

# History of Oncolytic Viruses: Genesis to Genetic Engineering

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Since the turn of the nineteenth century, when their existence was first recognized, viruses have attracted considerable interest as possible agents of tumor destruction. Early case reports emphasized regression of cancers during naturally acquired virus infections, providing the basis for clinical trials where body fluids containing human or animal viruses were used to transmit infections to cancer patients. Most often the viruses were arrested by the host immune system and failed to impact tumor growth, but sometimes, in immunosuppressed patients, infection persisted and tumors regressed, although morbidity as a result of the infection of normal tissues was unacceptable. With the advent of rodent models and new methods for virus propagation, there were numerous attempts through the 1950s and 1960s to force the evolution of viruses with greater tumor specificity, but success was limited and many researchers abandoned the field. Technology employing reverse genetics later brought about a renewal of interest in virotherapy that allowed the generation of more potent, tumor-specific oncolytics. Here, examination of early oncolytic virotherapy before genetic engineering serves to highlight tremendous advances, yet also hints at ways to penetrate host immune defenses, a significant remaining challenge in modern virotherapy research.

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## INTRODUCTION

The years leading up to the twentieth century are traditionally considered the beginning of modern medicine. This was the time of Virchow, Lister, Koch, Pasteur, and of dramatic advances in science and technology. In the treatment of malignant disease, however, there were no appreciable developments. Cancer therapy as a field in effect simply referred to surgery, and even that was primitive. Anesthesia was just beginning to be standard practice and chemotherapy meant arsenic or castor oil.

It appears that the use of viruses in the treatment of cancer was not the result of some perspicacious theory of an alternative therapy but rather just stemmed from the observation that, occasionally, cancer patients who contracted an infectious disease went into brief periods of clinical remission. In the case of leukemia, it was well recognized that contraction of influenza sometimes produced beneficial effects.<sup>1,2</sup> Although no cases were reported where an accompanying infectious disease led to complete cure of leukemia, it was anticipated that a treatment based upon the causal agent of infection would provide an alternative to the “hopelessness of the ordinary treatment of leukemia.”<sup>1</sup>

For more than a hundred years, viruses have been pursued as experimental agents of cancer destruction. Interest in the field has fluctuated during this time, reaching fever pitch in the 1950s and 1960s, followed by near-abandonment in the 1970s and 1980s, and a resurgence of interest in the past two decades, culminating in the first marketing approval of an oncolytic virus, granted by Chinese regulators for the genetically modified oncolytic adenovirus H101 in November 2005.<sup>3</sup>

Historical perspective here is provided on the numerous approaches that were explored before the modern era of virus engineering through reverse genetics to develop non-pathogenic viruses selectively destructive to human tumor tissue (**Figure 1**).

## CANCER THERAPY: THE PAST 150 YEARS

Until the early twentieth century, cancer therapy referred to excision of the tumor by surgery. However, new treatments comprising what we would now classify as radiotherapy, chemotherapy, and immunotherapy were shortly to be introduced. Progress in the field of virotherapy came, to a large extent, from the appreciation of deficiencies in these other forms of cancer treatment, and, perhaps because of the absence of viable alternatives, promising new therapies often superseded their antecedents as the preferred method of treatment shortly after introduction.

Surgery was the predominant form of treatment until the 1900s. The evolution of surgery, however, was a slow one, stalemated until the introduction of anesthesia in 1846 and progressing only slowly thereafter.<sup>4</sup> Overwhelming were the advances in pathology, histology, cytology, and physiology, and although surgery had certainly become more aseptic, it remained in technique chiefly static. Though a surgical cure to cancers was often reported, the likelihood of success depended greatly upon a tumor being quickly diagnosed and somewhat readily accessible. For those patients for whom this was not the case, the outlook was bleak. Perhaps for this reason, upon the discovery of X-rays by Roentgen in 1895 and radioactivity by Marie Curie in 1898 and

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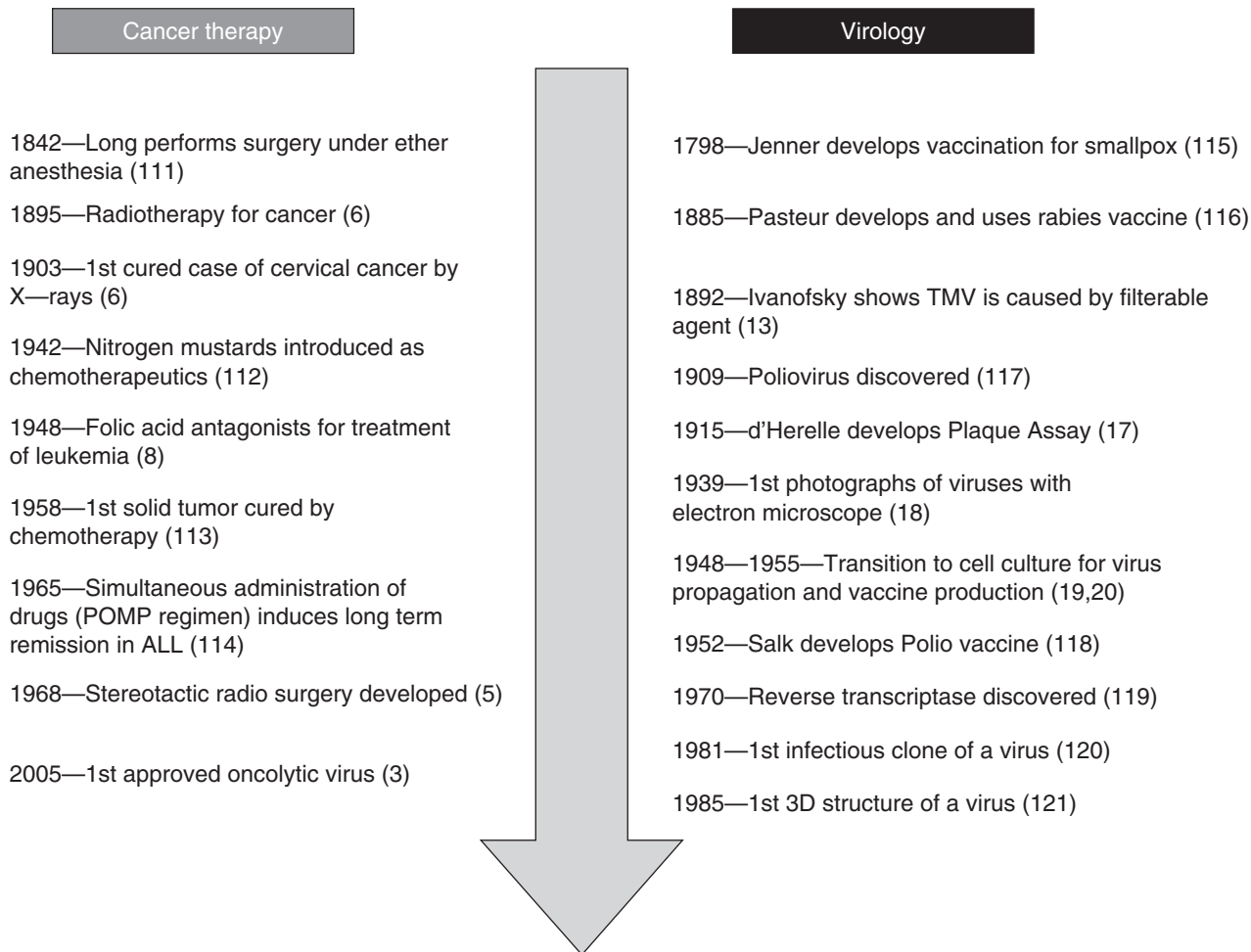


Figure 1 Milestones in the fields of virology and cancer therapy.

their subsequent application in treating cancer, radiation therapy became all the rage.<sup>5</sup>

Only three months after Roentgen published his report on X-rays, there was an account of X-rays in the treatment of breast cancer.<sup>6</sup> So fervent was the support for radiotherapy that it eclipsed surgery as the preferred method of treatment in many cancers, including head and neck.<sup>7</sup> Radiotherapy evolved from a horribly inexact science in which the large doses of radiation given were quantified by the extent of tissue damage to a targeted approach where the tumor was irradiated more specifically.<sup>6</sup> However, by the 1940s much of the initial enthusiasm for radiotherapy had diminished. Despite significant technical advances and some promising results, treatment of malignant disease with radiation had done little to improve long-term survival. Surgery, after several years on the backburner, was once again being advocated—as was chemotherapy.

Chemotherapy blossomed seemingly overnight with the introduction by Farber *et al.* in 1948 of the folic acid antagonist aminopterin for the treatment of leukemia.<sup>8</sup> Previously, heavy metals and nitrogen mustards had been used, but with no striking effects. As Farber's therapy was the first to bring about regular remissions, chemical compounds for the treatment of cancer became much sought after. Steroids such as cortisone<sup>9</sup> and

prednisone<sup>10</sup> were produced shortly thereafter, and antagonists of nucleic acids followed.<sup>11</sup> Chemotherapy was indeed an effective modality for leukemia treatment. It did not follow that this was the case for other cancers, however. Although hematological malignancies appeared susceptible to many chemotherapeutics, solid tumors were negligibly affected. As with its predecessor technologies, by the middle of the 1950s, there was uncertainty that chemotherapy alone would prove a panacea as a cancer treatment.

The early 1900s produced significant advances in cancer therapy—but no cures that were applicable to all tumors at all stages. This was a time of many scientific advances, but a growing skepticism about the future of cancer therapy. Tumors could not be removed all the time; they could not always be killed with high-energy beams of radiation or with poisons delivered intravenously; even the combination of these modalities proved insufficient. It was time for more investigation and a return to the pathogens that produced some of the first reports of remission.

#### VIROLOGY: THE PAST 150 YEARS

Viruses began to be employed for cancer therapy at the end of the nineteenth century, but despite the high number of

infectious diseases known to be of viral etiology at that time, there was no real concept of the nature of a virus. The “discovery” of viruses is difficult to pinpoint. Martinus Beijerinck reported in 1898 that after Chamberland candle filtration (through which bacteria could not pass), the agent causing tobacco mosaic disease could amplify itself in living, growing plant tissue.<sup>12</sup> His work built on the prior observations of Adolf Mayer and Dimitri Ivanofsky<sup>13,14</sup> and provided at least an operational definition of a virus, which he labeled a *contagium vivium fluidum*. Foot and mouth virus, in 1898, was the first “filterable agent” to be implicated in animal disease,<sup>15</sup> and yellow fever virus, in 1901, was the first to be implicated in human disease.<sup>16</sup> Biochemical analysis of viruses proceeded apace, but it was not until the advent of the plaque assay in 1917 that their particulate nature could be proven,<sup>17</sup> and electron microscopic images of viral particles were not obtained until 1939.<sup>18</sup> The first half of the twentieth century was something of a dark age for virology. Although agents that could pass through filters had been implicated in many diseases (e.g., polio, rabies, influenza), their precise identity was still unclear.

Understanding of viruses accelerated rapidly in the 1950s and 1960s, in large measure because of the advent of cell and tissue culture systems that allowed *ex vivo* virus propagation.<sup>19,20</sup> It is no coincidence that this was also a time of intensive virotherapy research when the oncolytic properties of numerous viruses were evaluated, first in human tumor cell lines, often implanted in immunosuppressed rodents, and subsequently in humans. During the past fifty years, viruses have been studied with such unparalleled intensity that their biology is now understood more thoroughly than that of any other organism in nature. Their genomes and proteins have been sequenced; their physical structures are known, as are many of the mechanisms whereby their genomes are regulated; their diversity is recognized; their replication cycles and pathogenetic strategies have been elucidated; and methods have been developed to manipulate their genome sequences to permit their further refinement as anticancer agents.

#### EXPERIMENTS OF NATURE: TUMOR REGRESSIONS DURING NATURALLY ACQUIRED VIRAL INFECTIONS

Since the mid-1800s, there has been a steady trickle of case reports where tumor regressions have coincided with natural virus infections.<sup>1,2,5,21</sup> Most often the patients in question were suffering from hematological malignancies such as leukemia or lymphoma, known to be associated with significant suppression of immune function. In addition, the remissions were short-lived, typically lasting only one or two months. In one very widely cited example, Dock<sup>1</sup> described a 42-year-old woman with “myelogenous leukemia” that went into remission after a presumed influenza infection. The report was made in 1896, 37 years before it was determined that influenza was a virus infection. The woman had a greatly enlarged liver and spleen, which shrank to nearly normal size, and a grossly elevated leukocyte count, which dropped more than 70-fold after the infection. In another case, chickenpox led to the regression of lymphatic leukemia in a 4-year-old boy.<sup>5</sup> Before the onset of chickenpox he was not receiving anti-leukemic therapy, his liver and spleen were enormous, he had enlarged cervical lymph nodes, and his leukocyte count was grossly elevated

(200 lymphoid cells/ $\mu$ l). Within a few days of his developing the classical varicella rash, his spleen and liver returned to near-normal size, his white count fell to normal (4.1 cells/ $\mu$ l), the differential count normalized, his platelet count and hemoglobin increased significantly, and examination of his bone marrow confirmed that he was in remission. Unfortunately, the remission lasted only one month, and his leukemia progressed rapidly until death.

More recent clinical reports have described the regression of leukemia,<sup>22,23</sup> Hodgkin’s disease,<sup>24,25</sup> and Burkitt’s lymphoma<sup>26</sup> concomitantly with measles infection. One can ascertain from these and similar case reports where remissions coincided with other naturally acquired viral infections, such as hepatitis, glandular fever, and measles,<sup>22–28</sup> the following: (i) under the right circumstances, certain viruses can destroy tumors without causing undue harm to the patient; (ii) virus-mediated tumor regression has most often been seen when the patient is young and has a compromised immune system, *i.e.*, is suffering from leukemia or lymphoma; (iii) virus-induced remissions have generally been short-lived and incomplete.

#### HUMAN PATHOGENS

Exploiting viruses for therapeutic gain was first suggested at the beginning of the twentieth century, yet attempts at implementation remained sporadic<sup>29–31</sup> until nearly fifty years later, when clinical testing began in earnest. In the context of current ethical standards, some of the clinical studies performed at that time seem quite alarming, as the therapeutic material administered to cancer patients often consisted of infectious body fluids or infected tissue harvested from patients with ongoing virus infections, some of which were quite serious. However, these were desperate times for those afflicted with cancer.<sup>32–34</sup>

Hepatitis viruses were among the first to be used for therapy. As early as 1897 it had been noticed that viral hepatitis had ameliorating effects on a variety of human diseases.<sup>28</sup> Then in 1949, when two patients with Hodgkin’s disease were observed to go into brief remission after contracting viral hepatitis, clinical trials were undertaken in which 22 patients suffering from Hodgkin’s disease were treated by administration of a total of 35 sera or tissue extracts containing “the hepatitis virus” (Table 1).<sup>32</sup> The patients who contributed sera and tissue samples for these studies were suffering either from infectious hepatitis, a self-limited picornavirus infection, or from serum hepatitis, most likely as a result of hepatitis B. Undissuaded by the death of the first patient being treated with the regimen of multiple inoculations of hepatitis serum, Hoster *et al.* expanded the experimental group to a further 21 patients. Of these, 13 developed hepatitis and 7 were reported to show improvement lasting at least one month in one or more aspects of their disease. Death directly attributed to viral hepatitis was reported to occur on occasion, yet the number of patients to whom this pertains is undisclosed.

Owing perhaps to the suggestion of success given by Hoster *et al.* and additional evidence pointing to remission of monocytic leukemia in a patient naturally infected with Epstein–Barr virus, clinical trials were undertaken in the United Kingdom using glandular fever serum for the treatment of acute leukemia.<sup>27</sup> In this case, the results were a little more encouraging, as three of five treated patients who acquired symptoms of glandular

**Table 1 Clinical virotherapy: four historically significant clinical trials**

Year(s)	Virus	Disease	No. of patients	Administration	Outcome	Side effect
1949	Hepatitis B virus <sup>32</sup>	Hodgkin's disease	22	Parenteral injection of unpurified human serum, tissue extract	14/22 developed hepatitis; 7/22 improved in clinical aspect of disease; 4/22 reduction in tumor size	Fever, malaise, death (1 confirmed)
1952	Egypt 101 virus (early passage West Nile) <sup>36</sup>	Advanced, unresponsive neoplastic disease	34	IV, intramuscular injection of bacteriologically sterile mouse brain, chick embryo, human tissue	27/34 infected; 14/34 oncotropism; 4/34 (transient) tumor regression	Fever, malaise; mild encephalitis (2 confirmed)
1956	Adenovirus adenoidal-pharyngeal-conjunctival virus (APC) <sup>50</sup>	Cervical carcinoma	30	IT, IA, IV injection of TC supernatant	26/40 inoculations resulted in localized necrosis	Vaginal hemorrhage; infrequent (3/30) fever, malaise
1974	Mumps virus (wild-type, non-attenuated) <sup>56</sup>	Terminal cancers; gastric, pulmonary, uterine account for more than 50%	90	External post-scarification; IT; IV; oral; rectal; inhalation of purified human saliva or TC supernatant	37/90 complete regression or decrease >50%; 42/90 decrease <50% or growth suppression; 11/90 unresponsive	7/90 adverse reactions: bleeding, fever

Abbreviations: IA, intra-arterial; IT, intratumoral; IV, intravenous; TC, tissue culture.



Chester Southam

fever did indeed go into remission, albeit briefly. Side effects attributed to the treatment were comparatively minor and of limited duration.

After these early clinical studies, many different human pathogens were administered to cancer patients during the next two decades. Also at this time, Alice Moore, who pioneered the testing of oncolytic viruses in animal cancer models (see below), teamed up with Chester Southam, now considered a rather overzealous clinical oncologist,<sup>35</sup> at Memorial Sloan-Kettering hospital. As the dominant players of that era in the field, Southam and Moore<sup>36-44</sup> contributed much in the form of both preclinical trials with animal models and clinical trials employing oncolytic viruses, but perhaps even more so in terms of cautionary tales of transitioning from bench to bedside.

With mosquitoes rampant across the United States and abroad, flavivirus infections such as West Nile, Uganda, dengue, and yellow fever were exceedingly common and were thus some of the first employed for virotherapy.<sup>36-38,40-42,45,46</sup> The Egypt 101 isolate of West Nile virus was used in more than 150 virus therapy

trials against a wide range of cancers (**Table 1**).<sup>36,38</sup> Viremia and intra-tumoral virus replication were confirmed in most patients, but tumor responses were rare. Immunosuppressed patients with leukemia or lymphoma were more likely to respond to therapy, but were also at higher risk of fatal neurotoxicity. Thus, of eight patients with leukemia or lymphoma, five experienced severe encephalitis. With limited success in controlling the neurotoxicity of the aforementioned agents, large-scale screens of putative oncolytics were carried out. Most were abandoned as lacking in efficacy or safety, however, and a gradual shift of emphasis occurred such that adenoviruses, herpes viruses, paramyxoviruses, picornaviruses, and the pox viruses eventually emerged as the new favorites.

Identified as an oncolytic agent in preclinical models in the 1950s, adenoidal-pharyngeal-conjunctival virus (APC, now known to be an adenovirus) quickly progressed to the bedside and was found to have relatively modest side effects: those who were administered APC occasionally developed inflammation of the eye or pharynx, but were encephalitis-free and, better yet, alive after inoculation.<sup>47</sup> Overcoming this first obstacle, APC rapidly made its way to clinical trials for the treatment of cervical cancer.<sup>48</sup> By intravenous, intravascular, or intra-arterial routes, APC was administered to 30 patients with advanced epidermoid carcinoma of the cervix (**Table 1**). In two-thirds of the cases, areas of necrosis were present in tumors within 10 days and, most remarkably, appeared to be confined to the cancerous tissue (though no biopsies were performed to test for viral recovery in the surrounding tissues). In those who responded to APC administration, cancerous tissue was shed in large amounts. Although the APC oncolytic produced striking effects, liquefying, causing severe hemorrhaging, and frequently a generalized necrosis specifically to the site of the tumor, infections were quickly eradicated by the host immune system and survival was not significantly prolonged. More than half of those who were treated with APC died within a few months of the beginning of the trial, all from the primary disease. As

expected, responses were diminished in patients with pre-existing anti-adenovirus antibodies, emphasizing the problem of premature immune-mediated virus elimination. Though investigation into adenovirus for the treatment of cervical cancer continued,<sup>49,50</sup> in the absence of any useful prolongation of survival, it slowed to a trickle.

Picornavirus implementation for oncolysis briefly came into vogue thereafter. In one study, published in 1957, tumor xenografts were established by intraperitoneal inoculation of HeLa cells in rats that had first been irradiated and treated with cortisone. After several adenoviruses, enteroviruses (Coxsackie A, B, and enteric cytopathic human orphan viruses), vaccinia, and vesicular stomatitis virus were tested for intra-tumoral amplification in this model, Coxsackie B3 virus emerged as the winner.<sup>51</sup> Poliovirus was later used and was shown to cause necrosis and regression in guinea pigs carrying HeLa tumors without any appreciable side effects,<sup>52</sup> yet data still were lacking for an oncolytic virus that was efficacious in human trials.

With enthusiasm for adenovirus as an experimental therapeutic starting to ebb, with no data on picornaviruses in clinical trials equal to what had been shown in rodent models, and with use of the neurotropic viruses of antiquity still out of the question to the circumspect a penchant emerged for a virus that was already widespread and with which there was a substantial amount of experience as a pathogen—thus, the paramyxovirus mumps.

Mumps virus was initially used not as an oncolytic agent but, similarly to some other viruses,<sup>53,54</sup> as an agent of immunotherapy to stimulate the immune system of those who did not respond to a combinatorial treatment of surgery plus chemotherapy plus BCG vaccine for metastatic melanoma.<sup>55</sup> Inoculation with killed mumps virus did appear to enhance tumor regression, but the virus garnered much more attention when it was allowed to replicate. In what was certainly a massive clinical trial for the time, Asada used non-attenuated mumps viruses in Japan to treat 18 types of tumors (Table 1).<sup>56</sup> Delivery methods ranged from the ubiquitous intravenous and intravascular administration to rather peculiar methods employing bread or pieces of tampon orally administered after soaking in virus-containing supernatant. They were extended to include topical application after scarification of the skin, rectal administration, and inhalation. Lest the results be too straightforward, the mumps virus itself was obtained from various sources, including the saliva of infected patients and infected cultures of monkey or human embryonic kidney cells. Despite the utter lack of controls for these studies, an admitted limited quantity of the virus itself, and the fact that most of the patients undergoing mumps therapy had neutralizing antibodies, Asada's results as reported were among the most dramatic ever seen. Minimal toxicity was reported in mumps therapy, and in 37 of 90 patients treated, the tumor regressed completely or decreased to less than half of the initial size. Indeed, all save 10% of the treated patients were reported to have some appreciable response to oncolytic mumps virus therapy. The treatment often produced these effects within a few days of the first administration, before the spread of the virus was brought to a halt by a strong anamnestic anti-mumps immune response. Boosting of anti-tumoral immunity was also reported in a number of cases. After the euphoria of Asada's initial clinical experience with

mumps virus therapy, the subsequent performance of mumps seemed lackluster by comparison.<sup>57-60</sup>

Perhaps because of the regulatory barriers that would have had to be confronted if non-attenuated pathogens were to become a standard anticancer therapy, there followed a slump in reported clinical trials employing oncolytic viruses, with the number decreasing rather dramatically in the 1970s and 1980s. Nonetheless, the dream of effective oncolytic virotherapy persisted, but now with focus shifting to viruses with diminished pathogenic potential.

## IN VIVO CANCER MODELS: VIROTHERAPY BECOMES A SCIENCE

*Ex vivo* culture of human cells had become possible in 1948 (ref. 61), and attempts to implant these cells into laboratory rodents followed, providing the first opportunity to test the *in vivo* anti-tumor activity of an oncolytic virus under controlled laboratory conditions. Moore, the first to investigate oncolytic viruses using newly developed rodent cancer models, used an *in vivo* tumor model to demonstrate conclusively that an oncolytic virus, in this case Russian Far East encephalitis virus, could selectively seek out and destroy cancer cells in a living animal, reporting her initial findings in 1949. She found that, in certain instances, the mouse sarcoma 180 could be completely destroyed,<sup>37</sup> a landmark for virotherapy. In an effort to extend these results to other tumors, Moore tested the virus against five mouse tumors of various origins<sup>40</sup> and found that it was indeed able to cause complete regression, provided the dose was sufficient. In addition, after infection, tumors could no longer be transplanted to other immunocompetent mice. Although infection with the Russian Far East encephalitis virus did eventually cause fatal encephalitis in the mice, proof of principle for its extraordinary oncolytic potency had been given and much interest was spurred in the field.

In the years that followed, many other human pathogens were investigated for oncolytic activity employing rodent models, including Bunyamwera, Ilheus, dengue, yellow fever, West Nile virus<sup>41,42</sup> and its Egypt 101 isolate,<sup>36</sup> Semliki Forest virus, mumps, vaccinia,<sup>62</sup> and adenovirus.<sup>48,63</sup> As previously discussed, many of these were also evaluated in clinical trials that served primarily to demonstrate that complete tumor regression was much more likely to occur in the mouse than in the patient,<sup>41,48,62,63</sup> bringing into question the relevance of the responses seen in heterotransplanted cancer tissues in the mouse model. Nevertheless, proof of activity in rodent models quickly became a necessary step to establish proof of principle for oncolytic activity of newly identified oncolytic viruses before clinical testing.

It is difficult to speculate on the impetus, but perhaps in an attempt to validate the mouse model, Southam and Moore abandoned briefly the use of mice and began transplanting human tumor cell lines into purported human volunteers before treating them with oncolytic viruses.<sup>39,64</sup> Tissue cultures of HeLa, HEP#1, HEP#2, HEP#3, J-111, and HS#1 cell lines were implanted by subcutaneous inoculation in the forearm, both in normal control patients and in 22 cancer patients. Subsequently, virus replication in tumor implants of cancer patients was compared with replication in healthy adults, and antiviral antibody responses were evaluated. The tumor cell lines were rejected by normal recipients, usually

within 3 weeks of implantation, but in 20 of the 22 cancer patients they survived and grew at the sites of implantation, although typically regressing completely by week 6. In a small number of cancer patients, regression was not seen and the tumors had to be excised, sometimes more than once as they tended to regrow at the site of excision. In at least one instance there was clear evidence of lymphatic spread to the axillary lymph nodes. The studies attracted quite harsh criticism from some quarters<sup>35</sup> and may have served to tarnish the entire field of research. It is unclear whether any of the results significantly advanced our understanding of the propagation of oncolytic viruses in cancer patients.

### CIRCUMVENTING PATHOGENICITY: USING ANIMAL VIRUSES FOR HUMAN THERAPY

In an effort to control virulence and at the same time avoid the problem of rapid virus elimination resulting from pre-existing antiviral immunity, it was initially hypothesized that a non-human animal virus might retain oncolytic activity even in a host not traditionally susceptible to that particular virus. In support of this theory was Moore's early work with Russian Far East encephalitis virus, a human pathogen showing activity against a murine tumor.<sup>40</sup>

In one early study, a high-throughput screen for non-human animal viruses that possessed oncolytic activity was conducted against a panel of human cancer cell lines. Adaptation by *in vitro* passing in this case was still assumed to be advantageous as the parental strain was either non-infectious or non-pathogenic in humans. Therefore, in the absence of normal cells, it was supposed that the viruses would acquire no additional tropism for cells other than those in which they were adapted.<sup>65</sup> Six animal viruses were identified that had the ability to propagate in human cell lines out of 24 candidates.<sup>65</sup> Even with the identification of these putative apathogenic oncolytics, Hammon *et al.* retained an air of despondency toward the use of viruses in cancer treatment. With chemotherapy still in its fledgling stages, however, the authors expressed a sense of obligation to explore virotherapy as it seemed as promising as any experimental therapy.

In a rigorous screening of the six viruses, Yohn *et al.* evaluated oncolytic potential by the ability to inhibit heterotransplanted human tumors from growing or by causing necrosis and regression of the tumors that did. Of these, two herpes viruses non-pathogenic in humans (equine rhinopneumonitis and infectious bovine rhinotracheitis) were identified as oncolytic for one or more human tumors.<sup>66</sup>

Arenaviruses have been little employed as oncolytics after the rather disappointing clinical performance of a virus referred to as the "M-P" virus (after the authors Molomut and Padnos), now identified as a strain of lymphocytic choriomeningitis virus. Par for the course in virotherapy, the M-P virus brought about dramatic tumor regressions in rodent models, increasing survival by more than 60% over controls in certain cases<sup>66</sup> yet offered little therapeutic benefit in human clinical trials, failing to prolong survival.<sup>67</sup>

In addition to herpes and arenaviruses, the use of avian viruses as oncolytic agents excited a great deal of interest in the 1960s, some of which has continued to this day. Among the first of the avian viruses used for cancer therapy was the avian plague

virus. Avian plague virus, presumed to be less pathogenic than many of the neurotropic viruses used by Southam and Moore,<sup>42</sup> was inoculated in sarcoma 97 or epithelioma tumors of the mouse. Although the virus brought about a reduction in tumor size and a transient remission of sarcoma 97 tumors, it rapidly localized to the brain in mice with epithelioma MI, ultimately resulting in death. The observation that nearly every virus identified to date had a neurotropism as strong or stronger than any tropism toward the tumor certainly gave reason to question what, if any, ameliorative effects viral therapy could really produce.

Hope for success, however, came with the testing of another avian virus, Newcastle disease virus (NDV). Although some complete tumor regressions had been seen in virotherapy previously, in nearly every instance the experimental animal died of infection caused by the virus.<sup>37,38,41</sup> In this instance, NDV was inoculated intraperitoneally in animals with Ehrlich ascites carcinomas and was found to be curative with no ill effects that could be attributed to the virus.<sup>70</sup> Even upon rechallenge, nearly 90% of mice were found to be immune to subcutaneous injection.<sup>71</sup> Newcastle disease virus continues to be used in cancer therapy and has been reported in follow-up studies to provide remissions lasting at least 10 years.<sup>72,73</sup>

Whereas much attention had been given to viral adaptation being advantageous for targeting, not much focus had yet been placed on adaptations that a non-human pathogen might acquire that could increase its virulence in a host not normally susceptible. Introduction of wild-type viruses in a traditionally naive host where populations have not evolved any resistance to the virus would today be considered quite risky. Indeed, one virus that had been used in virotherapy, feline panleukopenia virus,<sup>5</sup> later evolved independently to be transmissible to dogs, resulting in the pandemic canine parvovirus that is believed to have infected more than 80% of wild and domestic dogs between 1978 and 1979 across the world.<sup>74</sup>

For these and other reasons, few animal viruses are still pursued as oncolytic agents today. However, there are exceptions, such as vesicular stomatitis virus, an impotent pathogen of domestic cattle belonging to the Rhabdovirus family. This virus is selectively destructive to human tumor cells with interferon pathway defects<sup>75</sup> and centuries of human exposure to vesicular stomatitis virus-infected cattle has led to little more than occasional cases of conjunctivitis, implying it is quite unlikely to evolve to human pathogenicity when administered as an oncolytic. For similar reasons, NDV is also considered safe for human applications.

### ADAPTATION, ATTENUATION, AND ENGINEERING: BUILDING A BETTER VIRUS

Viruses appeared to have tremendous potential but needed manipulation to be targeted more specifically to cancerous cells. Thus began the era of adaptation and, ultimately, genetic engineering of viruses. It was recognized early that viruses were capable of adapting themselves for replication in specific tissues. Because direct manipulation of the viral genome was not yet possible, Southam, Moore, and others<sup>41,65,66</sup> tried to apply this property to a targeted evolution of sorts—making the virus more oncolytic or more tumor-specific. The technical foundation for this

concept came from the observation of Levaditi *et al.* in 1922 that a smallpox vaccine (vaccinia virus) was able to inhibit various tumors in rats and mice.<sup>30</sup> Subsequently, Pack reported in 1950 that he had observed a long remission of metastatic melanoma in a patient who was vaccinated against rabies after a dog bite.<sup>53</sup> He therefore went on to treat an additional 12 patients with metastatic melanoma by repeated intramuscular injection of rabies vaccine and saw two responses. The closing paragraph of his report states, "I am motivated in publishing this incomplete and unsuccessful story at this premature time by the unfortunate fact that news of this experiment has become widely disseminated. I have been besieged by innumerable telegrams, letters and telephone calls begging for more specific information." Clearly, the need for effective cancer therapy in 1950 was dire.

By 1952, Moore was already utilizing the adaptive capacity of viruses to enhance oncolytic activity. Perturbed, perhaps, by the differing oncolytic activity of a given virus in tumors of various origins, Moore hypothesized that successive passage of a virus with known oncolytic activity in a mouse tumor might increase its destructive capacity for that tumor. Indeed, this was found to be the case, as Russian encephalitis virus had greater oncolytic activity after 20–30 passages in sarcoma 180 tumors than the parental strain.<sup>41</sup> Moore had surmised that some progeny of the parental virus would acquire mutations that could be beneficial to replication specifically in those cells in which they were propagated. Thus, the era of viral manipulation had begun, even if at this time viruses themselves were doing the majority of it.

Even before recombinant DNA techniques became available, it was suggested that the alteration of the viral genome could provide improved targeting of oncolytic viruses.<sup>44</sup> Although Moore had developed a form of targeted evolution by propagation in a tumor, direct engineering of viruses was not yet possible. As there had been little success in excluding viruses from specific tissues, efforts began to interfere with their tropism for sites other than tumors—especially to the brain, which caused death in a number of cases. Chemical interference with neurotropism had been suggested previously,<sup>44</sup> and although there was some evidence to suggest that drugs and small molecules were somewhat efficacious in the treatment of viral disease,<sup>76–79</sup> exclusion of viral replication in only specific tissues was regarded as quite difficult and was little investigated.

In an effort to block the neurotropism yet retain the anti-tumor potency of a primary oncolytic virus, a second virus, NDV, was employed as an agent of interference.<sup>80</sup> The concept was that the brain, after infection with a non-pathogenic virus, would be resistant to invasion by an oncolytic virus administered concurrently. Although the data seemed to suggest that inoculation of NDV intra-cerebrally before infection with the active oncolytic agent did prolong survival by a short time, it offered little total protection. More disappointing, the virus that had been previously shown to be most efficacious in cancer therapy, the Egypt 101 isolate of West Nile virus, was negligibly attenuated by NDV.

With limited success in virus adaptation or with viral interference to reduce the pathogenicity of oncolytic viruses, focus shifted to the manipulation of the viral genome. Before the recombinant

DNA revolution, it was first demonstrated in 1968 that genetic alteration of a viral genome was possible when polynucleotides were added to the tobacco mosaic virus genome,<sup>81</sup> leading to expression of polylysine. However, it was not until the early 1990s, when recombinant DNA technology became standard, that virus engineering could provide any scientific furtherance of virotherapy. Not until more than thirty years after the suggestion by Southam of viral attenuation by genomic alteration of an oncolytic virus<sup>44</sup> did the first studies take place in which an engineered virus was employed for cancer therapy.

At the start of the 1990s, the engineering of viruses was in full swing, and virus-based gene therapy was underway for severe combined immunodeficiency,<sup>82,83</sup> liver failure, hemophilia,<sup>84</sup> and other diseases. Also, the specter of ethically questionable clinical trials employing oncolytic viruses was by now in the distant past. Enter Martuza and the treatment of malignant glioma using a mutant herpes simplex virus (HSV).

In contrast to "suicide" gene therapy that employed the thymidine kinase gene to render tumor cells sensitive to the pro-drug ganciclovir,<sup>85</sup> Martuza's approach was to completely remove it from the HSV genome. Although a mutant HSV lacking the enzymatic activity of thymidine kinase had been isolated almost twenty years previously,<sup>86</sup> not until Martuza *et al.*'s work was its potential as a cancer therapeutic appreciated. By extending on the observation that a thymidine kinase-negative HSV replicated in dividing cells but was crippled in non-dividing cells, Martuza was able to apply HSV-thymidine kinase to treat malignant gliomas by intra-cerebral inoculation in mice, thereby eradicating tumors completely.<sup>87</sup> Although the historical problem of encephalitis still lingered to some degree, nearly one-third of treated mice were spared.

Recombinant technology, when it arrived, focused predominantly on engineering adeno-,<sup>88,89</sup> paramyxo-,<sup>90,91</sup> herpes,<sup>92</sup> picorna-, and poxviruses.<sup>93,94</sup> Yet, even with the newfound ability to engineer viral genomes to produce a new generation of safer, specific oncolytics, a true therapeutic frontrunner has yet to emerge. This is likely due not to inherent problems with the viruses now in circulation, but rather to their rapid clearance by the host immune system.

## CONCLUSIONS

Under the right circumstances, viruses are capable of destroying tumor tissue in human cancer patients. Remarkably, for certain human pathogens, the damage inflicted on tumors is far more significant than the damage inflicted on normal host tissues. However, because of their pathogenicity, most human viruses cannot be considered suitable as drugs. Possible exceptions include Coxsackie virus A21, an oncolytic human picornavirus whose pathogenicity is limited to mild upper respiratory tract infections,<sup>95</sup> and human reovirus, which is oncolytic in preclinical models but apparently apathogenic in infected humans.<sup>96</sup>

Fortunately, most viruses can now be adapted or engineered to eliminate their pathogenicity without destroying their oncolytic potency. Some animal viruses lack pathogenicity in humans but are nevertheless capable of destroying human tumor tissue. The main impediment to using animal viruses for human cancer therapy is the ever-present risk of virus evolution giving

rise to a new human pathogen able to spread from the patient to non-immune contacts. This risk is not easy to quantify, and it is noteworthy that certain viruses of animal origin (e.g., NDV) have been administered so frequently to humans without adverse consequences that they are now considered to be safe platforms for the development of oncolytics.<sup>70–73,80,97</sup>

Most of the oncolytic viruses currently in clinical testing are attenuated derivatives of prevalent human pathogens. Typically, in recent years, they have been genetically engineered to further attenuate their pathogenicity, increase their oncolytic potency, or enhance their specificity for cancer tissue. Measles virus, for example, was first attenuated by serial passage in cultured cells, then genetically engineered to enhance its oncolytic potency and tumor specificity.<sup>98–102</sup> By and large, through a variety of mechanisms that have recently been reviewed<sup>103–110</sup> these clinically tested oncolytic viruses have shown strong specificity for neoplastic tissue.

With specificity addressed, a major remaining weakness of current oncolytics is their vulnerability to antiviral host defenses, in that they are usually eliminated from the body before they have had a chance to cause substantial damage to the tumor. Thus, the viruses run into resistance from innate and acquired antiviral immune responses, including the release of interferons, activation of natural killer cells, amplification of antigen-specific cytotoxic T cells, and the secretion of high-affinity antiviral antibodies. These host responses to virus invasion have long been recognized as major impediments to virus-mediated tumor destruction, but clinical trials of old suggest that oncolytic viruses can be effective provided the immune response is suppressed. Current scientific endeavors to develop strategies to control or circumvent these antiviral responses, therefore, seem highly prudent. With studies well underway to address specificity, delivery, and potency, there is a justified sense of optimism in the field and a feeling that the challenges of antiquity are finally (or soon to be) history.

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