Getting oncolytic virus therapies off the ground

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An international meeting was held on the development and application of replicating viruses for cancer therapy this past March in Banff, Alberta. In this review, using the presentations at this meeting as a backdrop, we discuss how recent scientific and clinical findings are reshaping the development of oncolytic virus therapeutics. Here we identify some of the obstacles that these therapeutics face and discuss evolving strategies, both preclinically and clinically, that are facilitating oncolytic virus development.

Oncolytic viruses are therapeutics that have either been naturally selected or engineered to specifically grow in and kill tumor cells. In general, oncolytic viruses derive their specificity by exploiting cell surface or intracellular aberrations in gene expression that arise in malignancies during tumor evolution. As David Kirn (Oxford University Medical School, UK) pointed out, while conventional chemotherapeutics are currently the best frontline treatments available, they are limited in their utility by relatively narrow therapeutic windows that rapidly close as tumors evolve drug resistance mechanisms. New small molecules targeted to oncogenic proteins have greatly reduced toxicity in normal tissues, but the very nature of their specificity may limit their long-term usefulness. Oncolytic viruses, on the other hand, are multimodality therapeutics that can be engineered to have the tumor specificity of a small molecule, the potent cell killing of a chemotherapeutic, the ability to arouse the host immune system against tumor antigens, and an innate capacity to stimulate the production of host cytokines that have potential anticancer activity. Perhaps the greatest advantage that the oncolytic virus platform offers over chemical agents is its ability to be tailored by in vitro genetic manipulation in response to preclinical and clinical findings.

Are current oncolytic viruses too attenuated?

Those developing oncolytic virus therapeutics are understandably concerned about the safety of these new agents. The clinical trials performed to date using a variety of oncolytic virus candidates demonstrate that these agents can be very safe; however, some oncolytic viruses are so attenuated that maximum tolerable doses, and perhaps efficacious doses, cannot be achieved (Markert et al., 2000; Varghese and Rabkin, 2002). The early strategies for attenuating viruses often involved deletion of entire genes, but in retrospect, this may have been overkill. Virus gene products are often multifunctional, and it is now recognized that it is desirable to engineer subtle mutations that delete only specific functions required for replication in normal cells but retaining activities required for overall efficient virus replication and spread. An example is the E1B deleted oncolytic adenovirus, Onyx-015. The best understood function of E1B is the inactivation of the cellular p53 protein, an observation that was thought to be the cornerstone of the Onyx 015 technology (Heise et al., 1997). The E1B gene product is also essential for the nuclear export of late transcripts that are required for robust adenovirus replication, including the L4 100K transcript (Babiss et al., 1985). The 5′UTR of L4 resembles that of the HSP 90 mRNA, and Frank McCormick (University of California at San Francisco) pointed out that the defect of L4 mRNA transport from the nucleus of Onyx 015 infected cells can be complemented by heat shock. Indeed, it has been shown previously that E1B deleted adenoviruses are cold-sensitive and replicate as well as wild-type adenovirus at 39°C (Goodrum and Ornelles, 1998). This provides an interesting new wrinkle to the therapeutic application of Onyx 015, as transient temperature spikes in patients may facilitate virus growth and killing of tumor cells. Whether this holds true or not, perhaps the best strategy is to create point or discrete mutations in E1B that abrogate p53 degradation but permit the other activities of E1B to remain intact (Shen et al., 2001). In an alternative approach, Heise and collaborators (Heise et al., 2000) have generated an adenoviral strain with a discrete E1A-CR2 deletion that outperforms other oncolytic viruses in preclinical models, underscoring the idea that perhaps "less is better" when it comes to attenuating viral therapeutics.

The initial clinical trials with HSV oncolytic therapeutics have proven safe, but in these studies, the maximum amount of virus that can be produced or delivered may be limiting efficacy, prompting Bob Martuza (Harvard Medical School) to coin the term "maximum affordable dose" as opposed to maximum tolerable dose. New engineered versions of HSV that have increased cytolytic or fusogenic properties are being created and appear more efficacious while remaining safe (Fu and Zhang, 2002; Todo et al., 2001).

Many of the adenoviral vectors used for oncolytic therapy have deletions in viral genes that function to suppress the host immune response or have impaired cytolytic activity. Terry Hermiston (Berlex Biosciences) pointed out, oncolytic viruses not only have to be safe and tumor-selective in their growth but, to be effective therapeutics, they must overcome obstacles of hypoxia, host immunity, physical barriers (e.g., normal stroma), and clearance and resistance mechanisms that may develop within the tumor milieu. This led to the idea of "arming" replicating oncolytic viruses with "payloads" that will enhance virus delivery, spread, and efficacy (Bauzon et al., 2003). As an example, Bill Wold (St. Louis University School of Medicine) and colleagues have shown that by reinserting the viral ADP gene (the "adenovirus death protein" which is required for efficient cell lysis) they have created an oncolytic virus that is more cytolytic (Doronin et al., 2000, 2003) and has an increased ability to spread between cells. Another approach is to include a transgene (e.g., TNFα or TRAIL, two apoptosis-inducing cytokines) that would enhance the cytolytic properties of the therapeutic virus (Lin et al., 2002; Rasmussen et al., 2002).

These are characteristics that, when coupled with tumor selectivity, are likely to create viruses that have increased therapeutic efficacy.

Targeting the tumor cell surface

Many strategies are being developed for targeting tumor cells with oncolytic viruses. One approach is to use a viral agent that binds to a very broadly or ubiquitously expressed cell surface antigen and restrict virus growth following entry into the cell (e.g., VSV, herpes, NDV or adenovirus). This strategy is a good one unless the virus receptor is lost during cancer cell evolution (Jee et al., 2002) or is so strongly expressed or accessible that one organ can serve as a sink and bind up the majority of a therapeutic dose. Several groups are developing ways to detarget the adenovirus from its normal cellular receptor (the CAR or "coxsackie/adenovirus receptor") and retarget tumor cell surfaces with single chain antibodies, growth factors, or peptides (Wu et al., 2002). Using the oncolytic measles virus platform, Stephen Russell and Roberto Cattaneo (Mayo Clinic) are close to achieving the goal of creating a virus that binds only to tumor cells. They have mapped and then mutated the amino acids required for effective interaction of measles virus with its two natural cellular receptors, CD 46 and Slam. This mutant virus is then engineered to express single chain antibodies (ScFVs) that direct the virus to bind only to tumor cell antigens (Bucheit et al., 2003; Peng et al., 2003; Schneider et al., 2002). While on the one hand this type of specificity holds great promise, it remains to be seen if, in the context of a tumor-bearing animal, such highly restricted viruses will be able to gain access to tumors (e.g., through the vascular endothelium).

Intracellular targeting

Over the last decade, a new understanding has emerged of the signaling pathways that mammalian cells use to resist virus infection (Samuel, 1994). It is now apparent that pathways controlling the first line of cellular defense against viral invasions often control aspects of cell growth and apoptosis. It follows that during tumor cell evolution, aberrations in cell growth control and apoptosis occur concomitantly with defects in cellular antiviral responses. Several oncolytic viruses are tumor-tropic, at least in part due to their ability to grow only in cells that have defective antivirus responses. As an example, normal cells treated with interferon can be completely protected from infection by vesicular stomatitis virus (VSV), but tumor cells lacking an interferon response are rapidly killed by VSV (Stojdl et al., 2000). We have found that VSV variants that robustly induce interferon production as a byproduct of infection are self-attenuating in normal tissues but grow unabated in tumor cells. Another interferon inducing virus, PV 701, derived from a vaccine strain of Newcastle disease virus (NDV), has entered clinical trials (see below) while influenza virus variants harboring mutations in the NS1 gene (Bergmann et al., 2001) can only propagate in interferon nonresponsive tumor cells. Grant McFadden (Robarts Research Institute, Canada) reported that rabbit myxoma virus, although unable to replicate in normal human cells, can infect and kill human tumor cells lacking components of the interferon response. In a similar way, Reovirus type III and certain herpes virus strains grow well in cells that have an activated Ras signaling pathway. We now know from Patrick Lee's (University of Calgary, Canada) work that Ras activates a phosphatase that antagonizes the antiviral kinase, PKR. Thus, tumor cells with an activated Ras signaling pathway have both increased proliferation and decreased antiviral activity.

Transcriptional targeting of viruses, by the substitution of viral promoters with cellular promoter elements, has been widely developed and reviewed extensively elsewhere (Bangma, 2000; Barnett et al., 2002; Liu, 2002; Logg et al., 2002; Nahde et al., 2001; Qiao et al., 2002; Savontaus et al., 2002). A new approach with plenty of potential is restricting virus growth to tumor cells based upon translational regulatory signals. Mathias Gromeier (Duke University) has detargeted poliovirus from nondividing neuronal cells and retargeted it to malignant glioma cells by swapping IRES elements between polio and rhinoviruses (Gromeier et al., 2000). It appears that the combination of a 3′ polio UTR sequence with a rhinovirus 5′ IRES element creates a chimeric virus that is incapable of efficient translation in neuronal cells but is well translated in malignant cells.

It's not only what you deliver, it's how you deliver it

While most oncolytic virus products are still in the early phases of clinical development, data from a handful of trials are providing important leads about the best way to deliver therapeutic viruses. Intratumoral injections of agents like Onyx-015 provided some early encouraging results (Khuri et al., 2000); however, the great hope of replicating oncolytic viruses is that they will spread and destroy systemic disease. While there have been some reports of virus spread from injected tumors to contralateral naïve tumors in preclinical models, this has not been the experience to date in the clinic. It is evident that for treatment of widespread disease, therapeutic viruses need to be delivered via the intravenous route. In the case of adenovirus, two clinical studies are of interest. John Nemunaitis (U.S. Oncology) and colleagues carried out a phase I study using intravenous injections of Onyx 015, and although it was found that the virus could be administered safely by this route, there was minimal demonstrated tumor response (Nemunaitis et al., 2001a, 2001b, 2003). In one anecdotal report, a patient that had achieved a complete response in a surface lesion following an intratumoral injection did not respond to systemic delivery of the virus. One obvious explanation is that neutralizing antibodies that preexist in the human population prevent effective dispersal of the virus, consistent with earlier published preclinical studies. If this were the case, it may be possible to overcome neutralizing antibodies by higher directed virus doses. In Tony Reid's study (discussed below), high doses of Onyx-015 were infused directly into tumor beds via the hepatic artery. In this study, significant tumor responses were reported, possibly because higher amounts of virus were delivered locally, overcoming antibody mediated virus neutralization. Interestingly, Hans de Haan of Medigene Inc. reported some unpublished results of a trial conducted by Yuman Fong and colleagues using intrahepatic artery delivery of an oncolytic herpes virus vector plus chemotherapy. Here again, some therapeutic efficacy was observed. These studies and supporting preclinical reports suggest that high doses of virus given in the face of preexisting immunity or early before neutralizing antibodies have been established will provide optimum therapeutic responses. In this vein, three successive clinical trials using PV701 (NDV) have provided new insights. In the first study, intravenous dose escalation revealed that high doses of virus can initiate cytokine-related flu-like symptoms upon first infusions; however, the frequency and severity of these effects were decreased upon subsequent treatments in the same patient. Scot Roberts and Bob Lorence (Wellstat Biologics) reported studies with mouse models that demonstrated low ini-

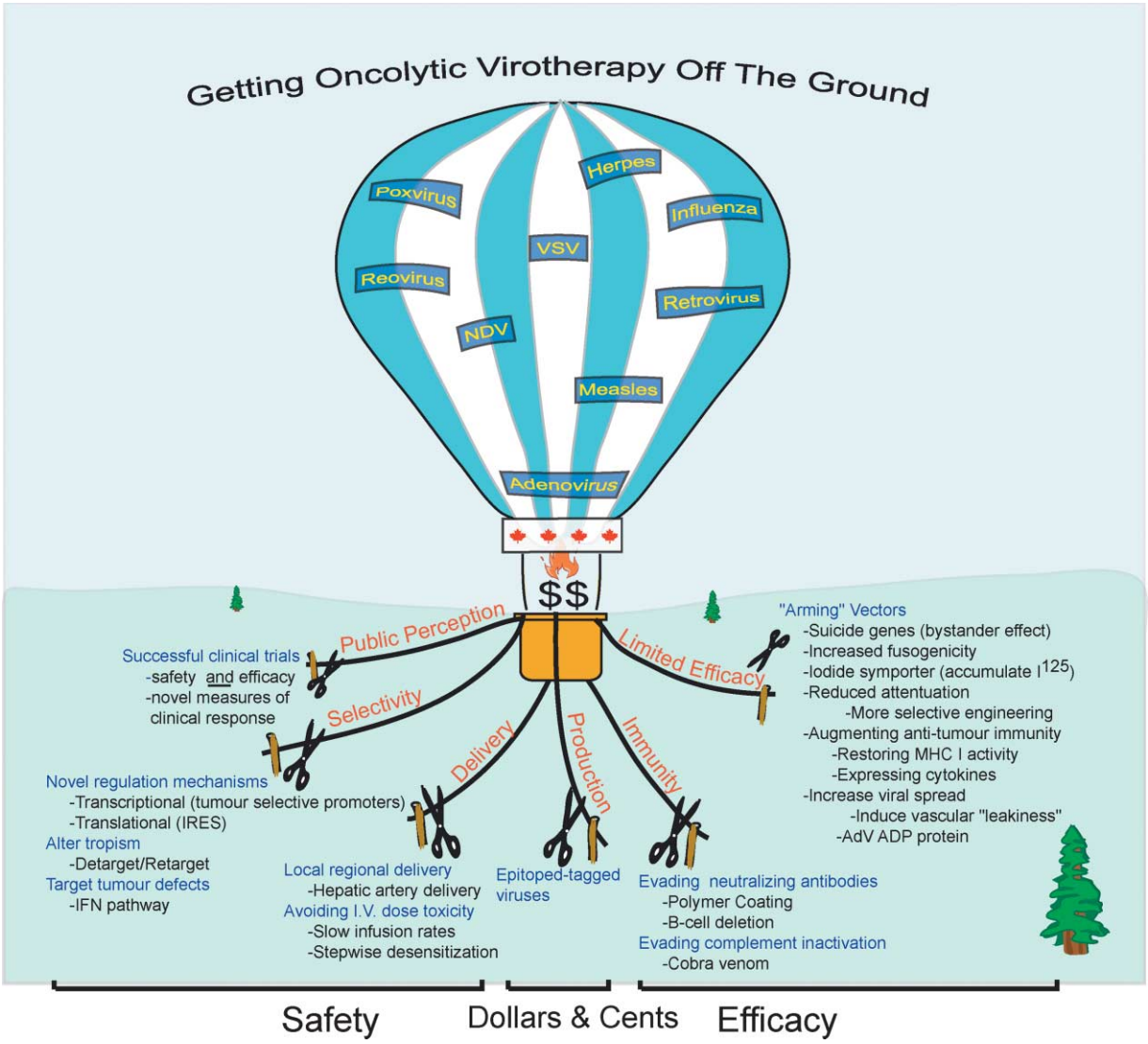

Figure 1. Getting oncolytic virotherapy off the ground

Several issues remain to be resolved before oncolytic viral products become approved therapeutics. Some of the problems are depicted here in three categories: Safety, Efficacy, and Dollars and Cents. The tethers, which bind the balloon to the earth, represent challenges to the development of oncolytic virus therapeutics, and beside each tether are some of the strategies groups around the world are developing to enable a successful launch into the clinic.

tial doses or slow infusions of higher doses substantially reduced these "flu-like" symptoms, a phenomenon they called "desensitization." These results led to two new intravenous phase I studies that showed that desensitization also occurs in humans and permits intravenous delivery without severe flu-like symptoms. Though still ongoing and with small numbers of patients, early results from these trials suggest that higher doses of slowly intravenously administered virus may have increased antitumor efficacy (five objective tumor responses in thirteen evaluable patients). Other barriers to effective delivery of virus may include antibody-independent innate host responses. For example, Nino Chiocca (Massachusetts General Hospital) has shown that complement seems to be an impediment to effective delivery of HSV by the intravenous route, a problem that may be overcome by therapeutic administration of cobra venom factor (Ikeda et al., 1999, 2000; Wakimoto et al., 2002). Another emerging theme in oncolytic viral therapy is that of combination therapy. For instance, combining viruses in clinical trials with standard chemo- and/or radiotherapy shows promise (Hecht et al., 2003; Reid et al., 2002; Toth et al., 2003). The use of prodrug converting enzyme genes expressed from oncolytic viruses in tumor cells is attractive, as this provides the double benefit of enhancing tumor killing while providing a means of controlling the infection (Freytag et al., 2002; Ichikawa et al., 2001; Morris and Wildner, 2000; Shariat et al., 2001). One concern here is the possibility that these nonessential suicide genes will be free to mutate as the virus replicates, thus allowing for virus escape mutants.

New paradigms in clinical evaluation of oncolytic viruses

Due to the unique biology of replicating viral therapeutics, it seems likely that clinical performance and biodistribution of

these agents need to be evaluated using tools that are different from those used for conventional therapeutics. This was brought into focus by work reported by Tony Reid from Stanford University Medical Center. In a recent phase I trial designed to evaluate the safety of intrahepatic artery administration of Onyx 015 (in combination with chemotherapy) for the treatment of colon cancer metastasis in the liver, Reid and colleagues noticed that, in most patients, tumor size increased following administration of Onyx 015 as determined by routine CT scans (Sze et al., 2003). While conventional wisdom would have led to the removal of these patients from the trial based upon tumor progression, Reid noted that many of the patients were physically feeling better, and some of their tumor-related signs were abating (e.g., decreased CEA levels). In fact, it was discovered that some 43% of patients on study had significant tumor shrinkage several months after initiation of virus therapy. Thus, while increased tumor size after treatment with a standard chemotherapeutic agent would normally be interpreted as disease progression, in the case of a virus therapeutic transient, increased tumor size may represent virus-induced inflammation and be a very positive indication. Reid argues that new diagnostic yardsticks need to be developed to assess patient response to oncolytic virus treatment, a sentiment that was echoed by Sebastien Hotte (Hamilton Regional Cancer Center, Canada) reporting on an ongoing phase I study with PV 701 (NDV). In this study and in an earlier one by Andrew Pecora and colleagues, intravenous administration of PV 701 often led to tumor site inflammation, making it difficult to assess changes in disease state by conventional radiographical techniques (Pecora et al., 2002). In future trials, alternative assessment criteria, possibly including the application of MRI or PET scanning to study the evolving physiology of a virus-infected tumor, need to be incorporated.

Cancer is a biologically complex disease, and treating it with sophisticated replicating viruses compounds the challenges that must be overcome to produce safe and effective therapies (Figure 1). Clinicians and scientists are rising to the occasion, however, and developing creative solutions to cut the tethers that bind oncolytic viruses in the realm of experimental therapeutics… a successful launch seems imminent.

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