Herpes simplex virus NV1020 as a novel and promising therapy for hepatic malignancy

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Abstract

Background—Patients with hepatic malignancy have a dismal prognosis with standard therapies. NV1020 is an oncolytic herpes simplex virus that has potential to be a safe and effective therapeutic agent for this disease.

Objective—We set out to discuss the development of NV1020 as an oncolytic agent and explore the potential role of this particular virus in the setting of human hepatic cancer.

Methods—The scope of this review includes an overview of preclinical experience with NV1020, as well as an examination of current standard and developing therapies for liver cancer. The primary focus, however, is on the safety and potential clinical efficacy of NV1020 against hepatic malignancy.

Results/conclusion—We have found that NV1020 is a safe, novel therapeutic agent for treatment of refractory hepatic malignancy.

Keywords

colorectal cancer; hepatic malignancy; herpes simplex virus; oncolytic viral therapy

1. Introduction

Colorectal cancer is the fourth most common cancer in men and the third most common cancer in women worldwide, with over 1.1 million new cases per year. It is also the fourth and fifth leading cause of cancer-related mortality in men and women worldwide, respectively, with an estimated 630,000 deaths in 2007 [1]. While surgical resection offers hope for cure to those patients diagnosed with early colorectal cancer, only about 39% of patients fall into this category. In fact, up to 50% of those patients who undergo surgery will ultimately develop recurrent disease.

Up to 25% of patients with colorectal cancer have liver metastases at the time of presentation. Untreated patients have a median survival of 6 – 13 months. Following hepatic resection, median survival is 24 – 46 months, with a 5-year survival of 20 – 37% [2]. Systemic adjuvant chemotherapy, such as with 5-fluorouracil (5-FU)-based regimens, have been shown to improve 5-year survival, although not drastically [3].

Of those patients who present with hepatic metastases, only a small percentage have resectable disease. For those with unresectable hepatic disease, systemic chemotherapy is one of few therapeutic options available. Regimens such as 5-FU, leucovorin, and oxaliplatin (FOLFOX) and 5-FU, leucovorin, and irinotecan (FOLFIRI) have shown response rates as high as 40 – 60%, but median overall survival with either regimen is still only 18 – 20 months. Hepatic arterial infusion (HAI) of chemotherapeutics, specifically floxuridine (FUDR), is another method developed to deliver chemotherapy directly to liver tumors, but is available only to patients with disease confined to the liver. HAI FUDR significantly improved both disease-free survival and 2-year survival when combined with systemic chemotherapy as opposed to systemic chemotherapy alone after liver resection [4]. Ongoing studies are evaluating the efficacy of HAI chemotherapy relative to systemic therapy alone and in conjunction with surgery.

Surgical resection remains the only possibility for cure for metastatic colorectal cancer (mCRC), as well as for other liver tumors such as hepatocellular carcinoma (HCC) and biliary neoplasms [5,6]. However, this procedure is limited by tumor burden within the liver and estimated degree of functional liver remaining after resection. A clinical risk score (CRS) system was developed to stratify patients by median survival, with implications toward who should be resected [7]. Prognostic factors include: node-positive primary tumor, disease-free interval < 12 months between colon resection and appearance of metastases, tumor > 5 cm, < 1 tumor, and carcinoembryonic antigen (CEA) level > 200 ng/dl.

An estimated 65% of patients with hepatic tumors will recur after surgical resection. These recurrences are thought to originate from microscopic foci of residual disease [5,8]. Hepatic resection results in a burst of hepatocyte regeneration in the immediate postoperative period. This local environment of high mitotic activity is thought to support and enhance growth of residual cancer cells [8]. Novel treatment strategies are necessary to target microscopic residual disease and prevent the development of recurrence after hepatic resection. Several candidate therapies are new in the clinic and in preclinical development.

2. Alternative available therapies for hepatic metastases

Monoclonal antibodies (mAbs) have recently been approved for treatment of mCRC as a therapy that targets the molecular pathways of cancer development. Bevacizumab (Avastin, Genentech, South San Francisco, CA) is an anti-vascular endothelial growth factor (VEGF) mAb that added significant benefit when combined with other chemotherapeutics in several Phase III trials. Based on the Bowel Oncology With Cetuximab Antibody (BOND) study, cetuximab (Erbitux, ImClone Systems, Inc., New York), an anti-epithelial growth factor receptor (EGFR) mAb, was approved for treatment of patients with metastatic disease expressing EGFR refractory to irinotecan-based therapy. Panitumumab (Vectibix, Amgen, Thousand Oaks, CA) is another anti-EGFR mAb approved for therapy in those patients refractory to other chemotherapeutics [9]. While no survival benefit has been shown with mAbs as monotherapy, use is based on the advantages of mAbs in combination with other chemotherapy regimens.

Another therapeutic option for patients with hepatic metastases is tumor ablation. Several ablative options exist, including radiofrequency ablation (RFA) and microwave coagulation. RFA demonstrated a higher recurrence rate compared with hepatic resection, but was associated with an acceptable morbidity rate. Experience with microwave coagulation is limited [2]. A new and promising therapy exists in the form of selective internal radiation therapy (SIRT), in which radioactive microspheres containing yttrium-90, a β-radiation emitter, are delivered via a hepatic arterial catheter. A Phase III trial found favorable results when combining SIRT with HAI therapy [10].

Much like surgical resection, ablative techniques are limited by size, location, and number of tumors in the liver. Chemotherapy and mAbs have associated toxicities that limit use. Despite improved response rates being achieved in mCRC with these therapies, overall 5-year survival for mCRC disease remains < 40%. Novel therapies are needed for patients with mCRC, especially those with recurrent disease, unresectable disease, and disease refractory to current available therapies.

3. Oncolytic viral therapy with herpes simplex virus type-1

Oncolytic viral therapy aims to direct the innate cytolytic ability of viruses to specific killing of cancer cells. A wide variety of viruses have been studied including adenovirus, vaccinia virus, myxoma virus, vesicular stomatitis virus, Newcastle disease virus, and herpes simplex virus [11]. All of these viruses display the ability to infect, replicate in, and lyse host cells, but they differ in innate tumor specificity and in their ability to tolerate genetic manipulation.

Herpes simplex virus type-1 (HSV-1) displays many innate characteristics that make it desirable as a gene therapy vector. The wild-type virus is naturally cytotoxic, destroying host cells by lysis in the course of its life cycle. HSV-1 is able to infect and replicate in a wide variety of cell types by interaction of its surface glycoproteins with cell surface receptors. For example, HSV-1 glycoprotein D (gD) binds the cellular receptor nectin-1 to achieve cell entry. Nectin-1 is not accessible in normal cells because it is present as a component of the adherens junction complex. In cancer cells with decreased intracellular adherence, however, nectin-1 is present in its free form and mediates herpes viral entry [12,13]. Because herpesvirus replicates in cancer cells prior to lysing them, the initial administered dose is progressively amplified over the course of therapy. The HSV-1 genome has an enormous coding capacity for gene delivery. One concern is that HSV-1 is capable of latent infection existing as an episome [14]. However, this virus is susceptible to antiviral therapies and thus has a safeguard against uncontrolled replication [11,15].

The HSV-1 viral genome consists of two unique sequences, unique long and unique short (denoted U_L and U_S, respectively), flanked by inverted repeat sequences, totaling approximately 152 kbp [16]. It has been found that of the 83 open reading frames (ORFs) expressing proteins in the HSV-1 genome, 38 are indispensable for successful viral infection and replication. The remaining 45, however, are not necessary for viral replication in at least some cell types, and can be deleted or modified to improve safety profile and to confer tumor specificity.

Recombinant HSV-1 mutants are under preclinical and clinical investigation for several gene therapy applications, including targeted gene delivery to the central nervous system, vaccination, and targeted anticancer therapy. Several replication-competent HSV-1 mutants have been shown to be effective against a wide variety of human cancer types, including brain, squamous cell, esophageal, gastric, colorectal, pancreatic, hepatocellular, lung, bladder, prostate, breast, melanoma, renal cell, ovarian, soft-tissue (rhabdomyosarcoma), and neural (neuroblastoma and peripheral nerve sheath tumors) [17–37]. These oncolytic herpes vectors have deletions of various viral genes. Host cell proteins that are equivalent or homologous to the missing viral gene products are abundant in malignant cells, but not in normal cells [38]. These deletions, therefore, render the engineered viruses cancer-specific. Table 1 summarizes oncolytic herpes simplex virus constructs that have gone to clinical trial [39–46].

4. Engineering of tumor specificity in HSV-1 mutant NV1020

NV1020 is an attenuated, replication-competent, recombinant virus derived from the HSV-1 strain R7020, and was originally designed as a potential HSV-2 vaccine [47]. The construction of the parent strain R7020 was described previously [48], and this virus was proven safe for administration in rodents and primates [48,49]. NV1020 was attenuated by deletion of a 15-kb region at the $U_{L/S}$ junction encompassing one copy of the diploid genes $\alpha0$, $\alpha4$, and $\gamma_1 34.5$ encoding the proteins ICP0, ICP4, and ICP34.5, respectively, and one copy of $U_L 56$, the protein product of which has not been fully characterized but is thought to be involved in neuroinvasiveness of HSV-1 [16,18,45,50]. ICP0 is one of the 45 dispensable gene products but mutants lacking this gene are debilitated at low multiplicity of infection (MOI, number of virus particles per cell) [16]. ICP4 is one of the indispensable gene products; at least one copy of the gene must be present for viral replication. ICP4 blocks apoptosis and positively regulates many other genes in the HSV-1 genome. ICP34.5 is a dispensable gene product. It functions to block the host cell protective mechanism of shutting off protein synthesis in response to viral infection by inhibiting a cellular protein kinase, PKR. Mutants missing this gene are therefore attenuated. In addition, $\gamma_1 34.5$ confers the ability of HSV to thrive, particularly in the central nervous system. The mechanism behind this neurovirulence effect of the gene is not known.

NV1020 is further attenuated by a 700-bp deletion encompassing the thymidine kinase (TK) gene locus and the promoter for the gene $U_L 24$. This deletion effectively prevents the expression of $U_L 24$, which is thought to encode a membrane-associated protein of unknown function [16].

In addition to these deletions, NV1020 carries an exogenous, inserted copy of the *tk* gene under restrictive control of the ICP4 promoter, and a 5.2-kb fragment of HSV-2 DNA as it was originally designed as an HSV-2 vaccine. Both of these insertions are located at the $U_{L/S}$ junction [18]. A schematic of the modified genome of NV1020 is illustrated in Figure 1.

These modifications render NV1020 highly attenuated, so that it can only propagate in transformed cells. Attenuated viruses rely on host cell proteins to compensate for the missing viral gene products, and many of these proteins are expressed only in post-mitotic, replicating cells in G1/S phase [51]. For example, cellular TK is not expressed in quiescent, normal cells, but is expressed abundantly in rapidly dividing malignant cells. Another common alteration in cells that have undergone malignant transformation is constitutive activation of the Ras signaling pathway. Ras activity directly blocks the effects of cellular PKR, so while the viral protein ICP34.5 is required for viral propagation in normal cells, it is not needed in cells with activated Ras [52].

5. Preclinical experience with NV1020

NV1020 has been shown to be efficacious in killing an enormous variety of cancer cell types *in vitro* and in animal models. It has been tested in pancreatic adenocarcinoma, mesothelioma, rhabdomyosarcoma, osteosarcoma, bladder, lung, gastric, and colorectal cancer, and in metastases to the liver and peritoneum [20,22,23,25,27,35,53,54] (Figure 2).

In 2000, Delman and colleagues reported a study designed to determine the effect of pre-existing immunity to HSV on oncolytic therapy with herpes vectors [6]. In this study, immunocompetent animals were immunized with HSV and tested for antibody titers. A model of hepatic metastasis was developed by splenic injection of CT-26 murine colorectal cancer cells. Animals were then treated with NV1020 administered intravenously or via the hepatic artery. The researchers found that pre-existing immunity somewhat abrogated the

oncolytic effect of intravenously administered NV1020, but had no effect when the virus was administered locoregionally. They concluded that when given in reasonable proximity to the tumor target, oncolytic therapy with HSV is still effective against hepatic malignancy in the setting of anti-HSV immunity.

Another animal study reported in 2002 investigated the safety profile of NV1020 after regional and systemic administration [20]. In this study, a xenograft model of carcinomatosis was developed in athymic mice with human gastric cancer cell lines. NV1020 administered intraperitoneally significantly reduced tumor burden (from 1380 ± 310 mg in control animals to 150 ± 40 mg in treated animals; $p < 0.005$). In addition, brain, liver, kidneys, and tumor were harvested from treated animals for evaluation for HSV biodistribution. There was no HSV staining by immunohistochemisty (IHC) and no necrosis noted in any non-tumor tissues.

Preclinical data up to this point clearly demonstrated NV1020 to be a safe, effective, and tumor-specific oncolytic agent *in vivo*, and warranted bringing this therapy to clinical trial. Investigators proposed the use of oncolytic HSV in combination with liver resection to enhance killing of hepatic malignancy. This proposal, however, raised the concern that highly metabolically active regenerating hepatocytes in the post-hepatectomy period may themselves be susceptible to infection by oncolytic HSV.

This question was addressed in a study by Delman and colleagues in 2004 [55]. This study demonstrated that in C57Bl/6 mice, hepatocyte mitotic activity peaked 24 – 48 h after 70% hepatectomy and returned to baseline by 96 h. NV1020 was administered via portal vein injection at various time points after hepatectomy. It was found that administration of virus within the peak period of hepatocyte regeneration (24 – 48 h) permitted transient productive viral infection of tissue that does not normally support viral growth. Virus administered after the regenerative peak ($\geq$ 96 h after hepatectomy) was not able to infect liver parenchyma. This data suggest that NV1020 can be administered safely in the adjuvant setting after partial hepatectomy, if given after the peak of hepatic regeneration.

6. Clinical experience with NV1020

Given such promising preclinical data and an excellent safety profile, NV1020 was taken to clinical trial in a Phase I study for administration to patients with refractory mCRC to the liver [43]. All patients had undergone partial hepatectomy and adjuvant chemotherapy prior to participating in this study. NV1020 was administered via HAI to four cohorts of three patients each at progressively escalating doses. The administered doses were 3×10^6, 1×10^7, 3×10^7, and finally 1×10^8 pfu. All patients received a chemotherapy infusion pump and cycles of FUDR plus systemic chemotherapy 1 and 2 months after administration of virus. No complications or adverse events occurred that were severe enough to define a maximum tolerated dose (MTD) for NV1020.

The most frequent adverse events attributable to virus infusion (occurring before pump placement) were pyrexia (50%), headache (50%), and rigors (25%). Only one serious adverse event occurred that was thought to be due to virus administration. This was a transient grade 4 elevation of γ-glutamyltransferase (GGT) in one patient, occurring 12 h after virus administration and normalizing by 24 h. There were no other significant abnormalities in blood chemistries, including liver function tests and serum cytokine levels. Additionally, serial monitoring of hepatic vein blood after virus infusion confirmed that the majority of virus was cleared by the liver. Very little virus reached systemic circulation at the higher doses of 3×10^7 and 1×10^8 pfu. There were no deaths in the study.

Patients underwent radiologic assessment for anti-tumor activity 28 days after administration of virus. Two patients in the higher-dose cohorts exhibited reduction in tumor size (39 and 20%), three exhibited progression of disease, and seven had stable disease.

Long-term follow-up of these 12 patients revealed that they all exhibited reduction in tumor size in response to subsequent chemotherapy. In one patient, NV1020 was detected by polymerase chain reaction (PCR) not only in tumor tissue from the virus-perfused lobe of the liver but also in a tumor from the non-perfused lobe. No patients developed reactivation of herpes or adverse effects as a result of treatment during the course of follow-up. The median survival for the group was 25 months. One patient with the greatest response to NV1020 is still alive at present (62 months after virus administration). The other 11 patients all died of disease progression (Y Fong, unpublished data, 2007).

An additional Phase I/II trial of NV1020 for colorectal cancer hepatic metastases with multiple dosing is underway. The Phase II component of this trial aims to determine the therapeutic effects of the optimally tolerated dose of NV1020 as determined by the Phase I component [56]. Results of this trial are not yet available.

7. Conclusion

In summary, there is demanding need for novel therapies for mCRC as well as for other hepatic malignancies. While surgical resection offers cure, a staggering number of patients are found to have unresectable disease on presentation or develop recurrent disease after resection. For these reasons, survival for hepatic malignancy is dismal.

Multimutated oncolytic herpes simplex viruses are in clinical trials as novel therapies for various malignancies (Table 1). While operating under the same principle of cancer-specific cell lysis, each herpes virus construct is different and each has a disease setting and route of administration with which it works best. NV1020 has been under investigation as an oncolytic agent for decades and has been shown to effectively kill a diversity of human cancer cell lines *in vitro* and *in vivo*. Even when given as early as 96 h after hepatectomy, NV1020 does not infect regenerating hepatocytes, which have upregulated metabolic pathways much like malignant cells. In a Phase I clinical trial, NV1020 was safely administered to the hepatic vasculature in 12 patients. The virus produced biologic changes in this toxicity study with single-dose administration.

8. Expert opinion

NV1020 holds great promise as a novel therapeutic agent for refractory hepatic malignancies. The results of the Phase I clinical trial demonstrate a clear biologic response to the virus without approaching a MTD. NV1020, unlike other therapies, remains biologically active once administered to tumor tissue. It is able to replicate *in vivo*, amplifying the initial administered dose, and is able to spread to tumor tissue adjacent to and even distant from the site of administration. This virus has been proven safe, even in the post-hepatectomy setting of hepatocyte regeneration. In addition, NV1020 has been shown to exhibit a synergistic cancer killing effect when used in combination with other therapies [22].

Preclinical studies have shown that oncolytic HSVs work best when administered in multiple doses [57,58]. Therefore, a logical next step in determining the full therapeutic potential of NV1020 against colorectal cancer liver metastases would be a clinical trial with multiple dosing regimens. The efficacy of NV1020 against other hepatic malignancies such as HCC and biliary neoplasms could also be explored in a similar manner. There could be roles for NV1020 as second- or third-line therapy for unresectable refractory hepatic disease,

as adjuvant therapy after hepatic resection, or as neoadjuvant therapy to prevent tumor progression while patients wait for liver transplant.

A foreseeable problem for use of NV1020 and any oncolytic virus in humans is delineating exactly what biologic effects result directly from virus administration, as oncolytic HSVs will be used for patients with refractory disease who have already received and are possibly still receiving standard therapies. In addition, it has been reported that oncolytic HSVs can mutate [59]. Such a mutation would likely further attenuate the virus, and may decrease therapeutic efficacy, but would not likely affect the safety profile, as the virus would still be missing important virulence genes.

If NV1020 is proven to be a safe therapeutic agent at doses that are clinically effective when administered intra-arterially, directly into the blood supply of a vital organ, it would follow that this virus would be safe in less potentially hazardous settings such as intratumoral administration into soft-tissue malignancies and intrapleural and intraperitoneal administration for chest and abdominal disease, respectively. The clinical settings in which NV1020 could potentially be efficacious are unlimited.

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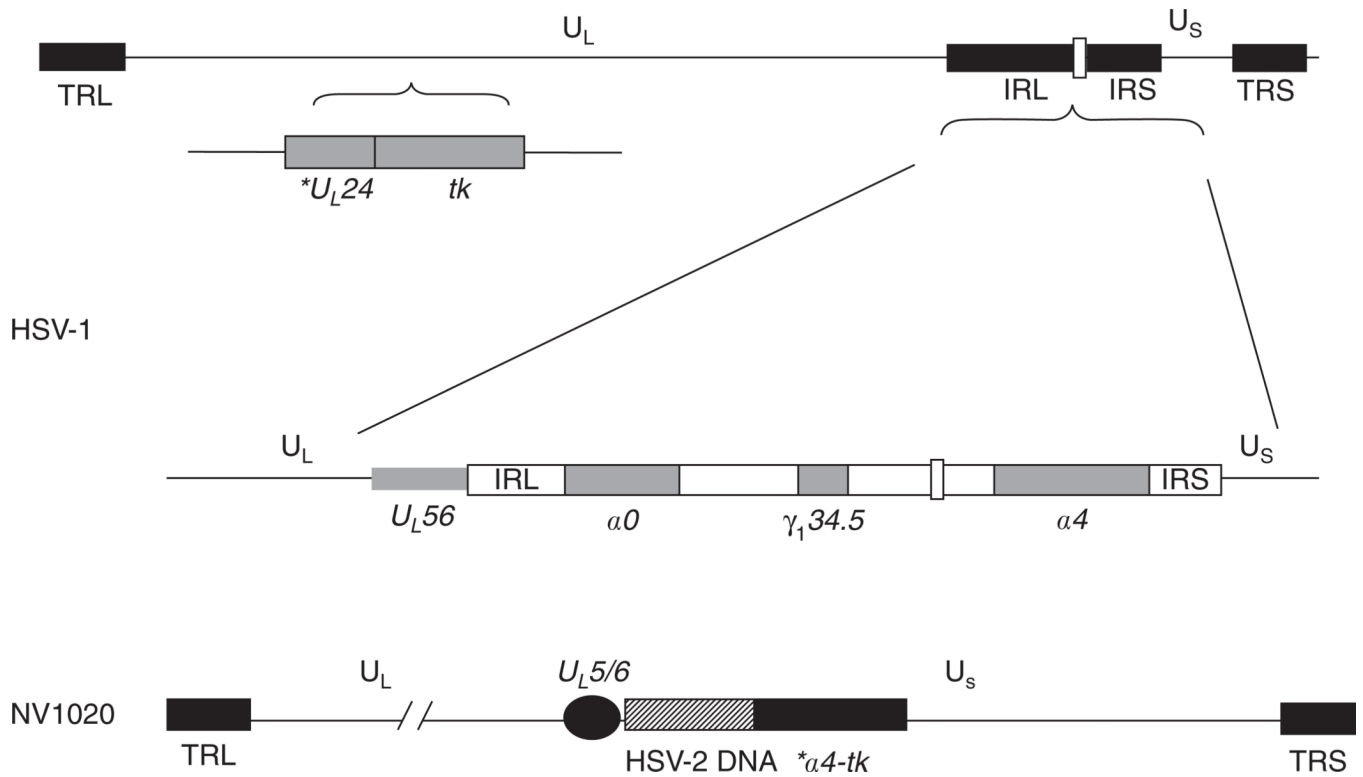
Figure 1. Construct of oncolytic herpes simplex virus recombinant NV1020
NV1020 was attenuated by deletion of a 15-kb region at the $U_{L/S}$ junction encoding the proteins ICP0, ICP4, and ICP34.5, and one copy of the gene $U_L 56$. In addition, NV1020 has a 700-bp deletion encompassing the thymidine kinase (TK) gene locus and the promoter for the gene $U_L 24$. The novel $U_{L/S}$ junction of NV1020 carries a 3.7-kb duplication of $U_L 5/6$, a 5.2-kb fragment of HSV-2 DNA, and an exogenous, inserted copy of the *tk* gene under restrictive control of the ICP4 promoter.
* Denotes *promoter* for *UL24* and *a4*, not entire gene.

1992

R7020 administered to 39 patients as HSV-2 vaccine with no toxic effects

2002

NV1020 shown to be effective against human NSCLC *in vitro* and in a pleural tumor model

2004

NV1020 proven safe when administered via the hepatic artery as early as 96 h after partial hepatectomy

1990

R7020 safely administered to immunocompetent and immunosuppressed primates

2000

NV1020 shown to be effective against human pancreatic cancer cell lines and xenograft tumors

2002

Human prostate cancer cell lines and xenograft tumors effectively killed with NV1020

2006

NV1020 shown to have synergystic efficacy when used in combination with 5-FU in human colorectal cancer both *in vitro* and in a xenograft model

R7020 as an HSV-2 vaccine
1988 – 1999

NV1020 as an oncolytic agent
2000 – Present

R7020 described in detail and evaluated in rodents

1988

Oncolytic therapy with NV1020 shown to be effective even in setting of preexisting immunity to HSV

2000

2002

NV1020 effective and safe for treatment of peritoneally disseminated cancer in an animal model

2006

NV1020 shown to have effective against human malignant pleural mesothelioma cell lines *in vitro*

NV1020 designed from HSV-1 R7020

Late 1990s

NV1020 shown to be effective against head and neck SCC *in vitro* and in flank tumors, and against bladder cancer in a syngeneic tumor model

2001

NV1020 shown to be effective in killing human rhabdomyosarcoma and osteosarcoma cell *in vitro*

2002

Distribution and efficacy of NV1020 in large, bulky tumors shown to be improved with fractionated intratumoral administration in rhabdomyosarcoma
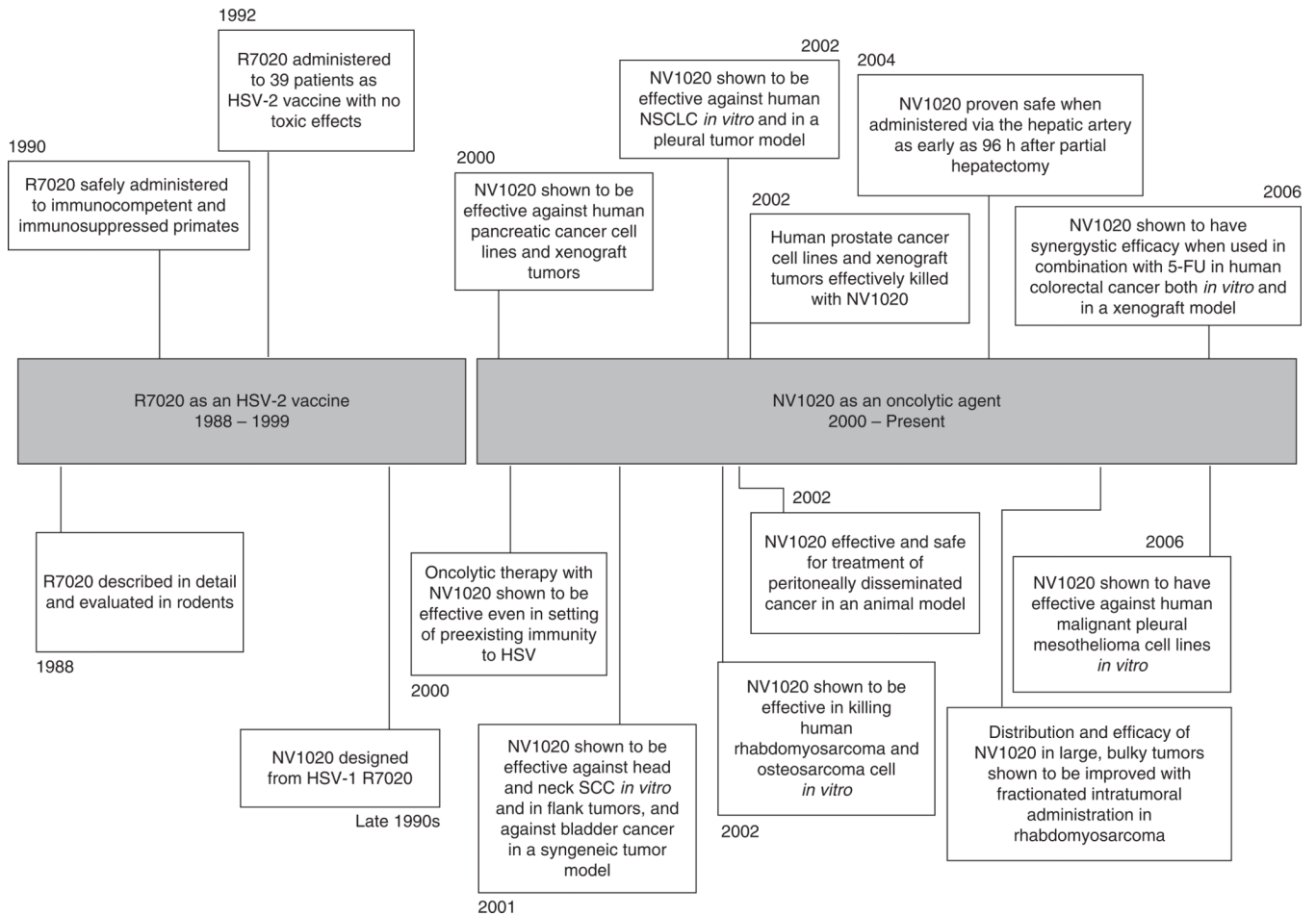
Figure 2. Timeline of development and preclinical investigation of NV1020
NV1020 was originally designed as a potential anti-HSV-2 vaccine. It was not until the year 2000, 12 years after the parent strain was designed, that NV1020 came to be investigated as an oncolytic agent. From 2000 to present, multiple preclinical studies have been performed demonstrating the efficacy of this virus against a variety of cancer types and establishing its safety profile.

Table 1

Oncolytic herpes simplex viruses in clinical trial.

Virus	Disease	Route of Administration and MTD	Toxicity
G207 [39]	Recurrent glioma	Intratumoral, tumor bed	MTD not determined, safe up to 3×10^9 pfu
HS VI716 [40–42]	Recurrent and high-grade glioma, melanoma	Intratumoral	MTD not determined, well-tolerated at single dose of 1×10^5 pfu and up to 4 doses of 1×10^3 pfu
NV1020 [43]	Liver metastases from colorectal cancer	Intraarterial (Hepatic artery)	MTD not determined; confirmed safe up to 1×10^8 pfu
OncoVEX^{GM-CSF} [44]	Cutaneous and subcutaneous metastases from melanoma, breast, head and neck, and colorectal cancers	Intratumoral (multiple dosing)	Dose-limiting local reaction with single admin of 1×10^7 pfu in seronegative patients; well-tolerated up to 1×10^8 pfu after seroconversion from 1×10^6 pfu dose (MTD not determined)
HF10 [45,46]	Cutaneous and subcutaneous breast cancer metastases	Intratumoral (multiple dosing)	MTD not determined, well-tolerated up to 3 doses of 5×10^5 pfu