

1. Scientific Abstract

Over 50,000 patients per year present with metastatic cancer from the colon to the liver in the United States (1). Surgical resection offers patients with primary liver malignancies a one-third chance of cure, but patients with unresectable tumors or microscopic disease have few effective treatment options (2). Systemic chemotherapy yields responses in only 15%-35% of patients and radiotherapy is generally ineffective. The direct infusion of fluorinated pyrimidines (FUDR) has resulted in higher local response rates (40%-60%), without a convincing improvement in overall survival. Median time to progression remains 6 to 9 months while overall survival in these patients ranges from 12 to 18 months (3).

There have been a number of attempts over the years to exploit the cytolytic properties of viruses for the treatment of cancer. Observed antitumor effects have been ascribed to a combination of viral lysis, local production of cytokines and infiltration of immune effector cells following infection. Recent advancements in molecular biology and the ability to engineer viruses with desired traits, with or without the incorporation of foreign genes, has led to the rational development of a new generation of oncolytic viruses, including derivatives of herpes simplex virus type 1 (HSV-1) (4, 5, 6).

HSV-1 is an enveloped double stranded DNA virus with a genome of 150 kb and codes for over 80 genes (7). In nature, HSV-1 is widespread in the human population and infections are usually mild or asymptomatic. Rare manifestations of HSV-1 induced systemic illness or encephalitis are usually restricted to newborns or highly immunosuppressed individuals. Fortunately, HSV-1 disease can be effectively controlled with the antiviral drugs such as acyclovir or foscarnet (8). Animal models exist for all aspects of the biology of HSV in humans. In particular, Aotus (owl) monkeys represent an exquisitely sensitive model for HSV infection of newborns and immunocompromised individuals.

HSV-1 strains have been isolated that have extraordinarily high degrees of attenuation in vivo yet are still able to replicate in cancer cells. Mutants that do not express the γ 34.5 protein, which is required for replication and spread in the nervous system, are among the most highly attenuated (9). G207 is a γ 34.5 mutant that has an additional attenuating mutation in the ribonucleotide reductase gene (10). G207 is extremely apathogenic in the CNS of experimental animals (11, 12). In a recently completed phase I dose escalation study inoculating G207 into recurrent brain tumors there has been no evidence of vector-related toxicity (NeuroVir G207 BB-IND 7393; Markert, J.M. et al. in press).

NV1020 is a replication-competent, genetically modified HSV-1 that has been highly attenuated for virulence by deleting 15 kb of the HSV-1 internal repeated sequences. It is a clonal derivative of R7020, developed as an HSV-1/2 vaccine candidate to be administered to healthy individuals based on its excellent safety profile and genetic stability (13, 14). NV1020 is less severely attenuated for growth in a number of human tumor cell lines compared to G207, and may therefore offer improved treatment options for tumors outside the brain (15).

Preclinical studies of NV1020 have shown the virus to be capable of killing or slowing the growth of multiple colorectal cancer cell lines in vitro. In vivo studies in a xenograft

mouse model of colorectal carcinoma have shown that a dose of 1×10^7 pfu of NV1020 injected directly into a flank tumor resulted in significant retardation of tumor growth and prolonged survival when compared to saline-treated controls. NV1020 has also been shown to retard the development of liver lesions in a Buffalo rat model of hepatoma at a dose of 1×10^8 pfu.

Extensive toxicology studies have been conducted on R7020, from which NV1020 was clonally derived (13, 14). In the highly sensitive Aotus monkeys, in contrast to wild-type virus that produces lethal infection with 100 – 1000 pfu, inoculation of greater than 10^6 pfu of R7020 only produced local lesions and limited viral shedding, but not disseminated disease (13).

Toxicology studies were conducted on NV1020 in mice, rats and monkeys, using higher doses than in the previously published studies for R7020. The neurovirulence potential of NV1020 was studied in mice. Balb/c mice receiving an intracranial injection of 5×10^6 pfu of NV1020 survived and were found to have only mild tissue destruction along the needle track compared to lethality in animals receiving 1×10^3 pfu wild-type strain F. However, in another study injection of 5.7×10^6 pfu into the brains of the more sensitive A/J strain lead to the death of 7/10 animals. To study the toxicology of virus delivered to the liver, NV1020 was administered by intraparenchymal injection to the livers of Balb/c mice at doses of up to 3×10^7 pfu. Mice were mildly ill at this dose level; however, no virus was detectable in the liver, and there were no histopathological findings by day 28 following injection. NV1020 has also been administered to the livers of Balb/c and C57Bl/6 mice via the portal vein at doses of up to 5×10^7 pfu. Although a dose of 1×10^7 pfu was well tolerated, 100% mortality was observed at doses of 2.5×10^7 pfu and 5×10^7 pfu in Balb/c mice. A dose of 5×10^7 pfu resulted in 10% mortality in C57Bl/6 mice. Thus, although NV1020 is well tolerated at lower doses, it can cause mortality in mice following intra-portal delivery at high doses. No mortality has been observed in Buffalo rats, which have been shown to tolerate up to 3 repeat doses of 1×10^8 pfu NV1020 delivered via the portal vein.

Toxicity was also studied in two Aotus nancymae that received a single injection of 3×10^8 pfu of NV1020 via the hepatic artery, to mimic the clinical protocol. Neither animal developed clinical signs or symptoms. Elevations of liver enzymes (ALT, AST) were noted during the period immediately following treatment. Histopathological examinations revealed no abnormalities attributable to NV1020. Although PCR analysis detected NV1020 DNA in multiple tissues including heart, lung, kidney, bone marrow, testes, and ovaries, no tissue was culture positive for NV1020. The starting dose for the proposed Phase I clinical trial described below is approximately 3000 times lower, on a per weight basis, than this no-effect dose in Aotus monkeys.

For this phase I study of NV1020 in patients with adenocarcinoma of the colon metastatic to the liver, the primary objectives are: (i) To assess the safety and tolerability of a single injection of escalating doses of NV1020 administered into the hepatic artery and to determine the maximum tolerated dose (MTD)/optimal biologic dose (OBD). (ii) To assess the safety and tolerability of multiple injections of NV1020 administered into the hepatic artery and to determine the MTD/OBD for multiple injections.