

## Viral Vectors for Cancer Immunotherapy

Richard Harrop<sup>1</sup> and Miles W. Carroll<sup>2</sup>

<sup>1</sup> Oxford BioMedica (U.K.) Ltd., Oxford Science Park, Oxford, OX4 4GA, United Kingdom, <sup>2</sup> MNLpharma Ltd., University of Reading Science and Technology Centre, Whiteknights, Reading, Berks, RG6 6AH, United Kingdom

### TABLE OF CONTENTS

1. Abstract	
2. Introduction	
2.1. History and successes of cancer immunotherapy	
2.2. Viruses and cancer therapy	
3. Identification of tumor associated antigens	
4. Ideal properties of an antigen delivery system	
4.1. Why are viral vectors suitable for delivering TAAs?	
5. Poxvirus vectors	
5.1. Vaccinia virus	
5.1.1. Use of vaccinia viral vectors to deliver tumor antigens	
5.1.1.1. Carcinoembryonic antigen (CEA)	
5.1.1.2. Prostate specific antigen (PSA)	
5.1.1.3. MUC-1	
5.1.1.4. 5T4	
5.2. Avian Poxviruses	
5.2.1. Fowlpox	
5.2.2. Canarypox	
6. Adenovirus	
7. Alphaviruses (Semliki forest, Sindbis, Venezuelan)	
7.1. Semliki Forest Virus (SFV)	
7.2. Sindbis virus (SIN)	
7.3. Venezuelan equine encephalitis virus (VEE)	
8. Miscellaneous viral vectors	
8.1. Herpes simplex virus (HSV)	
8.2. Newcastle Disease Virus (NDV)	
9. Heterologous prime boost approaches	
10. Perspective: The future of viral vectors for cancer immunotherapy and their potential limitations	
11. Acknowledgments	
12. References	

## 1. ABSTRACT

Over the last decade, immunotherapy approaches for the treatment of cancer have been investigated with renewed vigour, perhaps catalyzed by the clinical successes seen with monoclonal antibody and cytokine based therapies. The identification of tumor-associated antigens (TAAs) in multiple cancer types has enabled the development of targeted immunotherapies and allayed some of the safety concerns associated with the induction of deleterious autoimmune reactions. In addition to the TAA or therapeutic gene, the antigen delivery system is equally as important for the development of a successful cancer vaccine. One approach to induce a potent and targeted antitumor response is to use viruses to deliver the TAA to cells of the immune system. A diverse array of oncolytic viruses and recombinant viral vectors encoding numerous therapeutic genes or TAAs have been tested in pre-clinical studies and produced results which, in some cases, justify their clinical development as potential cancer immunotherapies. Within the last 5-10 years, many such recombinant vectors have made the transition from pre-clinical research to clinical development and it is these, which are given most weight in this review.

## 2. INTRODUCTION

### 2.1. History and successes of cancer immunotherapy

The concept that the immune system can be manipulated to reject existing tumors or prevent tumors from establishing is not new. Indeed, more than a century has passed since Coley attempted to treat cancer patients with bacterial extracts that were thought to act by boosting the immune response (1). Subsequently, Ehrlich suggested that the immune response could recognize malignancies and successfully vaccinated animals with tumor antigens (2). By the 1960s, it had been demonstrated conclusively that the immune system could recognize and reject tumors (3-5).

Cancer immunotherapies can broadly be divided into two categories, those which are tumor specific (e.g. a vaccine or antibody that targets a specific tumor antigen) and those which modulate the immune system but in a non tumor-specific way. An example of the latter is BCG, which has been used for many years in the treatment of bladder cancer and has been shown to have superior benefit than chemotherapy regimens in patients with a high risk of progression (6). While the precise mode of action of BCG

is not known, that it acts via modulation of the immune system is not doubted. Likewise, the cytokines IL-2 and IFN alpha have found widespread use in the treatment of different malignancies (e.g. renal cancer and melanoma).

It is only relatively recently that targeted therapies such as monoclonal antibodies have been used in the clinical setting. Since showing initial unfulfilled promise in the 1980s, monoclonal antibodies have had a renaissance in the treatment of different cancers and currently 8 therapeutic antibodies are approved by the FDA for sale in the United States (7). This passive form of immunotherapy may promote tumor cell death through a variety of mechanisms including: antibody-dependent cell-mediated cytotoxicity (ADCC), complement fixation, induction of apoptosis through receptor binding or simply by ligation of the ligand leading to the inhibition of growth. One of the recent successes of monoclonal antibody therapies has been Herceptin which is specific for the Her-2/neu tyrosine kinase receptor. Her-2/neu expression is elevated in approximately one-third of breast and ovarian cancers. Phase III clinical trials of Herceptin demonstrated tumor shrinkage in 10% of women with advanced breast cancer and a significant increase in median survival (8). Monoclonal antibodies conjugated to cytotoxic drugs, toxins or radioisotopes have also found use in the treatment of a number of malignancies. One such example is Non-Hodgkin's lymphoma which is a radiosensitive cancer and expresses a membrane protein (CD20) that can be targeted with a monoclonal antibody labelled with isotopes that emit  $\beta$  particles e.g. iodine-131 or yttrium-90 (9). Following internalization of the antibody, free radicals are generated which damage the DNA and ultimately kill the tumor cell.

The numerous successes achieved in cancer therapy through the diverse manipulation of the immune system, all point to the fact that immunotherapy can be effective. However, much optimization is still required to address issues such as toxicity and side-effects, lack of specificity, cost, and perhaps most importantly, efficacy.

### 2.2. Viruses and cancer therapy

The use of viruses to treat cancer has a long history which began with anecdotal reports of temporary cancer remissions in patients who had received a viral immunization or had a natural viral infection. In the early 1900s, the regression of cervical cancer in a patient vaccinated against rabies was reported and similar results were seen in cancer patients receiving smallpox vaccinations, or following natural virus infections such as mumps or measles. Based on these reports as well as pre-clinical data, live viruses were used to inoculate cancer patients as potential therapeutic treatments in the late 1940s and early 1950s (10). In 1957, Albert Sabin, who developed the live oral polio vaccine commented, "The most disappointing aspect is the fact that even when a virus is oncolytic and it punches a hole in a tumor, the immune response of the individual to the virus occurs so fast that the effects are quickly wiped out and the tumor continues to grow." By 1970, interest in the direct action of viruses was fading and attention began to shift to the use of oncolysates, or viral-infected tumors, as vaccines. Influenza was one of

the first viruses tested with sarcoma and malignant melanoma cells. Subsequently, Newcastle disease virus, vaccinia virus and vesicular stomatitis virus lysates were used to treat tumors. Although the use of viruses for cancer therapy has a long history, it is only relatively recently that potentially therapeutic viral vectors have been manipulated, refined, targeted and ultimately tested in humans in controlled clinical trials. Viruses can be utilized in two main ways to treat cancer, either directly to target and kill tumor cells (e.g. oncolytic viruses) or indirectly through the delivery of a therapeutic pay-load or tumor associated antigen. This chapter will focus primarily on the latter and predominantly those viral vectors that have been tested in clinical trials.

### 3. IDENTIFICATION OF TUMOR ASSOCIATED ANTIGENS

The genetic instability of cancer cells results in the production of mutated proteins which accounts for the differences in the antigenic profile of malignant and normal cells. Furthermore, aberrant functioning of the regulatory machinery of the cancer cell causes the tumor to produce proteins that are: (a) incorrectly glycosylated (b) expressed at dramatically higher levels or (c) expressed at a different developmental stage than in normal cells. Such changes in tumor cells provide potential targets for immunological intervention.

Before the identification of tumor associated antigens (TAAs), many of the initial immunotherapy clinical trials used whole tumor cells as a source of antigens. While this approach has certain advantages, the generation of immune responses against self-proteins has the potential to induce deleterious autoimmune reactions which could outweigh any beneficial anti-tumor effects. The identification of TAAs has enabled the development of improved targeted tumor therapies. Since their initial discovery, many tumor associated antigens have been identified, consisting of a diverse array of molecules with differing tissue distributions and characteristics.

However, despite the identification of TAAs and the detection of naturally occurring tumor-specific immune responses in patients with some cancer types (e.g. melanoma), tumors are notoriously poor at inducing immune responses. There are many reasons for the poor immunogenicity of tumors. Firstly, tumor antigens are rarely presented to the immune system in a manner which promotes a response. This is perhaps not surprising since tumor antigens are essentially self-proteins and therefore not recognized as foreign. Furthermore, tumors produce moieties which inhibit immune responses, lack co-stimulatory molecules essential for the priming of immune responses, and often have impaired antigen processing and presentation capabilities. Therefore, inducing a tumor-specific cellular or humoral response in a potentially immunosuppressed host, represents quite a challenge. Despite such hurdles, antigen delivery systems have improved greatly and are capable of eliciting potent immune responses to the target protein. In the field of cancer immunotherapy, a diverse array of antigen delivery

systems have been used, many of which are detailed in this and other chapters of this book.

#### 4. IDEAL PROPERTIES OF AN ANTIGEN DELIVERY SYSTEM

The optimal antigen delivery system has many different properties, however the ultimate goal of any antigen delivery system is to induce a safe, specific, efficacious and preferably, long-lasting immune response. For cancer immunotherapy to be successful, the key factors in any attempt to generate or boost antitumor immunity include the identification of an appropriate tumor antigen and its subsequent delivery to professional antigen-presenting cells. This latter step is fundamental and must occur in an environment which promotes rather than suppresses an immune response. Furthermore, for a therapy to be efficacious and lead to the destruction of established solid tumors, the antigen delivery system must be capable of inducing a high concentration of tumor-specific antibodies and T cells of high binding affinity. Once stimulated, the antibodies and/or T cells must then be able to traffic to, and infiltrate, the tumor to be able to mediate their effector role.

##### 4.1. Why are viral vectors suitable for delivering TAAs?

Viral vectors are an attractive choice of antigen delivery system for cancer immunotherapy since they mimic a natural infection and provide potent danger signals which are known to be important for the induction of an immune response. Furthermore, a recombinant virus encoding an antigen under the control of a strong promoter can be used to induce a targeted tumor-specific immune response. Numerous viral vector systems have been developed since the first recombinant vaccinia viruses were constructed more than 20 years ago (11). Recombinant viral vectors employed as experimental therapeutic cancer vaccines have been used to deliver immune modulators (e.g. cytokine or co-stimulatory molecules) or most frequently TAAs. In this chapter, we will concentrate primarily on the use of poxviruses, adenovirus and alphaviruses (for additional background information, see 12). Particular attention has been paid to those viral vectors which have been tested in clinical trials in cancer patients. Table 1 summarizes the results of clinical trials in which a number of viral vectors have been used to deliver different tumor antigens. The table also lists the trial phase, patient numbers and the immunological and clinical responses observed in the patients.

#### 5. POXVIRUS VECTORS

Poxviruses comprise a family of large and complex DNA viruses that replicate in the cytoplasm of vertebrate or invertebrate cells. The vertebrate poxviruses belong to the orthopox genus which includes variola, cowpox and vaccinia viruses.

Over 2 decades has passed since the first recombinant vaccinia virus was constructed (11, 13). During this time, poxviruses such as vaccinia, fowlpox and canarypox have found wide-spread use as vaccine vectors

in infectious disease and cancer research (the latter reviewed in 14) due to their good safety profile and efficient induction of both cellular and humoral immune responses.

##### 5.1. Vaccinia virus

Since there is antigenic cross-reactivity between variola (the causative agent of smallpox) and vaccinia viruses, the latter was used successfully in the smallpox eradication campaign in which millions of doses were administered. However, vaccinia virus is replication competent in human cells and was found to cause a relatively high incidence of vaccination complications (15). The safety profile of replication competent vaccinia was improved by the generation of attenuated strains such as NYVAC (16) and MVA (17). NYVAC was generated by deleting genes that restricted its replication in human cells, while MVA was attenuated by serial passage through chicken embryo fibroblast cells. MVA was used subsequently to vaccinate over 120000 people against smallpox, many of whom were thought to be at high risk from the complications associated with vaccinia virus immunization. In addition to its superior safety profile compared to replication competent vaccinia, MVA has been shown to be at least as efficacious in its ability to act as a delivery vector and induce potent immune responses. The reasons for these additional advantages are thought to include the reduced lytic activity of MVA in mammalian cells (18) and lack of immune evasion molecules (19-21).

Although vaccinia virus and its derivatives are the most widely utilized pox viral vectors, other members of the poxviridae family especially those from the avian poxvirus genus are currently undergoing extensive analysis. Both fowlpox and canarypox have been developed as recombinant vaccine vectors and shown to be safe and efficient in their ability to induce immune responses to the target antigen. Since millions of people were immunized with vaccinia virus as part of the smallpox eradication campaign, the presence of neutralizing antibodies may limit the ability of recombinant vaccinia viruses to boost immune responses to the expressed antigen. Even in vaccinia naïve individuals, the use of recombinant vaccinia viral vectors in homologous prime boost regimens can induce very high levels of circulating neutralizing antibodies which may limit efficacy. However, it has been reported that the prior exposure of mice to vaccinia virus did not have a detrimental impact on the generation of an therapeutic antibody response to a transgene delivered by a recombinant MVA vector (22). The potential problem of induction of high levels of neutralizing antibodies has, in part, been circumvented by the use of attenuated vaccinia virus vectors or through the use of heterologous prime-boost strategies (section 9).

##### 5.1.1. Use of vaccinia viral vectors to deliver tumor antigens

Recombinant vaccinia viruses have been evaluated in numerous cancer immunotherapy clinical trials, some of which are summarized in table 1. In the vast majority of cases, vaccination was not associated with serious adverse events. Furthermore, recombinant vaccinia viral vectors have been demonstrated to induce both antibody and cellular immune responses to many TAAs

**Table 1.** Summary of cancer immunotherapy clinical trials using different viral vectors.

Viral Vector	Clinical Trial Phase	Antigen	Patient Nos.	Product Name	Reported Antibody Responses	Reported Cellular Responses	Reported Safety / Clinical Observations	Ref
Vaccinia (Wyeth)	I	PSA	6	Prostvac	1 / 6 (16%)	N/A	Minimal toxicity One patient showed extended interval of undetectable serum PSA	27
Vaccinia (Wyeth)	I	PSA	33	Prostvac	1 / 33 (3%)	5 / 7 (71%)	Minimal toxicity 9 patients stable for 11-25 months. No evidence of clinical progression for $\leq 21$ months in some patients	28
Vaccinia (Wyeth)	I	PSA	42	Prostvac	0 / 42 (0%)	3 / 5 (60%)	Minimal toxicity No objective tumor responses	81
Vaccinia (MVA)	I	5T4	22	TroVax	13 / 17 (76%)	16 / 17 (94%)	Well tolerated. 5 patients showed periods of stable disease ranging from 3 to $>18$ months	38
Vaccinia (MVA)	I	MUC1	13	TG4010	0 / 13 (0%)	5 / 13 (38%)	Well tolerated. 4 of 13 evaluable patients showed disease stabilization for 6-9 months	30
Vaccinia (Copenhagen)	I	MUC1	9	TG1031	0 / 9 (0%)	1 / 9 (11%)	No adverse effects. Decrease in circulating CEA seen in 1 patient	29
Vaccinia (Wyeth)	I	CEA	20		0 / 20 (0%)	0 / 20 (0%)	Well tolerated. No significant toxicity attributable to the vaccine. No objective antitumor responses observed	26
Canarypox	I	CEA	20	ALVAC	N/A	7 / 9 (78%)	Well tolerated. No significant toxicity attributable to the vaccine. No objective antitumor responses observed.	38
Canarypox	I	CEA	18	ALVAC-CEA-B7.1	N/A	4 / 12 (33%)	Well tolerated. 3 patients had clinically stable disease that correlated with presence of CEA-specific precursor T cells	42
Canarypox	I	CEA	39	ALVAC-CEA-B7.1	2 / 31 (6.5%)	12 / 15 (80%)	Well tolerated. No significant toxicity attributable to the vaccine. Six patients showed decreases in circulating CEA for 4-12 weeks.	40
Canarypox	I	CEA	69	ALVAC-CEA-B7.1	N/A	12 / 21 (57%)	Well tolerated. Disease stabilization in some patients for up to 13 months	41
Canarypox and Vaccinia (Wyeth)	I	CEA	18	ALVAC	4 / 18 (22%)	8 / 11 (73%)	Well tolerated. No objective antitumor response observed	35
Fowlpox	I	gp100	46		N/A	23 / 38 (61%)	Well tolerated One partial regression in a patient receiving vaccine alone. Further objective responses seen when IL-2 administered in combination with the vaccine	37
Fowlpox and Vaccinia (Wyeth)	II	PSA	64		0 / 64 (0%)	14 / 30 (46%)	Minimal toxicity. 45% of eligible patients were free of PSA progression and 78% demonstrated clinical progression free survival at 19.1 months.	36
Adenovirus	I	gp100/MART-1	54		N/A	5 / 23 (22%)	No Significant toxic effects associated with the vaccines. 1 CR in patient receiving vaccine alone. 3 other CRs and 2 PRs seen in patients receiving vaccine + IL-2	48
NDV	I	Whole Tumor Cell	20		N/A	N/A	No serious adverse events Anecdotal evidence of encouraging 5 year survival figures	73

The table is grouped by viral vector and summarizes the antigen specific immune responses, safety and clinical responses observed in different clinical trials and reported in the tabulated reference

(e.g. CEA, PSA, MUC-1, 5T4). Some of the results summarized in table 1 are described in more detail in the following sections.

#### 5.1.1.1. Carcinoembryonic antigen (CEA)

Of all the tumor antigens which have been cloned into recombinant viral vectors, those expressing CEA have probably been tested in the greatest number of clinical trials. CEA is a 180 kDa oncofetal glycoprotein which is over-expressed in a large number of adenocarcinomas including those of the colon, rectum, stomach, pancreas, lung and breast (23). A large amount of pre-clinical development work using CEA expressed in vaccinia virus

(Wyeth strain), and other poxvirus vectors, led to the conclusion that the addition of 3 co-stimulatory molecules (B7.1, ICAM-1 and LFA-3; termed “TRICOM”) resulted in more potent immunological responses (24). The first clinical trial of a recombinant vaccinia encoding CEA was conducted in 1993 in 26 patients with metastatic adenocarcinoma (25). Since then, several phase I trials have been undertaken using vaccinia virus recombinants in both homologous and heterologous prime-boost approaches. Conry *et al* (26) reported details of a phase I trial of a vaccinia viral vector (NYVAC) encoding CEA (rV-CEA) given to patients with metastatic adenocarcinoma. Again, toxicity was found to be minimal, but unfortunately, no

objective clinical responses or CEA-specific immunological responses were observed.

### 5.1.1.2. Prostate specific antigen (PSA)

PSA is an androgen-regulated secreted serine protease which is produced primarily by normal prostate epithelial cells but which is over-expressed in prostate cancer. Vaccinia virus (Wyeth strain) has been used to deliver PSA to patients with prostate cancer in a number of clinical trials. In one phase I clinical trial, patients were vaccinated with rV-PSA following radical prostatectomy (27). Toxicity was found to be minimal in all patients at a dose level up to  $2.65 \times 10^8$  pfu. Unfortunately, a PSA-specific immune response was only detected in 1 patient following vaccination, although only a single injection was given to patients enrolled in this trial. A later dose-escalation study was undertaken in 33 patients with metastatic disease or rising PSA who received three monthly doses of rV-PSA at  $2.65 \times 10^6$ ,  $2.65 \times 10^7$  or  $2.65 \times 10^8$  pfu either alone, or in combination with GM-CSF (28). Again, virus-related toxicity was minimal with no patient experiencing anything greater than grade I cutaneous toxicity. PSA levels in nine patients remained stable for 11-25 months and there was some indication that this was more common in patients receiving the higher dose levels of rV-PSA and those receiving the higher dose levels of rV-PSA in combination with GM-CSF. Detectable PSA-specific immune responses were more encouraging in this study. Using the IFN $\gamma$  ELISPOT to analyze patient cellular responses to a 9-mer peptide derived from PSA, increases of at least 2-fold in PSA-specific T cell precursors were seen in 5 of 7 patients after 3 vaccinations. One out of 33 patients developed low-level PSA specific IgG antibodies. The authors stated that "rVV-PSA vaccination enhanced T cell responses to PSA after the first vaccination only, as opposed to the second or third. This is most likely due to host-immune responses to vaccinia proteins that limit replication of the vaccinia virus." They went on to suggest that "rV-PSA is best used in priming the immune system to a weak antigen such as PSA, and that another immunogen to be used to boost the immune response." Thus, a subsequent phase II trial used recombinant vaccinia and fowlpox vectors expressing PSA in a heterologous prime-boost protocol (see section 9).

### 5.1.1.3. MUC-1

MUC-1 is a highly glycosylated mucin of high molecular weight (> 500kDa). It is normally found at the apical surface of mucin-secreting epithelial cells in many types of tissues. In many human cancers (e.g. breast), MUC-1 is overexpressed and aberrantly glycosylated. Two phase I clinical trials have been reported in which vaccinia virus was used to deliver MUC-1 and IL-2 to patients with breast cancer (29) or MUC-1 positive tumors (30). Both studies demonstrated that the vaccine was safe and well tolerated even at the highest dose of  $1 \times 10^8$  pfu. A number of patients showed evidence of MUC-1 specific cellular responses and had periods of disease stabilization.

### 5.1.3. 5T4

The human oncofetal antigen 5T4 is a 72kDa surface leucine-rich glycoprotein that is expressed at high levels on the placenta and also on a wide range of human

carcinomas including colorectal, gastric and ovarian, but rarely on normal tissues (31,32). Human 5T4 is frequently expressed on metastases and such expression shown to be associated with poor prognosis (33). MVA has been used as a vaccine vector to deliver 5T4 and the recombinant viral vector (termed TroVax) has now been tested in both pre-clinical and clinical cancer immunotherapy settings. In a pre-clinical murine model, it has been demonstrated that CD4 $^{+}$  T cells were essential for the induction of a protective immune response and that antibodies specific for 5T4 were the likely effector moiety (Harrop & Carroll, unpublished). TroVax has been tested in a dose escalation phase I/II clinical trial in late-stage colorectal cancer patients. Patients received up to 5 immunizations of TroVax via intra-muscular or intra-dermal routes with doses ranging from  $5 \times 10^7$  to  $5 \times 10^8$  pfu. TroVax was well tolerated at all dose levels (34). Both cellular and humoral immune responses were detected in the majority of patients with some anecdotal evidence of a relationship between 5T4 specific antibody levels and overall patient survival. Furthermore, periods of disease stabilization ranging from 3 months to >18 months was observed in some patients. Phase II clinical trials in both colorectal and renal cancer are currently ongoing.

## 5.2. Avian Poxviruses

One of the advantages that the avian poxviruses have over vaccinia virus is that they are replication defective in human cells and do not undergo late stage gene expression of structural proteins. Therefore, anti-vector immune responses should have a less detrimental effect on the efficacy of subsequent vector immunizations. Indeed, in some patients, canarypox expressing CEA could be given up to 8 times with continued increases in CEA T cell precursors (35), suggesting that neutralizing antibodies have minimal impact on the ability to boost immune responses in some patients. It has been noted that the avipox viruses are non-pathogenic and offer safety advantages over other viral agents (36).

### 5.2.1. Fowlpox

Fowlpox is replication defective in mammalian cells and can express transgenes in infected cells for up to 3 weeks, in contrast to vaccinia virus-infected cells that express antigens for approximately 2 days before cell death. Since fowlpox viruses express antigen for a longer period than vaccinia virus, the T cell response may be significantly enhanced when using these vectors for immunization.

Rosenberg *et al* (37) selected fowlpox to deliver the melanoma associated antigen, gp100 to patients with metastatic melanoma. Three consecutive clinical trials were undertaken in which 3 different forms of gp100 were used: (a) native, full-length gp100, (b) a modified gp100 molecule and (c) a minigene construct encoding a single gp100 epitope. All 3 recombinant viral vectors were well tolerated. Immune responses to gp100 were detected in the majority of patients immunized with the modified gp100 or minigene recombinant viruses but not in those receiving fowlpox encoding the native gp100 molecule. One patient showed a partial response. However, when patients showed

evidence of progressive disease, they were eligible for IL-2 treatment alone or in combination with the recombinant virus. Of 13 patients receiving IL-2 and the vaccines encoding the full-length or modified gp100, none showed an objective clinical response. However, of 12 patients who were treated with IL-2 and the recombinant fowlpox encoding the gp100 minigene, 6 patients showed objective cancer regressions and 3 patients had complete regressions.

### 5.2.2. Canarypox

In the first clinical trial of a canarypox vector (ALVAC) in a cancer setting, CEA was delivered to patients with CEA-expressing advanced malignancies (38). A dose escalation was undertaken in which patients received  $2.5 \times 10^5$ ,  $2.5 \times 10^6$  or  $2.5 \times 10^7$  pfu ALVAC-CEA. The vaccine was shown to be well tolerated at all dose levels with no significant toxicity to the treatment. Although no objective tumor responses were observed, significant increases in CEA-specific CD8<sup>+</sup> T cells were detected in 7 of 9 patients evaluated. Subsequently, T cell cultures established from 7 patients were shown to be able to lyse the CEA expressing human tumor cell lines SW1463 and SW480 (39). A non-MHC matched CEA expressing tumor cell line was not lysed demonstrating the specificity of the response.

In a later trial, patients with CEA-expressing tumors were immunized with  $2.5 \times 10^7$ ,  $1 \times 10^8$  or  $4.5 \times 10^8$  pfu of ALVAC expressing CEA and the co-stimulatory molecule B7.1 (40). Therapy was well tolerated and of 15 HLA-A2 positive patients who could be tested for T cell responses to a CEA A2 peptide, 12 showed  $\geq 2$  fold increases in precursor frequency post-vaccination. A subsequent trial by the same group combined ALVAC-CEA with GM-CSF in patients with metastatic carcinoma (41). Encouraging CEA-specific T cell responses were observed in patients after 4 vaccinations and some evidence of disease stabilization was reported. In a further encouraging phase I trial, ALVAC was again used to deliver both CEA and B7.1 to patients with CEA-expressing adenocarcinomas (42). No significant toxicity was observed and 3 patients were reported to show clinically stable disease which correlated with increasing CEA-specific T cells as detected by IFN $\gamma$  ELISPOT. Additional vaccinations in the 3 patients resulted in augmented CEA specific T cell responses.

## 6. ADENOVIRUS

Adenoviruses are double-stranded DNA viruses with a genome which encodes approximately thirty proteins. There has been wide-spread interest in the use of adenoviruses for the treatment of cancer, although this has primarily been for gene therapy (43) or oncolytic virotherapy approaches (44). Conditionally replicating adenoviruses (CRAds) are being developed as a promising new tool for cancer therapy. CRAds specifically replicate in and kill cancer cells (45). Within a solid tumor mass, release of newly formed infectious particles from infected cancer cells allows additional cell layers to be infected and destroyed.

However, for cancer immunotherapy approaches, the ability of adenoviruses to efficiently transduce professional antigen presenting cells such as dendritic cells (DCs) has facilitated the *ex vivo* delivery of tumor antigens to DCs followed by their subsequent re-infusion into the host. Recombinant adenoviruses have a number of advantages for the active immunization of cancer patients (46). Genes encoding tumor associated antigens can be relatively easily inserted into replication incompetent adenoviruses and can be safely administered to humans. Furthermore, adenoviruses are highly immunogenic, although this can also limit the utility of the vector in situations where multiple boosts are required (47).

In a dose escalation phase I study, 54 patients with metastatic melanoma were immunized with recombinant adenovirus encoding either MART-1 or gp100 melanoma antigens (48). Both vaccines were administered safely. High levels of neutralizing antibodies were detected in the pre-treatment sera of patients, but further increases were induced following vaccination. Such high levels of neutralizing antibodies may have accounted for the low percentage of patients showing antigen specific immune responses. However, objective clinical responses were observed in a number of patients including one complete response.

## 7. ALPHAVIRUSES (SEMLIKI FOREST, SINDBIS, VENEZUELAN)

Alphaviruses such as Semliki Forest virus (SFV), Sindbis virus (SIN) and Venezuelan equine encephalitis virus (VEE) are positive-strand RNA viruses that have very broad host cell specificity (49). They have several characteristics that make them attractive for cancer therapy vectors such as their high-level expression of a heterologous gene. Since Alphaviruses replicate in the host cell cytosol without integration of the viral genetic material in the cellular genome, safety concerns about cell transformation are reduced. Alphavirus vectors have been engineered to express several therapeutic genes including HSV thymidine kinase (50), IL-12 (51) and GM-CSF (52) as well as HPV 16 E6 and E7 genes (53). Alphaviruses which have been used for cancer therapy are discussed in more detail in the following sections.

### 7.1. Semliki Forest Virus (SFV)

The SFV expression system has been developed for use in targeted cancer immunotherapy approaches. For example, SFV encoding HPV 16 E6 and E7 genes has been generated and shown to induce long-lasting cellular immunity in a pre-clinical model (53). These authors stated that “the SFV vector system would appear to be an ideal system for inducing optimal immunity, since, .... SFV infection results in apoptosis of infected cells that will be taken up by specialized antigen presenting cells”. The recombinant vector is now being developed for the treatment of HPV-induced cervical cancer.

### 7.2. Sindbis virus (SIN)

Another member of the Alphavirus genus is Sindbis virus which is a blood borne virus that is

transmitted to mammals by mosquito bites. Sindbis has a small RNA genome (< 12000 nucleotides) that is easy to manipulate and which is amplified many fold once in the cytoplasm of a cell. Sindbis virus has a high gene transfer efficiency into mammalian cells, thought to occur via the high-affinity laminin receptor (54), which is substantially upregulated in numerous human cancers e.g. breast (55) and colorectal (56). This confers on SIN vectors the ability to preferentially infect most tumor cells and, like SFV, the virus induces apoptosis in infected cells. New SIN vectors have been engineered that are capable of nonreplicative infection thus increasing the safety profile of the vector (49). Recent pre-clinical studies have shown very encouraging results whereby a Sindbis viral vector given via the intraperitoneal route was capable of specifically infecting and eradicating tumor cells grown subcutaneously, intrapancreatically, intraperitoneally and in the lungs without adverse effects (57). The efficacy of the vector has been enhanced further by the incorporation of cytokine genes such as IL-12 and IL-15 (58) which may enhance the induction of an immune response against antigens expressed on the tumor.

### 7.3. Venezuelan equine encephalitis virus (VEE)

Venezuelan equine encephalitis virus (VEE) is another Alphavirus which has potential utility in cancer therapy, especially given its tropism for dendritic cells. The tumor antigen Neu has been engineered into a VEE derived replicon vector system. The recombinant vector expressing rat Neu was able to break immunological tolerance to the self-antigen in a rat and conferred significant protection from challenge with a rat mammary tumor (59).

A different approach that has seen success has been the use of a naked DNA vaccine encoding an alphavirus replicon and the tumor antigen tyrosinase-related-protein-1 (60). This vaccine induced a more potent immune response, thought to be due to the recognition of the double stranded RNA produced by the replicon, leading to the production of type I IFN and heat-shock proteins. This hybrid construct induced protection against a melanoma challenge in a self-antigen murine model.

Despite the use of Alphaviruses in pre-clinical tumor models, few reports have addressed the safety or efficacy of such vectors in the clinic. Furthermore, prior to their use in the clinic, the development of packaging cell lines and large-scale GMP grade production of alphavirus vectors need to be investigated further.

## 8. MISCELLANEOUS VIRAL VECTORS

Several other viral vectors have been investigated for their ability to be used as viral vectors in cancer therapy settings, some of which have made the transition into clinical development. It is these vectors that will be discussed here.

### 8.1. Herpes Simplex Virus (HSV)

Herpes simplex virus has received more attention as a mediator of cancer gene therapy rather than immunotherapy. However, oncolytic strains of Herpes

simplex virus (HSV) have shown antitumor effects against a variety of human malignancies and offer promise for the treatment of solid tumors such as ovarian cancer. Oncolytic HSV can infect, replicate in and kill tumor cells by a direct cytopathic effect, while showing only restricted ability to replicate in normal cells (61-63). However, results in initial cancer clinical trials showed limited efficacy using the current generation of HSV (64, 65). Further research is ongoing to optimize the potency of these oncolytic viruses for use in cancer patients (66). One such approach is utilizing cancer specific promoters to regulate viral replication. For example, recent work reported the construction of a HSV1 in which the Gamma 134.5 gene was regulated by a DF3/MUC1 promoter. Regulation of HSV-1 Gamma 134.5 function resulted in preferential viral replication and oncolysis in cancer cells that expressed MUC1, restricted biodistribution *in vivo* and less toxicity (67). While the oncolytic properties of HSV can induce tumor specific immune responses, this is a by-product of tumor cell death rather than a targeted approach. However, the use of HSV to deliver immuno-modulatory molecules such as IL-12 (68), GM-CSF (69, 70) or tumor antigens (e.g. gp100, MART-1 and Tyrosinase) have received some attention. For example, HSV encoding GM-CSF (OncoVEX<sup>GM-CSF</sup>) is currently undergoing a Phase I study in several solid tumors including melanoma and breast, head and neck, gastro-intestinal, pancreatic, oesophageal and bowel cancers.

### 8.2. Newcastle Disease Virus (NDV)

NDV is an enveloped, negative strand RNA virus of the paramyxoviridae family that is the causative agent of Newcastle Disease in a wide variety of birds (most notably chickens). NDV can also infect humans, but causes only minor illness. In man, NDV replicates up to 10000 times better in cancer cells than it does in most normal cells (71). The NDV strains that have been evaluated most widely for the treatment of cancer are 73-T (lytic), MTH-68 (thought to be lytic) and Ulster (nonlytic). All 3 have shown little or no evidence of neurotropism. In human studies, NDV has been used indirectly as an oncolysate vaccine or a lytic strain of NDV has been used directly to immunize cancer patients enrolled into phase I and II clinical trials. In one study, the maximum tolerated dose a replication-competent strain of NDV was defined following immunization of 79 patients with advanced solid cancers (72). The most common adverse events were flu-like symptoms that occurred primarily after the first dose and decreased after subsequent doses. Some objective tumor responses were reported. In a more recent study (73), an NDV modified autologous tumor cell vaccine was used to immunize 20 patients with advanced head and neck squamous cell carcinomas. No severe side effects were observed and evidence of tumor specific cellular immune responses were observed which could still be detected 5 years after the last vaccination. More importantly, encouraging survival data was reported with 61% of vaccinated patients alive at 5 years compared to an expected figure of 38% (albeit from an historical cohort). In summary, recent results with NDV used in the clinical setting have shown encouraging anecdotal benefit and no serious side-effects. Such data

have caused renewed interest in the development of NDV as a possible cancer therapeutic (74), although additional research is still required to confirm these initial findings.

### 9. HETEROLOGOUS PRIME-BOOST APPROACHES

For cancer immunotherapy to be successful, it is highly likely that a vaccine would have to be given more than once to elicit a mature immune response and that continued boosters would be required to maintain the response at an efficacious level over a period of months and years in the presence of even residual disease. However, multiple exposure of the host to the same vector is likely to induce a potent virus-specific immune response which could potentially limit the utility of a single viral vector for cancer immunotherapy. The requirement for a heterologous prime-boost approach was summarized concisely by Leitner *et al* (60) who stated that “vaccine vectors have been used for many years, but the delivery of target antigens can be accompanied by unwanted side-effects. First, pre-existing antibodies can neutralize the virus before it is able to deliver its payload. Second, structural proteins from the virus can dominate T- and B- cell-mediated immune responses, diverting immunity away from the target immunogen. Hence, there is a critical need to develop vaccine vectors that are not only highly immunogenic, but also antigenically simple”. The optimal prime-boost scenario in terms of the induction of tumor-specific immune responses is shown schematically in Figure 1. In this ideal scenario, the first vaccine is able to prime a strong cellular response against the target antigen but the vector itself elicits feeble immunity. Subsequent boosting with a heterologous vector should induce a massive increase in the frequency of TAA-specific T cells, but again induce a weak primary anti-vector response (the 2 vectors should ideally show no antigenic cross-reactivity). Further boosts with the same vector should continue to increase the TAA-specific precursor frequency while eliciting no neutralizing anti-vector response. In the cancer immunotherapy field, many paired combinations of viral vectors have been tested in heterologous prime-boost scenarios and include vaccinia and canarypox (35), vaccinia and fowlpox (36), Sindbis and vaccinia (75) and DNA and vaccinia (76). The potential problem of neutralizing immune responses has been addressed by using heterologous booster protocols which employ two immunologically non-cross reacting poxvirus vectors e.g. FPV and MVA (19) and ALVAC and vaccinia (77). The optimal combination of two or more different vectors and the order in which they should be used to prime and subsequently boost the immune response remains under discussion. However, research in both cancer and infectious disease fields, has sought to optimize immune response to target antigens by using different heterologous prime-boost protocols.

In a pre-clinical model, recombinant vaccinia, MVA and fowlpox viral vectors encoding CEA and 3 co-stimulatory molecules (B7.1, ICAM-1 and LFA-3 (TRICOM)) were tested in various combinations (78). It was shown that the use of MVA-CEA/TRICOM in a heterologous prime-boost regimen with fowlpox-CEA/TRICOM induced significantly greater levels of

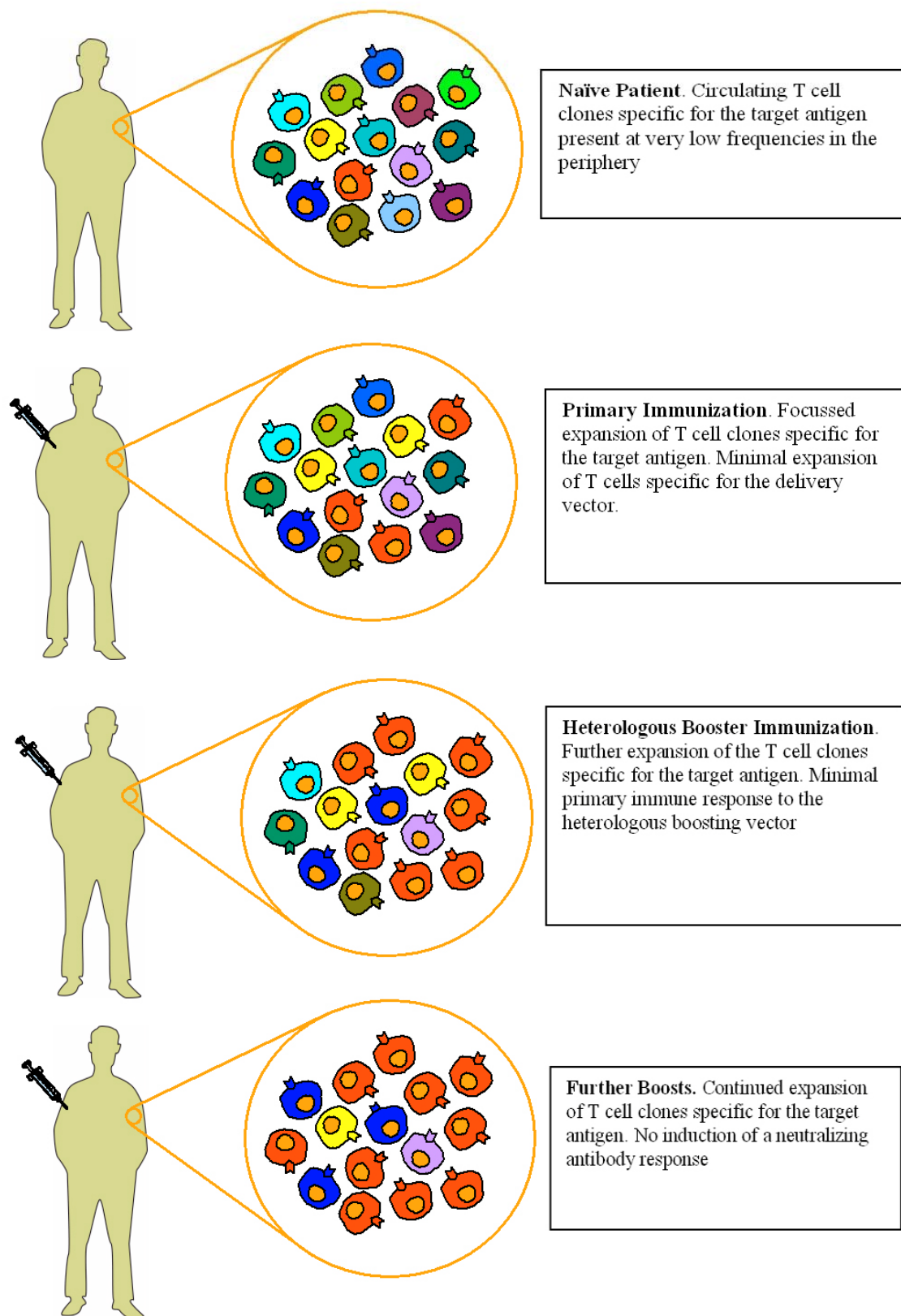
CEA-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells than seen with a vaccinia-CEA/TRICOM prime, fowlpox-CEA/TRICOM boost. Furthermore, in a murine self-antigen tumor model the MVA prime, fowlpox boost regimen resulted in longer survival than vaccinia prime, fowlpox boost. Additional pre-clinical research has indicated that the use of recombinant vaccinia and avipox vectors expressing CEA (rV-CEA and avipox-CEA respectively) in a heterologous prime-boost regimen generated a more potent T cell response than either vaccine alone (77). Indeed, it was shown that priming of the immune system with rV-CEA and boosting with avipox-CEA induced four times greater level of CEA-specific T cells than those achieved with 3 vaccinations with avipox-CEA alone. Furthermore, additional vaccinations with avipox-CEA were able to boost the CEA-specific T cell response.

Following such promising pre-clinical data, the use of diversified prime-boost regimens is now being transferred into the clinical setting. The first report of a heterologous prime-boost approach with recombinant viral vectors used vaccinia and avipox viruses to deliver CEA (rV-CEA and avipox-CEA respectively; (35)). Eighteen patients with advanced CEA expressing tumours were immunized with: (a) 1x rV-CEA followed by 3x avipox-CEA vaccinations or (b) 3x avipox-CEA followed by 1x rV-CEA vaccinations. In this setting, treatment was well tolerated and it was noted that rV-CEA was more effective at priming a CEA-specific immune response than avipox-CEA. Furthermore, avipox-CEA could be administered up to 8 times with continued increases in detectable CEA-specific immune responses following each vaccination. In a phase II clinical trial, recombinant vaccinia and fowlpox viruses expressing PSA were used to vaccinate patients with advanced prostate cancer (36). Patients were divided into three groups 3 groups which received: (a) 4 vaccinations with fowlpox-PSA (rF-PSA), (b) 3x rF-PSA followed by 1x vaccinia-PSA (rV-PSA) vaccinations or (c) 1x rV-PSA followed by 3x rF-PSA. Following *in vitro* stimulation, PSA specific T cell responses were detected by ELISPOT in 14 out of 30 patients and almost half of the treated patients showed encouraging trends in the levels of circulating PSA.

### 10. PERSPECTIVE: THE FUTURE OF VIRAL VECTORS FOR CANCER IMMUNOTHERAPY AND THEIR POTENTIAL LIMITATIONS

While pre-clinical tumor models using viral vectors have provided great hope that the observed therapeutic effects can be translated into the clinical setting, objective tumor responses in humans to date have been disappointing (79, 80). However, most viral vector based cancer immunotherapies have been evaluated in patients with late stage disease in which the expected median survival may be less than one year and in whom the induction of an efficacious immune response within this time-frame is challenging. Furthermore, cancer vaccines, unlike chemotherapy, may not induce immediate clinical responses but may reduce the rate of disease progression resulting in increased survival times. This latter parameter is rarely captured in early phase clinical trials. While





**Figure 1.** Schematic representation of the ideal properties of a prime-boost vaccination regimen in relation to the antigen-specific T cells which are stimulated.

immune responses to the target tumor antigen have been detected in many clinical trials (table 1), these are sometimes only seen in a minority of patients, may be of low magnitude and frequently do not correlate with observed clinical responses. There are many potential reasons for the disparity between detectable antigen-specific immune responses and the clinical responses observed in cancer patients. If such disparities are viewed purely from the properties of the induced immune response, potential reasons for the low incidence of therapeutic effects may occur because the antigen-specific immune responses are: (a) of insufficient magnitude, (b) too transient, (c) of poor avidity (d) inappropriate for efficient killing of tumor cells or (e) the effector moieties cannot migrate to, or penetrate large solid tumors. For such reasons, it has been suggested that cancer vaccines may be best placed as adjuvant to traditional therapy or in the management of residual disease following surgical resection. However, much translational work remains to be performed to understand the reasons behind the successes and failures achieved in both clinical and pre-clinical settings. Refinement of cancer vaccine approaches which may enable increased success rates in the clinic include: (a) identification of the optimal prime-boost combination of recombinant viral vectors, (b) identification of the optimal route, dosage and timing of immunization, (c) identification of the key immune effector arm(s) and (d) selection of the appropriate clinical target/setting for the vaccine. Over the next decade it is hoped that increased clarity on such issues and advances made our ability to predict whether a cancer patient is suitable for an immunotherapy approach will lead to much greater success rates and a higher profile for tumor vaccines in the expanding arsenal of therapies used to fight cancer.

## 11. ACKNOWLEDGMENTS

The authors would like to thank Dr. S. Marlow for her time and useful comments during the drafting of this chapter.

## 12. REFERENCES

1. W. Coley: Further observations upon the treatment of malignant tumors with the toxins of erysipelas and *Bacillus prodigiosus* with a report of 160 cases. *Bull Johns Hopkins Hospital* 7, 157 (1896)
2. P. Ehrlich: Uer den jetzigen Stand der Karzinomforschung. *Ned Tijdschr Geneesk* 53, 273-290 (1909)
3. E. J. Foley: Antigenic properties of methylcholanthrene-induced tumors in mice of the strain and origin. *Cancer Res.* 13, 835-837 (1953)
4. R. T. Prehn & J.M. Main: Immunity to methylcholanthrene-induced sarcomas. *J. Natl Cancer Inst.* 18, 769-778 (1957)
5. G. Klein, H. O. Sjogren, E. Klein & K. E. Hellstrom: Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. *Cancer Res.* 20, 1561-1572 (1960)
6. A. B Alexandroff & K. James: Immunotherapy of bladder cancer. In: *Cancer Vaccines and Immunotherapy*. Eds: P. L. Stern, P. C. L. Beverley & M. W. Carroll *Cambridge University Press* 19-46 (2000)
7. J. S. Ross, D. P. Schenkein, R. Pietrusko, M. Rolfe, G. P. Linette, J. Stec, N. E. Stagliano, G. S. Ginsburg, W. F. Symmans, L. Pusztai & G. N. Hortobagyi: Targeted therapies for cancer 2004. *Am. J. Clin. Pathol.* 122(4), 598-609 (2004)
8. D. J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga & L. Norton: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* 344(11), 783-792 (2001)
9. A. D. Zelenetz: A clinical and scientific overview of tositumomab and iodine I 131 tositumomab. *Semin. Oncol.* 30 (2 Suppl 4), 22-30 (2003)
10. N. J. Nelson: Viruses and Cancer. *J. Natl. Cancer Inst.* 91(20), 1709 (1999)
11. M. Mackett, G. L. Smith & B. Moss: Vaccinia virus: a selectable eukaryotic cloning and expression vector. *Proc. Natl. Acad. Sci.* 79, 7415-7419 (1982)
12. M. W. Carroll, G. Wilkinson & K. Lundstrom: Mammalian expression systems and vaccination. In: *Genetically Engineered Viruses* Eds. C. Ring & E. Blair *BIOS Scientific Publishers Ltd* Oxford, U.K. 107-158 (2001)
13. D. Panicali & E. Paoletti: Construction of poxviruses as cloning vectors: insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus. *Proc. Natl. Acad. Sci.* 79, 4927-4931 (1982)
14. M. W. Carroll & N. P. Restifo: Poxviruses as vectors for cancer immunotherapy. In: *Cancer Vaccines and Immunotherapy*. Eds P. L. Stern, P. C. L. Beverley & M. W. Carroll *Cambridge University Press*, 47-61 (2000)
15. J. M. Lane & J. D. Miller: Risks of smallpox vaccination complications in the United States. *Am. J. Epidemiol.* 93, 238-240 (1971)
16. J. Tartaglia, M. E. Perkus, J. Taylor, E. K. Norton, J. C. Audonnet, W. I. Cox, S. W. Davis, J. van der Hoeven, B. Meignier & M. Riviere: NYVAC: a highly attenuated strain of vaccinia virus. *Virology* 188, 217-232 (1992)
17. G. Sutter & B. Moss: Non-replicating vaccinia vector efficiently expresses recombinant genes. *Proc. Natl. Acad. Sci.* 89, 10847-10851 (1992)

18. M. W. Carroll & B. Moss: Host range and cytopathogenicity of the highly attenuated MVA strain of vaccinia virus: propagation and generation of recombinant viruses in a nonhuman mammalian cell line. *Virology*, 238, 198-211 (1997)
19. M. W. Carroll, W. W. Overwijk, R. S. Chamberlain, S. A. Rosenberg, B. Moss & N. P. Restifo: Highly attenuated modified vaccinia virus Ankara (MVA) as an effective recombinant vector: a murine tumor model. *Vaccine* 15(4), 387-394 (1997)
20. G. Antoine, F. Scheiflinger, F. Dorner & F. G. Falkner: The complete genomic sequence of the modified vaccinia Ankara strain: comparison with other orthopoxviruses. *Virology* 244(2), 365-396 (1998)
21. G. L. Smith, J. A. Symons, A. Khanna, A. Vanderplasschen & A. Alcamì: Vaccinia virus immune evasion. *Immunological Reviews* 159, 137-154 (1997)
22. I. Redchenko, M. Ryan & M. W. Carroll: Pre-existing immunity to vaccinia virus does not affect efficacy of a recombinant MVA vaccine: Poxvirus cross-reactivity. *12<sup>th</sup> International Congress of Immunology and 4<sup>th</sup> Annual Conference of FOCIS* 459-464 (2004)
23. D. M. Goldenberg, R. M. Sharkey & F. J. Primus: Carcinoembryonic antigen in histopathology: immunoperoxidase staining of conventional tissues sections. *J. Natl. Cancer Inst.* 57, 11-22 (1976)
24. J. W. Hodge, H. Sabzevari, A. G. Yafal, L. Gritz, M. G. Lorenz & J. Schlom: A triad of costimulatory molecules synergize to amplify T-cell activation. *Cancer Res.* 59(22), 5800-5807 (1999)
25. J. M. Hamilton, A. P. Chen & B. Nguyen: Phase I study of recombinant vaccinia virus (rV) that expresses human carcinoembryonic antigen (CEA) in adult patients with adenocarcinomas. *Proc. Am. Soc. Clin. Oncol.* 13, 961-969 (1995)
26. R. M. Conry, M. B. Khazaeli, M. N. Saleh, K. O. Allen, D. L. Barlow, S. E. Moore, D. Craig, R. B. Arani, J. Schlom & A. F. LoBuglio: Phase I trial of a recombinant vaccinia virus encoding carcinoembryonic antigen in metastatic adenocarcinoma: Comparison of intradermal versus subcutaneous administration. *Clin. Cancer Res.* 5, 2330-2337 (1999)
27. M. G. Sanda, D. C. Smith, L. G. Charles, C. Hwang, K. J. Pienta, J. Schlom, D. Milenic, D. Panicali & J. E. Montie: Recombinant vaccinia-PSA (PROSTVAC) can induce a prostate-specific immune response in androgen-modulated human prostate cancer. *Urology* 53(2), 260-266 (1999)
28. J. P. Eder, P. W. Kantoff, K. Roper, G. X. Xu, G. J. Bubley, J. Boyden, L. Gritz, G. Mazzara, W. K. Oh, P. Arlen, K. Y. Tsang, D. Panicali, J. Schlom & D. W. Kufe: A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. *Clin Cancer Res.* 6(5), 1632-1638 (2000)
29. S. M. Scholl, J. M. Balloul, G. Le Goc, N. Bizouarne, C. Schatz, M. P. Kieny, S. von Mensdorff-Pouilly, A. Vincent-Salomon, L. Deneux, E. Tartour, W. Fridman, P. Pouillart & B. Acres: Recombinant vaccinia virus encoding human MUC1 and IL2 as immunotherapy in patients with breast cancer. *J. Immunother.* 23, 570-580 (2000)
30. C. Rochlitz, R. Figlin, P. Squiban, M. Salzberg, M. Pless, R. Herrmann, E. Tartour, Y. Zhao, N. Bizouarne, M. Baudin & B. Acres: Phase I immunotherapy with a modified vaccinia virus (MVA) expressing human MUC1 as antigen-specific immunotherapy in patients with MUC1-positive advanced cancer. *J. Gene Med.* 5(8), 690-699 (2003)
31. N. Hole & P. L. Stern: A 72 kD trophoblast glycoprotein defined by a monoclonal antibody. *Br. J. Cancer* 57, 239-246 (1988)
32. K. A. Myers, V. Rahi-Saund, M. D. Davison, J. A. Young, A. J. Cheater & P. L. Stern: Isolation of a cDNA encoding 5T4 oncofetal trophoblast glycoprotein. *J. Biol. Chem.* 269, 9319-9324 (1994)
33. T. Starzynska, P. J. Marsh, P. F. Schofield, S. A. Roberts, K. A. Myers & P. L. Stern: Prognostic significance of 5T4 oncofetal antigen expression in colorectal carcinoma. *Br. J. Cancer* 69, 899-902 (1994)
34. M. W. Carroll, R. Harrop, N. Connolly, I. Redchenko, M. Ryan, N. Drury, M. Kelleher, S. Kingsman & R. Hawkins: Modified Vaccinia Ankara (MVA) expressing the tumour associated antigen 5T4 (TroVax) induces immune responses in late stage colorectal cancer patients in a phase I/II clinical trial. *Proceedings of the American Association for Cancer Research Annual Meeting* (2004) *Abstract*
35. J. L. Marshall, R. J. Hoyer, M. A. Toomey, K. Faraguna, P. Chang, E. Richmond, J. E. Pedicano, E. Gehan, R. A. Peck, P. Arlen, K. Y. Tsang & J. Schlom: Phase I study in advanced cancer patients of a diversified prime- and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J. Clin. Oncol.* 18(23), 3964-3973 (2000)
36. H. L. Kaufman, W. Wang, J. Manola, R. S. DiPaola, Y. J. Ko, C. Sweeney, T. L. Whiteside, J. Schlom, G. Wilding & L. M. Weiner: Phase II randomized study of vaccine treatment of advanced prostate cancer (E7897): a trial of the Eastern Cooperative Oncology Group. *J. Clin. Oncol.* 22(11), 2122-2132 (2004)
37. S. A. Rosenberg, J. C. Yang, D. J. Schwartzentruber, P. Hwu, S. L. Topalian, R. M. Sherry, N. P. Restifo, J. R. Wunderlich, C. A. Seipp, L. Rogers-Freezer, K. E. Morton, S. A. Mavroukakis, L. Gritz, D. L. Panicali & D. E. White: Recombinant fowlpox viruses encoding the anchor-

modified gp100 melanoma antigen can generate immune responses in patients with metastatic melanoma. *Clin. Cancer Res.* 9, 2973-2980 (2003)

38. J. L. Marshall, M. J. Hawkins, K. Y. Tsang, E. Richmond, J. E. Pedicano, M. Zhu Zhu & J. Schlom: Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J. Clin. Oncol.* 17(1), 332-337 (1999)

39. M. Z. Zhu, J. Marshall, D. Cole, J. Schlom & K. Y. Tsang: Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. *Clin. Cancer Res.* 6, 24-33 (2000)

40. M. von Mehren, P. Arlen, K. Y. Tsang, A. Rogatko, N. Meropol, H. S. Cooper, M. Davey, S. McLaughlin, J. Schlom & L. M. Weiner: Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin. Cancer Res.* 6(6), 2219-2228 (2000)

41. M. von Mehren, P. Arlen, J. Gulley, A. Rogatko, H. S. Cooper, N. J. Meropol, R. K. Alpaugh, M. Davey, K. Y. Tsang, J. Schlom & L. M. Weiner: The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin. Cancer Res.* 7, 1181-1191 (2001)

42. H. Horig, D. S. Lee, W. Conkright, J. Divito, H. Hasson, M. LaMare, A. Rivera, D. Park, J. Tine, K. Guito, K. Wong-Yok, J. Schlom & H. L. Kaufman: Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol. Immunother.* 49, 504-514 (2000)

43. M. Everts & D. T. Curiel: Transductional targeting of adenoviral cancer gene therapy. *Curr. Gene Ther.* 4(3), 337-346 (2004)

44. R. L. Chu, D. E. Post, F. R. Khuri & E. G. Van Meir: Use of replicating oncolytic adenoviruses in combination therapy for cancer. *Clin. Cancer Res.* 10(16), 5299-5312 (2004)

45. D. Oosterhoff & V. W. van Beusechem: Conditionally replicating adenoviruses as anticancer agents and ways to improve their efficacy. *J. Exp. Ther. Oncol.* 4(1), 37-57 (2004)

46. J. L. Imler: Adenovirus vectors as recombinant viral vaccines. *Vaccine* 13, 1143-1151 (1995)

47. S. K. Basak, S. M. Kiertscher, A. Harui & M. D. Roth: Modifying adenoviral vectors for use as gene-based cancer vaccines. *Viral Immunol.* 17(2), 182-196 (2004)

48. S. A. Rosenberg, Y. Zhai, J. C. Yang, D. J. Schwartzentruber, P. Hwu, F. M. Marincola, S. L. Topalian, N. P. Restifo, C. A. Seipp, J. H. Einhorn, B. Roberts & D. E. White: Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens. *J. Natl. Can. Inst.* 90(24), 1894-1900 (1998)

49. J. H. Strauss & E. G. Strauss: The alphaviruses: gene expression, replication, and evolution. *Microbiol. Rev.* 58, 491-562 (1994)

50. S. Loimas, M. R. Toppinen, T. Vasakoppi, J. Janne & J. Wahlfors: Human prostate carcinoma cells as targets for herpes simplex virus thymidine kinase-mediated suicide gene therapy. *Cancer Gene Ther.* 8, 137-144 (2001)

51. C. Asselin-Paturel, U. Lassau, J-M. Guinebretiere, J. Zhang, F. Gay, F. Bex, S. Hallez, J. Leclere, P. Peronneau, F. Mami-Chouaib & S. Chouaib: Transfer of the murine interleukin-12 gene *in vivo* by a Semliki forest virus vector induces B16 tumor regression through inhibition of tumor blood vessel formation monitored by Doppler ultrasonography. *Gene Ther.* 6, 606-615 (1999)

52. A. H. Klimp, E. van der Vaart, P. O. Lansink, S. Withoff, E.G. de Vries, G. L. Scherphof, J. Wilschut & T. Daemen: Activation of peritoneal cells upon *in vivo* transfection with a recombinant alphavirus expressing GM-CSF. *Gene Ther.* 8, 300-307 (2001)

53. T. Daemen, J. Regts, M. Holtrop & J. Wilschut: Immunization strategy against cervical cancer involving an alphavirus vector expressing high levels of a stable fusion protein of human papillomavirus 16 E6 and E7. *Gene Therapy* 9, 85-94 (2002)

54. K. S. Wang, R. J. Kuhn, E. G. Strauss, S. Ou & J. H. Strauss: High-affinity laminin receptor is a receptor for Sindbis virus in mammalian cells. *J. Virol.* 66, 4992-5001 (1992)

55. S. Martignone, S. Menard, R. Bufalino, N. Cascinelli, R. Pellegrini, E. Tagliabue, S. Andreola, F. Rilke & M. I. Colnaghi: Prognostic significance of the 67-kilodalton laminin receptor expression in human breast carcinomas. *J. Natl. Cancer Inst.* 85, 398-402 (1993)

56. X. Sanjuan, P. L. Fernandez, R. Miquel, J. Munoz, V. Castronovo, S. Menard, A. Palacin, A. Cardesa & E. Campo: Overexpression of the 67-kD laminin receptor correlates with tumor progression in human colorectal cancer. *J. Pathol.* 179, 376-380 (1996)

57. J. C. Tseng, B. Levin, A. Hurtado, H. Yee, I. Perez de Castro, M. Jimenez, P. Shamamian, R. Jin, R. P. Novick, A. Pellicer & D. Meruelo: Systemic tumor targeting and killing by Sindbis viral vectors. *Nat. Biotech.* 22(1), 70-77 (2004)

58. J. C. Tseng, A. Hurtado, H. Yee, B. Levin, C. Boivin, M. Benet, S. V. Blank, A. Pellicer & D. Meruelo: Using

- Sindbis viral vectors for specific detection and suppression of advanced ovarian cancer in animal models. *Cancer Res.* 64, 6684-6692 (2004)
59. E. L. Nelson, D. Prieto, T. G. Alexander, P. Pushko, L. A. Lofts, J. O. Rayner, K. I. Kamrud, B. Fralish & J. F. Smith: Venezuelan equine encephalitis replicon immunization overcomes intrinsic tolerance and elicits effective anti-tumor immunity to the 'self' tumor-associated antigen, neu in a rat mammary tumor model. *Breast Cancer Res. Treat.* 82(3), 169-183 (2003)
60. W. W. Leitner, L. H. Hwang, M. J. De Veer, A. Zhou, R. H. Silverman, B. R. G. Williams, T. W. Dubensky, H. Ying & N. P. Restifo: Alphavirus based DNA vaccine breaks immunological tolerance by activating innate antiviral pathways. *Nat. Med.* 9(1), 33-39 (2003)
61. C. A. Bolovan, N. M. Sawtell & R. L. Thompson: ICP34.5 mutants of herpes simplex virus type 1 strain 17syn+ are attenuated for neurovirulence in mice and for replication in confluent primary mouse embryo cell cultures. *J. Virol.* 68, 48-55 (1994)
62. J. Chou & B. Roizman: The  $\gamma$  1(34.5) gene of herpes simplex virus 1 precludes neuroblastoma cells from triggering total shutoff of protein synthesis characteristic of programmed cell death in neuronal cells. *Proc. Natl. Acad. Sci. (USA)* 89, 3266-3270 (1992)
63. E. A. McKie, A. R. MacLean, A. D. Lewis, G. Cruickshank, R. Rampling, S. C. Barnett, P. G. Kennedy & S. M. Brown: Selective *in vitro* replication of herpes simplex virus type 1 (HSV-1) ICP34.5 null mutants in primary human CNS tumors: evaluation of a potentially effective clinical therapy. *Br. J. Cancer* 74, 745-752 (1996)
64. R. Rampling, G. Cruickshank, V. Papanastassiou, J. Nicoll, D. Hadley, D. Brennan, R. Petty, A. MacLean, J. Harland, E. McKie, R. Mabbs & M. Brown: Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther.* 7, 859-866 (2000)
65. J. M. Markert, M. D. Medlock, S. D. Rabkin, G. Y. Gillespie, T. Todo, W. D. Hunter, C. A. Palmer, F. Feigenbaum, C. Tornatore, F. Tufaro & R. L. Martuza: Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther.* 7, 867-874 (2000)
66. X. Fu & X. Zhang: Potent systemic antitumor activity from an oncolytic herpes simplex virus of syncytial phenotype. *Cancer Res.* 62, 2306-2312 (2002)
67. H. Kasuya, T. M. Pawlik, J. T. Mullen, J. M. Donahue, H. Nakamura, S. Chandrasekhar, H. Kawasaki, E. Choi & K. K. Tanabe: Selectivity of an oncolytic Herpes Simplex Virus for cells expressing the DF3/MUC1 antigen. *Cancer Res.* 64, 2561-2567 (2004)
68. M. Toda, R. L. Martuza, H. Kojima & S. D. Rabkin: *In situ* vaccination: An IL-12 defective vector/replication-competent herpes simplex virus combination induces local and systemic antitumor activity. *J. Immunol.* 160, 4457-4464 (1998)
69. R. J. Parkinson, S. Mian, M. C. Bishop, T. Gray, G. Li, S. E. McArdle, S. Ali & R. C. Rees: Disabled infectious single cycle herpes simplex virus (DISC-HSV) is a candidate vector system for gene delivery/expression of GM-CSF in human prostate cancer therapy. *Prostate* 56(1), 65-73 (2003)
70. P. T. Loudon, C. S. McLean, G. Martin, J. Curry, M. Leigh Shaw, C. Hoogstraten, E. Verdegaal & S. Osanto: Preclinical evaluation of DISC-GMCSF for the treatment of breast carcinoma. *J. Gene Med.* 5(5), 407-416 (2003)
71. K. W. Reichard, R. M. Lorence, C. J. Cascino, M. E. Peebles, R. J. Walter, M. B. Fernando, H. M. Reyes & J. A. Greager: Newcastle disease virus selectively kills human tumor cells. *J. Surg. Res.* 52(5), 448-53 (1992)
72. A. L. Pecora, N. Rizva, G. I. Cohen, N. J. Meropol, D. Stermna, J. L. Marshall, S. Goldberg, P. Gross, J. D. O'Neil, W. S. Groene, M. S. Roberts, H. Rabin, M. K. Bamat & R. M. Lorence: Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. *J. Clin. Oncol.* 20(9), 2251-2266 (2002)
73. J. Karcher, G. Dyckhoff, P. Beckhove, C. Reisser, M. Brysch, Y. Ziouta, B. H. Helmke, H. Weidauer, V. Schirmacher & C. Herold-Mende: Antitumor vaccination in patients with head and neck squamous cell carcinomas with autologous virus-modified tumor cells. *Cancer Res.* 64, 8057-8061 (2004)
74. N. J. Nelson: Scientific interest in Newcastle disease virus is reviving. *J. Natl. Cancer Inst.* 91 (20), 1708-1710 (1999)
75. C. T. Lin, C. F. Hung, J. Juang, L. He, K. Y. Lin, T. W. Kim & T. C. Wu: Boosting with recombinant vaccinia increases HPV-16 E7-Specific T cell precursor frequencies and antitumor effects of HPV-16 E7-expressing Sindbis virus replicon particles. *Mol. Ther.* 8(4), 559-566 (2003)
76. C. L. Smith, P. R. Dunbar, F. Mirza, M. J. Palmowski, D. Shepherd, S. C. Gilbert, P. Coulie, J. Schneider, E. Hoffman, R. Hawkins, A. L. Harris & V. Cerundolo: Recombinant modified vaccinia Ankara primes functionally activated CTL specific for a melanoma tumor antigen epitope in melanoma patients with a high risk of disease recurrence. *Int. J. Cancer* 113(2), 259-266 (2005)
77. J. W. Hodge, J. P. McLaughlin, J. A. Kantor & J. Schlom: Diversified prime and boost protocols using recombinant vaccinia virus and recombinant nonreplicating avian pox virus to enhance T-cell immunity and antitumor responses. *Vaccine* 15(6/7), 759-768 (1997)

## Cancer immunotherapy

78. J. W. Hodge, D. J. Poole, W. M. Aarts, A. G. Yafal, L. Gritz & J. Schlom: Modified Vaccinia Ankara recombinants are as potent as vaccinia recombinants in diversified prime and boost vaccine regimens to elicit therapeutic antitumor responses. *Cancer Res.* 15, 7942-7949 (2003)

79. S. A. Rosenberg, J. C. Yang & N. P. Restifo: Cancer Immunotherapy: moving beyond current vaccines. *Nat. Med.* 10 (9), 909-915 (2004)

80. S. Mocellin, S. Mandruzzato, V. Bronte, M. Lise & D. Nitti: Part I: Vaccines for solid tumours. *Lancet Oncol.* 5(11), 681-689 (2004)

81. J. Gulley, A. P. Chen, W. Dahut, P. M. Arlen, A. Bastian, S. M. Steinberg, K. Tsang, D. Panicali, D. Poole, J. Schlom & J. M. Hamilton: Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. *Prostate* 53(2), 109-117 (2002)

**Key Words:** Cancer, Tumor, Neoplasia, Immunotherapy, Vaccine, Tumor Antigen, Viral Vectors, Review

**Send correspondence to:** Dr Richard Harrop, Oxford BioMedica (U.K.) Ltd., Oxford Science Park, Oxford, OX4 4GA, United Kingdom, Tel: 44 1865 783000 , Fax: 44 1865 783001, E-mail: R.Harrop@oxfordbiomedica.co.uk or Miles Carroll: miles.carroll@mnpharma.com

<http://www.bioscience.org/current/vol11.htm>