed viral therapy has been extensively studied over the past few decades. Creating a tumor-specific cytotoxic virus that has minimal toxicity to the host has long been considered the “holy grail” amongst researchers and clinicians alike. There are several viruses that are currently being studied as possible vectors for tumor directed therapy including adenovirus, herpes simplex, reovirus, Newcastle disease virus, and the vaccinia virus. The use of vaccinia as a cancer treatment modality is a focus of many ongoing laboratory and clinical research projects. Several avenues are
currently being explored including: (1) vaccinia as vector for gene delivery; (2) vaccinia as a tumor vaccine; and (3) vaccinia as an oncolytic agent.

2. CHARACTERISTICS

Vaccinia virus is a member of the pox family of viruses. It comprises a family of complex DNA viruses that replicate in the cytoplasm of both vertebrate and invertebrate cells. They can be classified into two subfamilies based on host species: chordopoxvirinae (vertebrate poxviruses) and entomopoxvirinae (insect poxviruses) (1–3). Its clinical use dates back more than 150 yr to 1798, when Edward Jenner demonstrated that vaccination with the cowpox virus offered protection from smallpox. Vaccinia was used for widespread vaccination against smallpox until 1978 when smallpox was declared eradicated.

Vaccinia virus has many attributes which make it an attractive vector for tumor directed therapy including: (1) a quick lifecycle with the ability to form mature virions within 6 h of infection; (2) the ability to spread from cell to cell efficiently; (3) a large genome which allows it to accept large fragments of DNA without deletion; (4) the ability to achieve high levels of transgene expression; and (5) most importantly, it can infect a large range of human tissues without causing any known human disease (4). Another very interesting quality of vaccinia virus is its affinity to replicate selectively in tumor tissue making it potentially valuable for tumor targeting strategies. This tumor affinity combined with the properties mentioned above make the vaccinia virus the focus of ongoing anticancer research worldwide.

3. BIOLOGY OF VACCINIA VIRUS

The vaccinia virus exists in two infectious forms. The intracellular mature virus (IMV) is released upon cellular disruption during the viral purification process, which is the form found in vitro. It has a single outer membrane derived from the trans-golgi network membrane. The extracellular enveloped virus (EEV) is responsible for cell–cell spread in vivo. It incorporates an outer membrane derived from the cell membrane. The EEV is not able to be purified in vitro as a result of the fragility of its outer envelope which does not withstand the purification process (5).

The mechanism of attachment and uptake of the vaccinia virus remains unclear because of its various infectious forms and variety of cellular receptors and viral proteins (1,9,10). No definite cellular receptor for vaccina virus has been identified. Confocal microscopy has shown that IMV and EEV enter cells by different mechanisms (11). Both attach to cells via different proteins allowing the virus to enter by membrane fusion. The IMV envelope proteins A17L, A27L, and D8L may be instrumental in viral attachment. The D8L protein was one of the earliest IMV membrane proteins identified and is thought to mediate IMV binding to cell surface chondroitin sulfate (12,13). The A27L protein may mediate vaccinia attachment through cell surface heparin sulfate; this was shown by demonstrating a 60% viral inhibition in the presence of soluble heparin sulfate (7). The EEV is the infectious form responsible for cell–cell transmission in vivo. To date, 6 EEV specific membrane proteins (A33R, A34R, A36R, A56R, B5R, and F13L) have been identified (1). It has been found that the A56R protein can be mutated without affecting infectivity (14).

Vaccinia have been engineered to circumvent the normal cell receptor requirements by binding to alternate cell-surface molecules. Expression of an ScFv to erbB2 on the
surface of the EEV (created as a fusion with A56R) was shown, by enzyme linked immunosorbent assay (ELISA), to bind erbB2 (14). Fusions of other surface proteins including B5R have been reported (15).

Like all poxviruses, vaccinia spends its entire life cycle within the cytoplasm of the host cell and does not integrate into the host genome (see Fig. 1). Because of its ability to rely on its own encoded proteins for life activities, the virus can rapidly and efficiently replicate without restrictions from the host cell defense. Following entry into the cell, vaccinia releases enzymes required for the initiation of early transcription. The virus then undergoes a process of early DNA uncoating and initiates early transcription. Early, intermediate, and late transcription utilize each its own specific promoters and transcription factors. Within 4 to 6 h of infection there is almost complete inhibition of host protein synthesis allowing for efficient expression of viral genes and replication (8).

In the initial early phase a DNA dependent RNA polymerase is released from the virus into the cytoplasm, which induces the transcription of early mRNA. Translation of the mRNA forms early proteins, which are involved in the uncoating and replication of the viral DNA. These early proteins also induce the transactivation and transcription of intermediate messenger RNA (mRNA). Intermediate mRNA encodes for late transactivators leading to late mRNA synthesis. The proteins synthesized in the late phase of viral replication constitute membrane structural proteins and early transcription factors and enzymes that will be incorporated into new viral particles. The relatively small number of proteins required for DNA synthesis make the system very simple and self-sufficient (1,2,16). Ten thousand copies of the viral genome are created within 12 h of infection, half of which are incorporated into mature virions and released (8).

Vaccinia virus DNA replication occurs in the areas of cytoplasm enclosed by the endoplasmic reticulum (ER). These areas have been termed mini-nuclei (17). The replication takes place in the form of multiple concatemers of the DNA. These concatemers are then resolved into individual genomes, which are then encapsulated along with the early transcription factors by Golgi-derived membranes.

The next stage in the formation of infectious particles is the development of viral crescents. These crescents are composed of a single lipid bilayer, which has no contact to the cellular membranes and viral protein, however, the source of the lipid bilayers remains, so far, enigmatic (18). The crescents then coalesce into a noninfective form of immature virus. Only after condensation of core proteins and the addition of two additional membranes do these virus precursors become IMVs. The necessary membranes are derived from modified trans-Golgi network membranes. Further modifications entail the inclusion of virus-encoded proteins into these membranes, which then become part of the outer envelope of the EEV. Once the viruses are fully wrapped they move to the cell surface where the outer membrane fuses with the plasma membrane, exposing the mature virus on the cell surface.

3.1. Strains of Vaccinia Virus

The widespread use of vaccinia virus in the eradication of smallpox led to the development of multiple strains with various characteristics, pathogenicity, and host range. The New York City Board of Health strain was obtained from England in 1856 and was originally used for smallpox vaccination in the United States (3). The Western Reserve (WR) strain is a laboratory derivative of this strain and is one of the more virulent strains in laboratory animals and nonhuman primates. Another derivative, the Wyeth strain is used clinically for smallpox vaccination. The modified vaccinia Ankara (MVA) strain was
created through multiple rounds of infection in avian cells. This strain is highly attenuated and does not replicate in human or other mammalian cells (19). Numerous other attenuated strains of vaccinia have been produced through deletional mutations.

4. CONSTRUCTION OF RECOMBINANT VIRAL VECTORS

The creation of recombinant vaccinia vectors is relatively simple because of the homologous recombination, which occurs naturally during its viral replication and allows for efficient insertion of foreign DNA. The issues to be considered when creating recombinant vaccinia vector include choosing a site for the desired recombination, choosing a method to select the recombinant vaccinia vector and choosing a promoter for the transgene (foreign gene).

Four approaches have been developed to create new recombinants. The traditional and widely used method utilizes the homologous recombination taking place inside cells. Homologous recombination leads to the insertion of foreign genes into 0.1% of progeny viral genomes (20). Permissive cells such as CV-1 cells are transfected with the parental virus and a transfer plasmid containing an expression cassette with a viral promoter and the gene of interest. The gene of interest is flanked with a few hundred pairs of viral DNA derived from the insertion locus of the parental virus. The new recombinant

Fig. 1. Vaccinia virus replication cycle. A diagram of the infected cell is shown. The major stages of the virus lifecycle are listed. Following late gene expression, previrion forms assemble to form the IMV. The IMV is targeted to the trans-Golgi Network (TGN) and following envelopment, the IEV is formed. IEV’s are propelled to the cell surface by the polymerization of actin filaments. Once released, the virus may remain attached to the membrane as a cell associated enveloped virus (CEV) or be released into the medium as an extracellular enveloped virus (EEV). Reprinted with permission from ref. 2.
arises when homologous recombination takes place between the viral sequences in the transfer plasmid and the parental virus. Another approach involves in vitro ligation of a foreign gene into the vaccinia virus genomic DNA (21). The third approach employs the viral genome as part of a bacterial artificial chromosome (BAC). The rVV containing BACs allow the generation of mutant or recombinant viral genomes in bacteria, without the need for recombination or plaque purification in mammalian cells (22). Finally, a newly described innovative method utilizes the high-frequency recombination and reactivations catalyzed by the Shope fibroma virus (SFV) which is coupled with SFV promoted reactivations to rapidly construct rVV in high yields (23).

The homologous recombination method is the most widely used and begins with the creation of a transfer plasmid. The transfer plasmid contains the foreign gene expressed from a vaccinia promoter and flanked by vaccinia DNA sequences. Care should be taken to ensure that the foreign gene of interest should not contain the vaccinia transcription termination signals for early promoters (TTTTTNT) (24). The most common site of recombination has been the vaccinia thymidine kinase (TK) gene. Insertion of genes into the TK locus eliminates functional viral TK and leads to attenuation of the virus in normal tissues in vivo (24). Recombination into other loci has also been performed, including intergenic DNA segments, such that no functional deletion occurs (25,26).

A wide range of vaccinia promoters are available for expression of transgenes. It is necessary to use vaccinia promoters for the creation of recombinant vectors, as these are specific for vaccinia polymerase. Eukaryotic promoters are not functional in vaccinia infection, as the host cell polymerase is not present in the cytoplasm where vaccinia transcription occurs. Several natural and synthetic early and late promoters have been described with various levels of activity (27–29). The native vaccinia promoters are generally very strong and compare favorably to other viral promoters used in other viral vectors. The synthetic early/late promoter described by Chakrabarti et al. (29) has led to consistent, reliable high levels of gene expression in numerous systems tested.

Once the shuttle plasmid has been constructed, it can be cotransfected with vaccinia into permissive cells. Recombinant vaccinia virus (rVV) can then be selected based on the selection marker inserted into the viral genome. Growth in the presence of the thymidine analog BrdU can be used to select the TK negative phenotype of the permissive cells after recombination into the TK locus (30). Others have commonly used the selection gene xanthine-guanine phosphoribosyltransferase (XGPRT) which allows for selective growth in media containing mycophenolic acid (31). Positive selection through replacement of an essential gene previously deleted from a backbone virus grown in permissive cell lines is another commercially available selection tool (32).

### 5. HOST RESPONSE TO VACCINIA

Vaccinia virus has mechanisms to avoid detection and clearance by the immune system. The vaccinia has evolved expression of immunosuppressive proteins (38). Viral surface proteins act as complement inhibitors and the extracellular envelope is known to be almost completely resistant to antibody neutralization (39).

Understanding vaccinia’s immune evasion strategies may help optimize the virus as a vector for clinical use. The virus is effective in suppressing both innate immunity and the development of T-helper cells. Vaccinia virus has adopted genes whose product can block the function of the interferon family members interferon- \( \alpha/\beta/\gamma \) or that can inhibit chemokines, which are some of the earliest substances produced during the initiation of a viral host immune response (42–47) (Table 1).
Cellular immunity to the vaccinia virus is an important element in the clearance of vaccinia virus and animal models suggest that it may be more potent than antibody mediated viral clearance. In T-cell deficient tumor bearing nude mice, vaccinia is able to replicate and express genes within tumor cells for greater than 30 d, whereas in immunocompetent hosts the window of gene expression lasts only 8 d with high levels lasting only 4 d (40,41).

Vaccinia also encodes for the interleukin (IL)-18 binding protein, which is a naturally produced soluble factor that blocks the binding of IL-18 to its cognate receptor. IL-18 binding protein has been shown to be one of the most potent inhibitors of the development of a T-helper cell type 1 (Th1) biased immune response (48–51). Vaccinia virus also encodes for several other immunosuppressive factors such as IL-1B and tumor necrosis factor (TNF) receptor blockers. These factors are involved in blocking complement activation (46). These findings suggest that blocking the early Th1 response may be important in the efficacy of vaccinia-mediated therapy.

Balancing the body’s immune response is paramount in utilizing vaccinia virus in the treatment of cancer. The vigorous immune response to Vaccinia is desirable as a vaccine but is also detrimental because it results in the rapid clearance of the virus before adequate replication can occur.

Because the vaccinia virus is not endemic to humans, patients who have not had prior smallpox vaccination will not have preformed circulating antibodies. However, most cancer patients were born before 1970 and have had smallpox vaccination and, with the recent fear of bioterrorism, younger patients may also undergo smallpox vaccination. The prior smallpox vaccination intensifies the clearance of systemically administered vaccinia, limiting the amount of virus available for replication in tumor tissue.

Other studies have confirmed the critical role of Th1 response to clearance of vaccinia viral infection. Van den Broek et al. examined the effect of Th1 (IFN-γ, IL-12) and Th2 (IL-4, IL-10) cytokine balance in the clearance of vaccinia virus in mice using cytokine knockouts (52). Vaccinia viral replication was enhanced in IL-12 and IFN-γ knockout mice with IL-12 knockout mice demonstrating greater susceptibility to infection. IL-12 knockout mice had complete abrogation of anti-vaccinia cytotoxic T-lymphocytes whereas IFN-γ knockout mice had normal T-cell function. In contrast, IL-4 and IL-10 deficient mice showed marked enhancement of vaccinia viral clearance suggesting that

| Gene Products which Inhibit the Immune Response |
|-----------------------------------------------|
| **Vaccinia open reading frame** | **Function** |
| B13R(SPI-2) | Inhibits IL-1β converting enzyme |
| E3L | Inhibits PKR activation by dsRNA |
| K3L | Inhibits phosphorylation of eIF2α by PKR |
| A53R | Soluble TNF receptor |
| B8R | Soluble IFN-γ receptor |
| B18R | Soluble IFN-α/β receptor |
| B29R | Soluble chemokine binding protein |
| C3L | Inhibits Complement (C4B, C3B) |
| B5R | Inhibits Complement |
| B16R | Soluble IL-1β receptor |
| A44L | Steroid synthesis |
these cytokines naturally suppress the host response to vaccinia. IL-10 knockout mice thereby exhibited greater inhibition of viral replication than IL-4 deficient mice. When the effects of each cytokine was examined in the infection with recombinant vaccinia virus constructs, local expression of IL-4 showed a much greater inhibition of host responses. In fact, whereas the absence of IL-10 resulted in improved clearance of IL-6 and IL-1, the local expression of IL-10 had little to no effect on viral clearance. Similarly, Deonarain et al. have shown that IFN-α/β knockout mice demonstrate markedly enhanced susceptibility to vaccinia viral infection (53). Other studies have shown that IL-12 and IL-18 act synergistically to clear vaccinia infection and that virus clearance involves NK and T-cells (54).

6. STRATEGIES IN VACCINIA GENE THERAPY TO EVADE IMMUNE CLEARANCE

The clearance of the virus in vivo needs to be overcome in order to deliver an adequate amount of virus to the tumor and allow time for viral replication. Several strategies have been tested to overcome this barrier. The first strategy is to create a virus that is less recognizable by the immune system. The problem with this method is that the vaccinia virus presents a broad spectrum of antigens to the host. Hence, one or two mutations in the viral envelope would probably not be sufficient to avoid detection by the immune system. The other issue with altering the viral envelope is that the alteration may result in decrease infectivity of the virus.

The second strategy involves developing other pox viruses that are able to selectively infect and lyse human tumor cells without crossreacting with vaccinia. Examples include yatapox virus, Yaba like disease virus, and Avian poxvirus. The problem with these alternative viruses is that they do not replicate in human cells and are less efficient vectors (55–57).

The third strategy involves using immunosuppressive agents to increase the viral load and the time of expression in tumor cells. Unpublished studies from our group have found that immunosuppressive therapy increased viral recovery and tumor response in animal models without increasing the pathogenicity. Because of the knowledge gained from transplantation, specific agents are now available that allow for targeting of the immune system selectively on various effector pathways. The use of immune modulation to overcome preformed antibodies may be useful in the future for treating patients with prior smallpox vaccination.

7. CLINICAL SAFETY

There is extensive data regarding the overall safety of the vaccinia virus, which was generated during its use in the eradication of smallpox. The complications associated with vaccinia virus include encephalitis, vaccinia necrosum, and eczema vaccinatum. These complications are more prevalent in immunocompromised individuals and infants (see Fig. 2) (34–36).

Vaccinia associated encephalitis results from infection of the central nervous system (CNS). Studies have shown viral recovery from the CNS of patients suffering from vaccinia associated encephalitis (35). This dreaded complication can be avoided by use of a tumor selective vaccinia virus.

Vaccinia necrosum is a progressive necrotic ulcer caused by the vaccinia virus. It is more common in immunosuppressed patients and can destroy significant amounts of
tissue producing significant morbidity. The extensive tissue loss may require reconstruction with tissue grafts and can sometimes require amputation. Surprisingly, this dramatic local infection does not cause a systemic viral spread.

Eczema vaccinatum originates from the infection of eczematous skin throughout the body by vaccinia. It causes a large viral load that induces viremia with fever and malaise and can sometimes progress to death. Although rare, the side effects of vaccinia virus have been the focus of multiple laboratory experiments and animal models suggest a role of inflammatory cytokines in the pathogenicity of viral infection.

8. VACCINIA AS A CANCER VACCINE

The experience with vaccinia in the eradication of smallpox led to research into its use as an antitumor vaccine. Vaccinia was engineered to express tumor antigens and serve as a cancer vaccine. The size of the potential transgene that can be put into the vaccinia vector allows for flexibility in engineering, such that immune enhancing genes and antigenetic genes can be recombined into the genome. The immunostimulatory effects and efficient transcriptional machinery of the virus were utilized to create various cancer vaccine vectors (Table 2).

Recently, a phase I clinical trial of vaccinia expressing prostate specific antigen (PSA) in prostate cancer patients was published. In this trial the Wyeth strain virus was delivered intradermally every 4 wk for three doses without producing significant systemic toxicities. A cutaneous reaction, consistent with viral replication was seen in all patients treated with the virus at a dose of $2.65 \times 10^7$ pfu. Several patients developed T-cell immune responses to PSA associated with prolonged periods before disease progression (55). Another phase II clinical trial by the NCI examines the potential of three strains of recombinant vaccinia virus expressing either PSA, B7.1, or of the fowlpox virus expressing PSA. Vaccinia expressing the tumor antigen carcino embryonic antigen (CEA) has been studied clinically as a priming vaccine followed by a boost with avipox expressing CEA. This regimen consisted of $1 \times 10^7$ pfu Wyeth strain vaccinia.
injected intradermally. Although specific T-cell immune responses were generated and the regimen was well tolerated, there were no positive clinical responses noted (56). Rochlitz et al. published their phase I trial of a modified vaccinia (MVA strain) expressing human MUC1 for antigen specific immunotherapy in patients with advanced MUC1 positive cancers (60). They found that patients tolerated repeated doses of the virus with minimal side effects and 1 of the 13 patients with advanced cancer showed a marked decrease in the size of his metastasis that lasted 14 mo. Greiner et al. developed a vaccinia expressing a triad of costimulatory molecules including B7.1, intercellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 combined with CEA to produce a vaccine against CEA expressing cancers. This virus, known as rV-CEA TRICOM or a recombinant vaccinia vector that carries a triad of costimulatory molecules, has been encouraging in preclinical studies and is now the focus of clinical trials (57).

### 9. VACCINIA AS A VECTOR FOR TUMOR DIRECTED GENE DELIVERY

The properties that make vaccinia attractive as vector for gene delivery were described earlier and the use of vaccinia as a vector for gene delivery is now being investigated in clinical trials. Mastrangelo et al. have reported their phase I clinical trial using vaccinia expressing granulocyte-macrophage colony-stimulating factor (GM-CSF). Patients underwent intratumoral injections of up to $2 \times 10^7$ pfu per lesion and $8 \times 10^7$ pfu per session twice weekly over 6 wk. Systemic toxicities were limited to mild flu-like symptoms and local inflammation at the injection site with doses greater than $10^7$ pfu/lesion. All patients were vaccinated against the vaccinia within weeks prior to receiving the vaccinia-GM-CSF. Interesting positive responses were reported in five of the seven patients treated. Three patients had mixed responses with complete regression of treated and untreated dermal metastases, one patient had partial response with regression of injected and uninjected regional dermal metastasis and one patient had complete remission of multiple dermal metastasis (58,59).

Vaccinia has also been engineered to express suicide and tumor suppressor genes. The suicide gene therapy involves the combination of a nonmammalian enzyme such as cytosine deaminase and the nontoxic prodrug 5-fluorocytosine, which is catalyzed to 5-FU by the cytosine deaminase. Vaccinia expressing the suicide genes cytosine deaminase and 5-fluorocytosine has shown promising results in both in vitro and mouse

### Table 2: Recent Clinical Trials

| First author | Vector         | Results                                      |
|--------------|----------------|----------------------------------------------|
| Mastrangelo (58) | Vaccinia-GM-CSF | Regression of injected lesions.               |
| Marshall (56)   | Vaccinia-CEA   | No clinical response.                        |
| Mukherjee (68)  | Vaccinia-IL-2  | No clinical response.                        |
| Eder (55)       | Vaccinia-PSA   | Stabilization of PSA levels.                  |
| Sanda (69)      | Vaccinia-PSA   | Stabilization of PSA levels.                  |
| Conry (70)      | Vaccinia-CEA   | No clinical response.                        |
| Tsang (71)      | Vaccinia-CEA   | No clinical response.                        |
| Adams (67)      | Vaccinia-HPV   | Response in cervical cancer.                  |
| Rochlitz (60)   | MVA-Muc1       | Response in metastatic disease.               |
| Greiner (57)    | rV-CEA TRICOM  | Safe.                                        |
models (64). Another approach in which vaccinia vectors were used to transfer the tumor suppressor gene \( p53 \) into gliomas and bladder tumors that expressed mutated \( p53 \) induced apoptotic cell death and showed some antitumor efficacy (65,66).

10. VACCINIA AS AN ONCOLYTIC VIRUS FOR CANCER THERAPY

The concept of a tumor selective oncolytic virus that can be safely administered is very appealing and is the focus of current research by multiple laboratories. The advantages of the vaccinia virus as an oncolytic virus have been described earlier in the chapter. The most important advantage is the efficiency of viral replication, cell to cell spread and ability to destroy tissue.

Development of an oncolytic virus has focused on genetic alterations of the WR strain of virus to achieve a tumor selective replicating virus (4). It has been previously demonstrated that an intradermal injection of \( 10^6 \) pfu of the wild-type WR strain of vaccinia into nonhuman primates leads to a necrotic ulcer of \( 108 \) cm\(^2\) in only 8 d without systemic spread of the virus (unpublished data). This ability to quickly spread and its ability to produce high levels of transgene expression is extremely promising, as one of the limiting factors in antitumor gene therapy is the limitation of vector distribution throughout fibrinous tumors. Animal models studying the distribution of a systemically delivered tumor-selective mutant vaccinia virus have shown the highest levels of virus in the tumor whereas little to no virus has been detected in other organs. The most promising mutant has been a virus deleted of the TK and vaccinia growth factor (VGF) genes (see Fig. 3).

LTK is important for vaccinia nucleotide and DNA synthesis and it is almost essential in normal cells where the host nucleotide pool is low. VGF is a protein that is expressed early by vaccinia virus and is secreted by infected cells. It binds growth factor receptors on surrounding resting cells and stimulates them to proliferate. This increases the available nucleotides and primes them for vaccinia infection. By deleting both the VGF and TK genes the replication of vaccinia in normal cells can be completely abrogated without decreasing the ability of the vaccinia to replicate in tumor cells. This double deleted virus has been tested and found to preferentially replicate in tumor cells and ovarian tissue with little or replication in nontumor tissue (4). Experiments using nude mice injected systemically with \( 1 \times 10^9 \) pfu of the double deleted vaccinia showed a marked response in established tumors with no pathogenicity (61). Primate studies showed no pathogenicity of \( 10^9 \) pfu of vaccinia delivered intravenously (unpublished data).

Puhlman et al. demonstrated that systemic administration of a TK-deleted vaccinia virus expressing the suicide gene purine nucleoside phosphorylase in combination with 6-methylpurine deoxyribose treatment led to a complete response in 50% of mice with hepatic metastases (62,63). We are currently exploiting another strategy by deleting the antiapoptotic genes \( spi-1 \) and \( spi-2 \) to improve tumor selectivity and oncolysis. Ultimately, the combination of genetic deletions and expression of antitumor genes may prove to be more successful to inhibit tumor growth than the strains available at present.

11. SUMMARY

Vaccinia virus is a member of the pox family of viruses. Viral recombinants are made with relative ease and their natural tumor affinity make them attractive vectors for tumor directed therapy. It has a proven safety profile from its use as a smallpox vaccine and its
immunogenic and oncolytic properties combined with its effectiveness to spread through tissues make it an attractive vector for future development of tumor directed therapies. Ongoing clinical trials focusing on these properties are beginning to show its potential in the treatment of cancer.

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Fig. 3. Differential viral recovery. Vaccinia titers recovered from brain and tumor 4 d after injection of virus intraperitoneally in MC38 subcutaneous tumor bearing mice. Data represents median of 5 values. No recoverable titers are seen in brain tissue form the double deleted virus (VVDDEGFP) whereas the tumor has equivalent titers to wild type.
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