Vesicular stomatitis virus: An exciting new therapeutic oncolytic virus candidate for cancer or just another chapter from *Field's Virology*?

Selected mutant strains of vesicular stomatitis virus (VSV) are described that are unable to combat endogenous IFN-β signaling within infected normal cells and as a result are dramatically more selective for productive growth in tumor cells having a defective antiviral response. The VSV mutants may have the potential to be used clinically as a systemic oncolytic agent for the treatment of distal and metastatic cancers.

Viruses from several families are being developed and tested clinically as oncolytic agents for selected cancers. The use of viruses as oncolytic agents for cancer therapy (virotherapy) was first described several decades ago, using a variety of viruses, including adenovirus (Ad), influenza virus, mumps virus, and Newcastle disease virus (NDV), in a number of diverse indications (reviewed in Kirn et al., 2000; Stanziale and Fong, 2003). Although sporadically successful, this field lay essentially dormant for many years, but received a kick-start with the application of two DNA viruses, first herpes simplex virus (HSV) and then Ad, having deletions in genes that conferred specificity for cancer cells. While response rates in early clinical trials have been encouraging, there still are no phase III clinical trials with any oncolytic viruses. Thus, while the concept of viruses that propagate selectively within tumor cells is tantalizing, there continues to be ample room in the field for novel approaches in order for oncolytic viruses to become a major paradigm of cancer therapy.

In this issue of *Cancer Cell*, John Bell, David Stojdl, and colleagues (Stojdl et al., 2003) present exciting results with a relatively new player in the virotherapy field: vesicular stomatitis virus (VSV), an enveloped virus containing a single-stranded RNA genome. Particular VSV mutants showed a dramatic preference for productive infection in tumor cells, as compared to normal interferon (IFN)-responsive cells. The VSV mutant strains could be administered systemically to athymic or immunocompetent mice bearing human tumor xenografts or disseminated tumors and confer significant long-term durable survival, importantly without treatment-associated toxicity.

Historically, many RNA viruses were tested clinically as cancer therapeutics (Russell, 2002). More recently, their use lagged behind work with DNA viruses, perhaps in part due to poor understanding of how the natural biology of RNA viruses could be exploited to target cancerous cells. A key to preferential replication was the discovery that cancer cells commonly acquire defects in IFN-α/β signaling pathways, a frontline innate response that host cells utilize to combat viral infection. Through dsRNA intermediates, infection by both DNA and RNA viruses induces host cells to produce interferon and both have evolved mechanisms to inhibit the effects of IFN induction. RNA viruses, such as VSV, are far more sensitive to the antiviral activity of IFN induction. Over time, it became conceptually apparent that the defective interferon response in cancer cells presented a therapeutic window for oncolytic RNA virus therapy. Previous work by the authors of this manuscript and by others have demonstrated both the efficient lytic growth of VSV in several human tumor cell types and the potent antitumor efficacy of unmodified VSV in both immune-competent and xenograft models (Huang et al., 2003; Balachandran and Barber, 2000; Stojdl et al., 2000).

What VSV-mediated virotherapy has lacked to date—an effective oncolytic agent with a therapeutic window big enough to allow systemic administration—has been addressed in this investigation. The breakthrough resulted from characterizing the underlying mechanisms by which VSV combats the host innate antiviral response. The biologic properties of three VSV mutants, AV1, AV2, and AV3 (Desforges et al., 2001), each having a small plaque phenotype in interferon-responsive cells, were compared to wild-type VSV. The AV variants are all mutants of the viral M gene, which encodes one of the structural proteins that constitute the enveloped "coat." Compelling data are presented demonstrating that the AV mutants are powerful inducers of the host antiviral response: (1) supra-high levels of IFN-α were induced following infection of epithelial cells; (2) primary mouse embryo fibroblasts (MEFs) were refractory to infection with the VSV variants (while MEFs were refractory to infection with wild-type VSV only when interferon was added exogenously); and (3) high levels of systemic IFN-α were also observed in mice injected with the AV mutants. Furthermore, coadministration to mice of an AV variant dramatically lowered the toxicity of wild-type VSV, presumably due to induction of protective levels of interferon by the VSV M protein mutants. The AV variants and wild-type VSV were equally toxic in Type 1 IFN receptor knockout mice, demonstrating the requirement for an intact interferon signaling system in order for the AV mutant phenotypes to be revealed. Taken together, these experiments demonstrated that unlike wild-type virus, high levels of the VSV variants could in fact be safely administered to mice systemically. As wild-type VSV is neurovirulent in mice, the mutant strains utilized in this investigation should have a major impact on pre-clinical development of VSV virotherapy. An alternative approach with a similar result was recently reported by Glen Barber and colleagues (Obuchi et al., 2003), demonstrating that recombinant VSV encoding IFN-β was nonlytic in normal human cells, but highly productive for growth in tumor cells, and could be given safely to tumor-bearing immunocompetent animals by intravenous administration, resulting in significant antitumor efficacy.

Why do the AV mutants induce high levels of IFN-α production within interferon-responsive cells? The investigators addressed this question through a combination of microarray and RT-PCR analyses of infected cells. The induction of essential transcription factors, like IRF-3 (together with NF-κB and c-JUN/ATF-2), which triggers the activation of antiviral responses, was equivalent in

Table 1. Selected RNA virus clinical trials

Parental virus	Strain name	Indication	Trial	Regimen	Results	Toxicity	Ref.
Reovirus	REOLYSIN	Advanced cancer with palpable lesions	I (n = 18)	1×10^7–1×10^{10} PFU intralesional single versus 3 × (q2d)	Rising antibody titers in all pts	Grade 2 or less; transient flu-like symptoms, headache	1, 2
				1 CR and 1 PR (local lesion)			
	REOLYSIN	T2 Prostate	I (n = 45) ongoing	Single intralesional injection prior to resection	Viral activity in 5 of 6 treated patients; limited to cancerous lesion	No AEs or DLTs	3
	REOLYSIN	Recurrent glioma	I/II	Single intra-lesional, image-guided surgery	4 of six treated patients alive at end of 6 month follow-up period	Well tolerated	4
Newcastle disease virus (NDV)	Ulster	Colorectal liver mets	I/II (n = 23)	Oncolysate vaccine	Increase in recurrence-free interval; no impact on survival	Transient elevated temperature	5
	MTH-68/N	Advanced cancer	I/II (n = 33)	4000U/day inhalation	8 PR	Fever in 8/33	6
	PV701	Advanced solid cancer	I (n = 79)	5.9–24×10^9 PFU/m2 (IV)	14 SD, 1 CR, 1 PR	Fever, flu-like syndrome, leucopenia, thrombo-cytopenia, increase in liver enzymes, diarrhea	7
	PV701	Advanced cancer ongoing	11 enrolled	3 week cycle of 6 doses/2 wks 3 hr IV (dose 1;12×10^9 PFU) versus 1 hr IV (dose 2–6; 24–120×10^9 PFU)	Of 8 evaluable pts, 2 PR, 3 SD, 1 PD	3 hr IV: fever, hypotension, fatigue and chills 1 hr IV: tumor site AE, inflammation	8
	PV701	Peritoneal cancers ongoing	No report	Determine optimal first IV dose (desensitizing) and MTD of IP treatment dose	No report		9
Measles virus (MV-Ed)	TC-adapted MV strain	Advanced cancers	n = 90	IV, IT, PO, rectal, or inhalation	37/90 treated with >50% reduction in tumor burden	Febrile reactions	10
		Gynecological cancers	n = 22	Priming SC (1×10^8 PFU), followed by IP or intrathoracic (1×10^9 PFU)	Response limited to local MV delivery of primed patients		11

Abbreviations: PO, oral administration; IV, intravenous; IT, intraperitoneal; CR, complete response; PR, partial response; SD, stable disease; q2d, every other day; AE, adverse event; DLT, dose-limiting toxicity.

[1]Morris et al. (2002). ASCO abstract and presentation #92; [2]Coffey et al. (1998). Science *282*, 1332–1334; [3]Morris et al. (2003). Presented at "Oncolytic Viruses as Cancer Therapeutics," 28 March 2003, Banff, Alberta; [4]http://www.Oncolyticsbiotech.com; [5]Schlag et al. (1992). Cancer Immunol. Immun. *35*, 325–330; [6]Csatary et al. (1993). Cancer Detect. Prev. *17*, 619–627; [7]Pecora et al. (2002). J. Clin. Oncol. *20*, 2251–2266; [8]Hotte et al. (2003). ASCO abstract and presentation #791; [9]Spriggs (2003). http://www.clinicaltrials.gov.; [10]Asada (1974). Cancer *34*, 1907–1928; [11]Shimizu (1988). Cancer Detect. Prev. *12*, 487–495.

cells infected with either wild-type VSV or the AV variants. In contrast, the level of IFN-β message in the cytoplasm (and production of IFN-β protein) was strikingly lower in cells infected with wild-type VSV. This result suggests that the native VSV M protein somehow prevents transport of IFN-β mRNA to the cytoplasm; indeed, other investigators have shown that VSV M protein does in fact complex with the nuclear pore proteins, suggesting a possible mechanism in which M "clogs" the nuclear pore (Petersen et al., 2000). In turn, autocrine and paracrine signaling by IFN-β through the JAK/STAT pathway activates IRF-7 and the production of IFN-α. Based on comparative microarray analyses from wild-type VSV and AV mutant infected cells, the authors propose a model for an antiviral response cascade, in which IFN-β is an integral "gatekeeper" that activates (via IRF-7) induction of a full tertiary antiviral response, including production of IFN-α. According to this model, unlike wild-type VSV, the AV mutants are unable to suppress production of IFN-β, and full induction of the antiviral response occurs, resulting in a "cytokine cloud" that protects normal surrounding tissue in the host.

It is now clear that defects in IFN signaling pathway are common among cells from diverse cancer types. In part, the selection for these defects appears to result from a resistance to induction of apoptosis. Other selection pressures may be operable as well: induction of type I IFN leads to induction of IFN-γ and upregulation of class I molecules—the precise response that tumor cells *don't* want in the face of ever-vigilant immunosurveillance. Thus, IFN-β signaling seems to play an essential role in both the innate antiviral as well as the adaptive immune response: signaling through the JAK/STAT pathway by IFN-β promotes the antiviral response to directly attenuate virus infection through activation of PKR and RNase L (Balachandran et al., 2000); activation of IFN-α and sub-

sequent IFN-γ expression facilitates the adaptive immune response. It turns out that viruses are not unique in activating the production of IFN-β. Our laboratory is utilizing genetically defined attenuated mutants of the facultative intracellular bacterium, *Listeria monocytogenes*, to deliver antigens related to infectious and malignant disease. It was recently demonstrated that productive cytosolic propagation during *Listeria* infection is accompanied by host cell production of IFN-β (O'Riordan et al., 2002). Since protection against *Listeria* infection is mediated by $CD4^+/CD8^+$ T cells, it is reasonable to speculate that the production of IFN-β in response to both viral and *Listeria* infection is an innate mechanism to alert the adaptive immune response.

How have RNA viruses fared clinically? There have been multiple phase I/II clinical trials using RNA viruses from diverse families, and other clinical trials are planned (reviewed in Russell, 2002; Table 1). Only NDV, and to a lesser extent measles virus, have been safely administered intravenously. Both of these systemic virotherapies have reported objective responses across a wide range of solid tumors, importantly without acute toxicity. While encouraging, a fully controlled efficacy trial that is sufficiently powered will be required to determine whether the clinical responses with any of the RNA oncolytic viruses are significant. As has been shown with Ad, combination with either established chemotherapy regimens and/or radiation may significantly augment the efficacy of oncolytic RNA viruses.

Finally, is the notion of VSV as an oncolytic agent simply another chapter—among many—out of *Field's Virology*? The answer would appear to be certainly not. While it is too early to tell whether VSV will be effective clinically, it is clear that RNA viruses have many desirable features compared to Ad and HSV. For example, the comparatively short reproductive cycle of VSV could translate into high viral titers in the tumor microenvironment and broad lytic infection throughout the tumor. By understanding the mechanisms through which VSV combats the antiviral response, Stojdl and colleagues have developed a VSV variant with a sufficient degree of preferential replication in tumor cells to permit systemic delivery and treatment of metastatic disease. Additional improvements may still be needed (e.g., systemically administered VSV may be inactivated by human serum; DePolo et al., 2000), and virus-neutralizing immune responses upon repeated administration remains to be a key issue for virotherapy. Nevertheless, VSV mutant strains unable to prevent the IFN-β-mediated signaling of host cell innate antiviral responses represent a promising new therapeutic oncolytic virus candidate.

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