Targeting tumours with HSV

NeuroVir Therapeutics (Vancouver, British Columbia, Canada) have filed an investigational new drug application to the Food and Drug Administration (FDA) for a Phase I/II clinical study to use a herpes simplex virus (HSV)-derived product as a treatment for colorectal cancers that have metastasized to the liver. This tumour-killing HSV technology has also been used in a Phase I clinical study to treat patients with glioblastoma multiforme, the most common malignant primary brain tumour. This trial, which was completed in the second quarter of 1999, marks the first time the FDA have allowed the use of replicating herpes viruses in a clinical study to treat cancer.

The strategy behind this virus-based anticancer therapy is to develop viruses that can infect and destroy malignant tumour cells without deleterious effects on normal tissues. By introducing key alterations to its DNA, the virus loses the capacity to cause disease in non-cancerous tissues. 'In animal models of the disease, tumour-killing HSV spreads from tumour cell to tumour cell in a chain reaction, killing the cells as it progresses', says Frank Tufaro, Chief Scientific Officer and a Vice-President of NeuroVir. 'The technology adds a significant new tool to the cancer arsenal. Each new mode of tumour killing leads to a real advance in our ability to treat disease. With tumour-killing HSV, you do not have to stop practicing current standards of cancer care as the agent is compatible with, and in most instances enhanced by, surgery, radiotherapy and chemotherapy.'

Mechanism of action

This mechanism of reducing tumours *in vivo* using a genetically engineered replication-competent herpes virus was first shown by Martuza and coworkers[1]. These workers used a thymidine kinase (TK)-negative mutant of HSV-1 (dlsptk) in a human glioma cell line (U87) implanted into nude mice. However, TK mutants are not ideal as they are resistant to commonly used anti-HSV drugs and do not have a sufficiently reduced toxicity profile.

NeuroVir's first recombinant virus product, G207, is produced by deletion of both copies of the gamma 34.5 gene from the HSV genome, together with some additional modifications. It is the gamma 34.5 gene that enables the virus to grow in normal cells. Normally, infection of a cell with a gamma 34.5-deficient virus initiates apoptosis, thereby inhibiting protein synthesis and preventing the viral replication. This effect is caused by the fact that gamma 34.5 blocks the phosphorylation of the α-subunit of the cellular translation initiation factor eIF-2, thereby preventing the premature blockade of protein synthesis. When this gene is deleted from the viral DNA or 'knocked out', the host's non-malignant cells are destroyed before the virus has had a chance to replicate. Cancer cells, however, lose their ability to undergo cell death. In part, it is this phenomenon that enables the 'knockout' virus to discriminate between tumours and non-tumours.

The virus kills the tumour cells by shutting down the normal host genetic program by inhibiting RNA synthesis and preventing the production of its own proteins. Meanwhile, several toxic proteins are expressed from the viral genomes and the cell is converted into a 'factory' producing new HSV virions, which are released from the infected cell within hours of infection and continue to be released for several days. HSV-infected malignant cells then stimulate the production of specific cytotoxic T lymphocytes that recognize the tumour and, hence, are consequently destroyed by the immune system. This has been further demonstrated by the fact that, in animal models, two tumours can be killed by injection into only one of the tumours. 'So it appears that tumour-killing HSV produces a dual effect, killing cancers both directly as well as indirectly via the immune system', says Tufaro.

In pre-clinical studies, G207 was avirulent on intracerebral inoculation of mice and HSV-sensitive non-human primates, whilst killing human glioma cells in monolayer cultures[2]. In nude mice harbouring subcutaneous or intracerebral U87MG gliomas, intraneoplastic inoculation with G207 decreased tumour growth and/or prolonged survival.

Possible therapeutic applications

G207 has been designed to treat malignancies of the CNS. The second HSV product, NV1020, was initially developed as a treatment for colorectal cancer that has metastasized to the liver. NV1020 (developed by Bernard Roizman, University of Chicago, IL, USA) is more efficacious than G207 (developed by Robert Martuza at Georgetown University, Washington, DC, USA) because it was designed to act outside of the CNS, thus avoiding the problems associated with the penetration of the blood–brain barrier. By contrast, the deletion of gamma 34.5 from the gene in G207 makes HSV safe for delivery to the brain.

Four methods of delivery have been developed to enable tumour-killing HSV to reach its target sites. Because of the difficulty of getting the virus across the blood–brain barrier, HSV is injected directly into the tumours in the brain. For colorectal cancers that have metastasized

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to the liver, HSV is given by direct infusion into the hepatic artery, as these tumours require blood from the hepatic artery to grow. With ovarian cancer, the virus is delivered into the peritoneal cavity, as in animal models, the virus then infuses into the peritoneal fluid and selectively infects and kills ovarian tumour cells. Finally, methods of intravenous injection of G207 are being explored for the treatment of prostate cancers.

Clinical trials

Clinical studies with G207

The Phase I study of G207 in 21 patients with glioblastoma multiforme (performed at Georgetown University Medical Centre and at the University of Alabama, Birmingham, AL, USA) involved single and multiple stereotactic injections into the tumours. To qualify for inclusion in the study, patients had to have failed at least one course of surgery and radiation, have tumours that were progressing at the time of treatment and have an expected survival time of three to six months. In fact, most of the patients had failed two or three courses of treatment and had very large tumours at the time of treatment.

In the study, 18 patients received a single injection of escalating doses of G207, ranging from 10^6 virus particles and increasing to 3×10^9 virus particles, and the three patients treated with the highest dose of G207 received five injections, all of which revealed no evidence of toxicity. Of the 17 patients for whom follow-up data are available, three had stable disease at, or beyond, three months, one patient was stable at five months and one patient was stable at nine months. 'It is difficult to draw any firm conclusions regarding efficacy in a Phase I trial because we enrolled only three patients at each dose and have yet to analyze the data from the patients who received the highest doses and multiple injections. In addition to the unexpected stabilization of the disease in several patients, there is anecdotal MRI evidence that some patients appear to have holes in the tumour following a single delivery of the agent, suggesting a biological effect', says Tufaro.

The Phase II study – currently being planned – will involve delivering the agent to 30–40 newly diagnosed, previously untreated glioblastoma multiforme patients at the time of tumour resection. The end point of the study will remain the time to progression and survival.

Clinical studies with NV1020

NeuroVir is also planning a Phase I/II trial of NV1020 in association with the Memorial Sloan Kettering Cancer Centre (New York, NY, USA) for patients with liver metastases of colorectal cancer. Estimates suggest that there are approximately 138,000 cases of colorectal cancer that metastasize to the liver per year in North America and Europe. Approximately 40% of these patients with less than four tumour lesions are cured by surgical resection. The remaining 60% of patients have more than four lesions and are considered inoperable by most surgeons, as the removal of so many lesions is likely to damage liver function. These patients are therefore treated with systemic or intra-arterial chemotherapy and have a mean incremental survival of between eight and 16 months.

The Phase I/II study of NV1020 will recruit patients with multiple liver tumours who have failed conventional therapy following promising results in preclinical studies of NV1020 in liver tumours. Yuman Fong, a liver cancer surgeon at Memorial Sloan Kettering Cancer Centre demonstrated that oncolytic HSV can infect, kill and spread between cells in several different human colon carcinoma cell lines[3]. Furthermore, in a National Cancer Institute cell screen, NV1020 (and G207) killed most of the colon cancer cell lines they were exposed to.

In nude mice that have been implanted with human tumours and syngistic rat and mouse models of cancer (where the animal has a normal immune system), a single dose of oncolytic HSV can eradicate most colorectal tumours in the liver, and can cure a significant number of animals. In addition to evaluating safety, the planned Phase I/II study in 30 patients (due to commence in 1999) will examine the biological response, time to progression of the tumour and survival. 'From the outset, we are gathering the data needed for rapid approval of the drug', says Tufaro.

Conclusions

These studies have demonstrated synergistic effects of combining HSV with both chemotherapy and ionizing radiation. In most instances, treating with a combination of chemotherapy or radiation and HSV augments the beneficial effects of either treatment alone. 'Herpes has a different mode of killing than radiation and chemotherapy, and the incidence of cells developing resistance to the killing effect of HSV is extremely low. Moreover, it has been demonstrated that HSV will kill chemotherapy- and radiotherapy-resistant cancer cells', says Tufaro. 'Oncolytic virus therapy offers the possibility of extending the duration and quality of life for those with essentially incurable disease.'

REFERENCES

1 Martuza, R.L. *et al.* (1991) Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science* 252, 854–856

2 Mineta, T. *et al.* (1995) Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. *Nat. Med.* 1, 938–943

3 Kooby, D.A. *et al.* (1999) Oncolytic viral therapy for human colorectal cancer and liver metastases using a multi-mutated herpes simplex virus type-1 (G207). *FASEB J.* 13, 1325–1334

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